PROCEEDINGS

WORKSHOP:
Diagnostics in honeybees: from sampling to data analyses

August 30 - September 1, 2010
Ghent University, Ghent (Gent/Gand), Belgium

organized by the BEEDOC Diagnostics Department
(Prof. Dr. Dirk de Graaf, UGent and Dr. Peter Neumann, ALP)

PROGRAMME

Monday August 30

Start	End	Topic	Speaker	Abstract
13.30	14.00	Reception		
14.00	14.10	Welcome	D. de Graaf	
14.10	14.50	EU projects/networks	P. Neumann	O-1
14.50	15.30	Quality control	H. Imberechts	O-2
15.30	15.45	Break		
15.45	16.00	Transport to apiary		
16.00	17.30	Demonstration: sampling a beehive	F. Jacobs and D. Laget	
18.30	-	Dinner		

Tuesday August 31

Start	End	Topic	Speaker	Abstract
09.00	09.10	Announcements	P. Neumann	
09.10	09.50	Honeybee viruses	J. de Miranda	O-3
09.50	10.30	Presence and distribution of honeybee viruses in Spanish colonies	M. Vicente	O-4
10.30	10.45	Break		
10.45	11.25	Nosemosis: old story, new issue	D. de Graaf	O-5
11.25	12.05	BEEDOC diagnostics department	L. De Smet	O-6
12.05	-	Lunch		
14.00	14.10	Announcements	P. Neumann	
14.10	14.50	AFB and EFB	M. Watkins	O-7
14.50	15.30	Harmonizing AFB diagnosis	D. de Graaf	O-8
15.30	15.45	Break		
15.45	16.00	Transport to the lab		
16.00	17.00	Demonstration field test AFB	M. Brunain	
19.00	-	Social Dinner (reservation required)		

Wednesday September 1

Start	End	Topic	Speaker	
09.00	09.10	Announcements	D. de Graaf	
09.10	09.50	Belgian epidemiological study	B.K. Nguyen	O-9
09.50	10.30	Strategy to control bee diseases in the Netherlands	J. van der Steen	O-10
10.30	10.45	Break		
10.45	11.25	Small hive beetle	P. Neumann	O-11
11.25	12.05	Invasive parasites and bumblebee diversity loss: a plea for molecular detection of bumblebee parasites	I. Meeus	O-12
12.05	12.30	Discussion and closure		

ABSTRACTS

Oral presentations:

Abstract O-1:

EU projects / networks

Peter Neumann

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COLOSS (Prevention of honey bee COlony LOSSes) is a COST funded network currently consisting of 212 scientists, extension specialists, beekeepers and industry partners from 52 countries with the aim to understand and limit losses of honey bee colonies. For that purpose, conferences, workshops and Short Term Scientific Missions are organized to foster collaboration between laboratories, thereby limiting redundancy and instead promoting mutual enrichment. Initially, the development of international standards in monitoring and research is the focus. COLOSS is linked to various national and international ongoing European, North- and South-American programs, which ensures global visibility and fosters dissemination of the findings to a wide range of stakeholders for honey bee health. Within the FP7, two large-scale research projects have been granted, which are both closely linked with COLOSS:

The **BEE DOC** (BEes in Europe and the Decline Of Colonies) comprises a network of 11 partners from honeybee pathology, chemistry, genetics and apicultural extension aiming to improve honey bee colony health. The BEE DOC will empirically and experimentally fill knowledge gaps in honey bee pests and diseases, including the 'colony collapse disorder' and quantify the impact of interactions between parasites, pathogens and pesticides on honey bee mortality. The BEE DOC will develop new diagnostic methods and sustainable concepts for disease prevention.

STEP (Status and Trends in European Pollinators) consists of 20 partners with a strong focus on ecology and will document recent trends in pollinators and insect-pollinated plants as well as assess the role of different drivers in causing such trends. STEP will also consider the ecological and economical impacts of these changes and of potential mitigation actions that may be taken. STEP will take a wide-ranging approach by considering both managed pollinators (honey bees and some bumblebee and solitary bee species) and wild ones (bumblebees, solitary bees, hoverflies and butterflies).

Abstract O-2:

Quality control aspects of diagnostic tests for infectious diseases in honeybees

H. Imberechts

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Quality control in the laboratory dealing with diagnosis of animal infectious diseases has become a prerequisite, not only for the owner and the laboratory itself, but also for the competent authority who has to manage possible outbreaks. The main objectives of quality control systems are the tracing of the sample flow and of all events that may influence the outcome of the tests. Validation of tests for bacterial, parasitic or viral infections depends in large on internal quality controls and proficiency testing. In addition, also the laboratory's management, the recruitment and training of personnel, the purchase and maintenance of infrastructure, and the management of contaminated waste, all play a role in the quality assurance. Confirmation of suspected or positive test results, the evaluation of new diagnostic assays, the acquisition and evaluation of reference material and other aspects are specific tasks that should be done by reference laboratories that guarantee the best quality of the diagnostic tests.

Abstract O-3:

Honey bee viruses

Joachim R. de Miranda

Honeybees are hosts to many viruses, several of which can cause serious diseases. Most of these are RNA viruses, and most are generally insignificant in healthy, productive colonies. However, new stresses and exotic parasites have significantly altered this perspective and viruses are increasingly responsible, or co-responsible for colony deaths. Currently the most critical viruses for bee health are the picorna-like viruses (the iflaviruses and dicistroviruses) since several of these are transmitted by Varroa destructor and others are linked, either directly or indirectly, with rapid adult de-population (dwindling or Colony Collapse Disorder). Yet others are associated with Nosema and other internal parasites. The accurate diagnosis of the virus status of colonies and apiaries is therefore of major importance for bee health management. Viruses are the ultimate opportunistic pathogens, capable of rapid, exponential proliferation under the right circumstances. This logarithmic tendency of virus replication is critical for evaluating disease status, transmission risk, sampling strategy, assay development and data analysis. The need for an optimized sampling strategy suitable for all major viruses, as well as the progress towards a robust, practical field assay for adult and brood virus diseases will be discussed.

Abstract O-4:

Presence and distribution of honeybee viruses in Spanish colonies

Marina Vicente, Deborah Kukielka and J.M. Sánchez- Vizcaíno

Animal Health Department, Center VisaVet, Complutense University of Madrid, Spain

In the recent years the number of honeybees *Apis mellifera* has decreased dramatically in a worldwide phenomena called Collony Collapse Disorder (CCD).The direct importance of the honeybee productions and the indirect effect of this insect as a pollinator of the main crops in USA and UE, where Spain is the first honey producer, has provided numerous studies of CCD. However, the causes of this syndrome are still unknown, although most authors point a multifactorial problem where pathogens play an important role. Among which, viruses are the most unknown and their presence in CCD events alone or in co- infection with other pathogens such as *Varroa* and *Nosema* need further researches.

The aim of this work was to perform the first epidemiological description of the presence of honeybee viruses in Spain and their relationship with CCD and other honeybee pathogens.

On the one hand, different real time RT-PCR-based procedures developed or adapted by us were used to analyze the presence of 7 viruses (Deformed Wing Virus (DWV), Black Queen Cell Virus (BQCV), Sacbrood Virus (SBV), Kashmeer Bee Virus (KBV), Chronic and Acute Paralysis Virus (CBPV and ABPV respectively) and Israeli Acute Paralysis Virus (IAPV)), finding most of them in the Spanish bee colonies. DWV was the most prevalent virus and was highly detected in co-infection with BQCV. Besides, the presence of IAPV was described for the first time in Spain. To update and complete information, new samplings and analysis were performed this year, detecting other IAPV positive samples in the South of Spain.

On the other hand, results were interpreted with the epidemiological survey information of each sample so as to relate the presence of these viruses alone or in co-infection with other pathogens with the CCD.

This work could be useful for the implementation of surveillance programs to control bee losses in Spain.

Abstract O-5:

Nosemosis: old story, new issue

Dirk C. de Graaf

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The microsporidian parasite *Nosema ceranae* jumped recently from the Asian honeybee *Apis cerana* to the European honeybee *Apis mellifera*. This was discovered almost simultaneously and indepently from each other in two different research groups. The beekeeping sector has been confronted already for centuries with another microsporidian parasite, *Nosema apis*, but the beekeepers have learned how to deal with it. Although the information of the emerging *Nosema* disease with respect to its impact on the colony losses was rather conflicting, the sensitivity of the *N. ceranae* spores for freezing temperatures possibly provides a simple explanation why the *N. ceranae* disease is mainly a problem in warmer climates. Although the spores of the two species differ in size and shape slightly, microscopy does not provide a reliable differentiation tool for mixed samples. On the contrary, several PCR-based techniques can distinguish these two species very well. Haplotyping based on rRNA genes seems no reliable marker for differentiation. And recently a methodology to quantify the spore-loads by real-time PCR has also been developed.

Abstract O-6:

BEEDOC DIAGNOSTICS DEPARTMENT

L. DE SMET, F.J. JACOBS, D.C. DE GRAAF

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Insects, with solitary and social bees as most important group, play a crucial role in the reproduction of many crops and wild plants. Unfortunately, beekeeping is a declining industry. One of the principal reasons for the decline in managed honeybee colonies is extensive and unpredictable colony death. These losses are defined by a rapid loss of adult worker bees in colonies and the lack of apparent symptoms, leading to the nebulous label of 'Colony Collapse Disorder' (CCD). When apiculturist identifies symptoms at the colony level, and then starts diagnostic procedures to identify the disease and initiate a treatment, it often comes too late to save or cure the colony. Consequently, there is a clear need for fast, reliable, sensitive and cheap diagnostic tools that alert the beekeeper to potential problems before colony level symptoms appear.

The aim of the BEEDOC DIAGNOSTIC DEPARTMENT is to develop diagnostic tools which detect for pathogens and pesticides exposure. These will cover three levels of application: research grade; extension grade and field grade. For the **research grade** diagnostic tool we will develop a DNA chip for rapid screening of gene expression related to honeybee detoxification, nutritional and immune status and to a broad range of pathogens. This will be an extension of the BEE-PATH developed by Evans J (2006) with targets discovered in WP3 and WP4 and specific probes for different viruses. We will develop a colorimetric DNA chip to avoid the need for expensive instruments such as a laser scanner. Because of its lower cost and simplicity, a colorimetric DNA chip is technically feasible for diagnostic applications. The **extension grade** diagnostic tool will be based on PCR. Here we would like to develop an multiplex ligation-dependent probe amplification (MLPA®) based method. This method makes a nucleic acid sample suitable for a multiplex PCR reaction, in which up to 45 specific nucleic acid sequences are amplified simultaneously, using a single PCR primer pair. The third assay which will be developed is **a field grade** diagnostic tool such as dip-and-read-sticks which will be based on qualitative immune-chromatography. The last assay will be developed in collaboration with VITA®.

References:

Evans J (2006) Beepath: an ordered quantitative-PCR array for exploring honey bee immunity and disease. J. Invertebr. Pathol. **93**, 135-139

Abstract O-7:

Max Watkins

Abstract O-8:

Harmonizing AFB diagnosis

Dirk C. de Graaf

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Worldwide, American foulbrood (AFB) is the most devastating bacterial disease of the honey bee (*Apis mellifera*). Because the distinction between AFB and powdery scale disease is no longer considered valid, the pathogenic agent has recently been reclassified as one species *Paenibacillus larvae*, eliminating the subspecies designations *Paenibacillus larvae* subsp. larvae and *Paenibacillus larvae* subsp. *pulvifaciens*. The creamy or dark brown, glue-like larval remains of infected larvae continue to provide the most obvious clinical symptom of AFB, although it is not conclusive. Several sensitive and selective culture media are available for isolation of this spore-forming bacterium, with the type of samples that may be utilized for detection of the organism being further expanded. PCR methods for identification and genotyping of the pathogen have now been extensively developed. Nevertheless, biochemical profiling, bacteriophage sensitivity, immunotechniques and microscopy of suspect bacterial strains are entirely adequate for routine identification purposes. Recent proficiency tests demonstrate the need to further implement quality control measurements in the routine diagnosis of bee diseases. The number of techniques permitted for the identification of the AFB pathogenic agent has been reduced significantly since the last revision of the OIE Manual, but the work is not finished yet. The forthcoming publication of the BEEBOOK will be a excellent opportunity to further harmonize our diagnostic tools, especially with respect to AFB.

Abstract O-9:

Bach Kim Nguyen

Abstract O-10:

Strategy to control bee diseases in the Netherlands

J. van der Steen, B. Cornelissen, T. Blacquière

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Introduction

In the Netherlands the beekeepers themselves are responsible for honeybee health. Except for American Foulbrood which, must be reported to the Ministry of Agriculture, there are no legal regulations for the control of honeybee diseases.

Two research programs are currently performed:

1. “Maatregelen tot verbetering van de productie en afzet van producten van de bijenteelt”. This program, implemented by PRI bees@wur, is focused on sustainable control for *Varroa destructor* in relation to other bee diseases, environment and apicultural practice. Within this program all beekeepers can send in bee samples for bee disease checks (Varroa, Nosema spp, *Malpighamoeba* Prell, EFB and AFB). This program is funded by the EU / Ministry of Agriculture

2. BIJ-1. This program is funded by the Ministry of Agriculture (Min LNV) and is implemented by a consortium of NCB, PRI bees@wur and Alterra/EIS and the help of beekeepers. It focuses on monitoring of colony losses, factors that affect colony losses, particularly Nosema spp and environment, and the impact of apiculture and changing environment on wild bees.

The Dutch beekeepers are informed about Best Apicultural Practice by way of lectures, publications in the beekeeping magazines and work shops of the researchers of PRI bees@wur and NCB, a two-month e-newsletter by PRI bees@wur and the website of PRI bees@wur (bijen.wur.nl).

Varroa destructor

The recommended control technique for *Varroa destructor* is based on the scientific studies performed by PRI bees@wur and the international recognized bee institutes abroad.

The objective of the sustainable control of *Varroa destructor* is to have a fit healthy, virtually mite free winter population. To achieve this the beekeepers are strongly advised to apply brood removal in spring, an oxalic acid treatment in brood less colonies in May in combination with the making of artificial swarms, treatment of the colonies in the period ½ July till ½ August with formic acid or thymol products and a winter treatment in December with oxalic acid.

In 2010 PRI bees@wur published an updated Varroa control manual. This manual was sent to all registered beekeepers and can be downloaded from the PRI bees@wur website (www.bijen.wur.nl).

Nosema apis and Nosema ceranae

The recommendation to control Nosema spp. is focused on prevention. No medical treatment is allowed in the Netherlands. The essence of the advice is hygiene and continuous adequate pollen flow to have strong fit colonies that can cope with a Nosema infection. In 2010 an updated Nosema leaflet with the latest relevant scientific information and practical hints was sent to the beekeepers via the e-newsletter and can be downloaded from the PRI bees@wur website.

EFB

The prevalence of European Foulbrood increased the last decade. The beekeepers are advised to apply the shook swarm method to control EFB.

AFB

American Foulbrood is, contrarily to surrounding countries, rare in the Netherlands. AFB was reported in 2006 and 2008

Dutch beekeepers are compelled to report a suspect of AFB in their apiary to the Ministry of agriculture. An American Foulbrood infection must be legally confirmed by CVI (Centraal Veterinair Instituut)

The beekeepers, organized in beekeepers associations, are compelled to control AFB. Regionally bee health coordinators (trained and skilled beekeepers) coordinate the control, assisted by PRI bees@wur. In practice a “cordon sanitaire” is laid around the AFB infected apiary. A cordon sanitaire has a radius of 3 km around the apiary. No colonies may be removed and taken in or out the cordon sanitaire. This is checked by the AID (inspection service of the Ministry of Agriculture)

All colonies within this ‘cordon sanitaire” are checked by the health coordinators using the VITA AFB test. A positive outcome of the VITA test must be confirmed by the CVI. When the infected apiaries are charted, the colonies showing clinical symptoms are killed and destroyed in the regional incinerator. The hive and frames are cleaned by washing with natron (sodium bicarbonate) solution 6% and subsequently rinsed with water. The colonies showing no clinical symptoms are treated with the shook swarm method. Six weeks after the removal of the colonies and the application of the shook swarm methods all apiaries in the cordon sanitaire are checked again. In case new infections are detected the procedure starts all over again. In case no colonies showing clinical AFB symptoms are detected the cordon sanitaire is cancelled.

Results Nationwide Monitoring bee diseases in 2008

In June 2008 a nationwide monitoring study as conducted. In total of 170 apiaries colonies were sampled and pooled to apiary samples. The study revealed that

- *Paenibacillus larvae* < 1% (1 of 170)

- *Melissococcus pluton* 36%
- *Nosema apis* 10%
- *Nosema ceranae* 87%
- DWV: 16%
- BQCV 92%
- SBV 40%

Aethina tumida* and *Tropilaelaps clareae

Honeybee queens imported in the Netherlands are registered by the customs office. The beekeeper who imported the queen can introduce the queen in a hive with a closed flight entrance. The accompanying worker bees must be sent to PRI bees@wur for check of *Aethina tumida* and *Tropilaelaps clareae*. In case no *Aethina* and *Tropilaelaps* of detected the hive can be opened. In case the parasites are detected the colony will be destroyed. The results are reported to the Ministry of Agriculture. There is no contingency plan in case *Aethina tumida* or *Tropilaelaps clareae* have entered the Netherlands unnoticed. The control will be regulated on EU level.

Results free disease checks at PRI bees@wur

The number of samples increased from 45 in 2006 to 106 in 2010.

In 2010 samples of 91 dead colonies and 15 of living weakened colonies were sent in.

In 2010, 64% of the dead colonies had a Varroa infestation > 6%. Serious nosema-infection were detected in 21% of the dead colonies. Of the weakened colonies the infection rate of Varroa (> 6%) and nosema spp was 7% and 60% respectively.

In the 14 brood samples, 75% - 100% appeared to be infested by *Varroa destructor*.. In 87.5% EFB was detected. No AFB and chalk brood was detected.

Remark

Extension service based on scientific data and tested in the apicultural field is a requirement of best apicultural practice. This re-enforces on one hand the combination of fundamental and applied research and on the other hand a well equipped extension service. Nowadays information both reliable and not-reliable is easily available. This implies that beekeepers must be well informed to make a considered choice about the control of honeybee diseases and parasites. .

Abstract O-11:

Small hive beetles

Peter Neumann

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Small hive beetles, *Aethina tumida*, are an invasive pest species originating from sub-Saharan Africa and have become well established in North America and Australia, where they can cause substantial damage to local apiculture under suitable environmental conditions. While diagnosis of eggs and larvae is only possible with molecular tools, the adults can be precisely determined to *A. tumida*. Nevertheless, differential diagnosis of adults requires morphometric details due to considerable variation in coloration and body size and similarity to other Nitidulidae such as *Cychramus luteus*. Quantitative diagnosis outside of hives can be achieved with baited traps to attract the free-flying adults. In hives, quantitative diagnosis is feasible via complete colony screenings, traps and diagnostic strips. The strips have been validated and offer a reliable, fast and cheap method, which might be considered as a COLOSS standard.

Abstract O-12:

Invasive parasites and bumblebee diversity loss: a plea for molecular detection of bumblebee parasites

Ivan Meeus

Vakgroep Gewasbescherming, Universiteit Gent

Bumblebees are commercially reared and transported worldwide mainly for the pollination of greenhouse tomatoes. Pathogen spillover from reared bumblebees to native bumblebees has been reported. The introduction of these non-native pathogenic species is regarded as a serious threat for wild life biodiversity. We will discuss the impact of different bumblebee parasites on native bumblebees and the design and pitfalls of molecular detection techniques for different bumblebee parasites. We make a plea for the integration of cost-effective diagnostic techniques in bumblebee rearing facilities and governmental organizations to assure pathogen-free bumblebees.

Poster presentations:

Abstract P-1:

Histological features of honey bee: Diagnostic method for honey bee diseases?

[1]*Maiolino P.*, [1]*Carbone S.*, [1]*Rinaldi L.*, [2]*Martano M.*, [1]*Ilsami R.*, [1]*Cringoli G.*

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A broad spectrum of specific pathogens affect the honey bee colony including bacteria, viruses and fungi, and internal and external parasites. Many of these pathogens are responsible for disease outbreak.

The use of novel diagnostic techniques has increased during recent years offering a selection of powerful tools for laboratories involved in honey bee disease diagnostics and research. Despite recent advances, including molecular techniques, histological examination remains often the most important diagnostic method for many human and animal diseases. The authors emphasize the importance of this method also in the diagnosis of the most common diseases of honey bee, through the comparison with the normal histology of this insect.

Abstract P-2:

Exploring the impact of venom from the ectoparasitic wasp *Nasonia vitripennis* on host immunity with an agar diffusion test

Danneels, Ellen L. (Ellen.Danneels@UGent.be) ; Formesyn, Ellen; Jacobs, Frans J.; de Graaf, Dirk C.

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Adult females of *Nasonia vitripennis* inject a venomous mixture into its host flies prior to oviposition. Parasitization has an enormous impact on host physiology of which its effect on immune responses is of crucial importance. Hosts will react to the invasion of foreign agents by producing antimicrobial peptides and reactive oxygen species by contact epithelia, fat body and hemocytes. On the other hand, the host can not become highly susceptible to external microbial threats, so immune systems can not be shut down completely.

The afar diffusion test is a basic assay to measure the impact of *N. vitripennis* venom on immune reactions in the hemolymph of its host organism after first challenging its immune system with bacteria.

Abstract P-3:

Recombinant expression of *Nasonia vitripennis'* unknown venom proteins as the key to resolve their mystery

Formesyn, Ellen M. (Ellen.Formesyn@UGent.be); Danneels, Ellen L.; Jacobs, Frans J.; de Graaf, Dirk C.

Ghent University, Department of Physiology, B-9000 Ghent, Belgium

Nasonia vitripennis is an ectoparasitoid that uses host flies as a food source for its progeny. The venom of adult females is used to subdue the host and affects the immune responses, physiology and biochemical profile of parasitized fleshflies. Recently the genome of this wasp was sequenced and an in depth investigation of the venom composition was able to identify 79 venom proteins[1]. The possible functions and interactions of these 79 venom proteins with host pathways remain largely speculative. The main challenge now is to discover the functions of a subset of 23 venom compounds that do not display similarities to any known protein. The first step to determine their function starts with their recombinant production in *E.coli* BL21 (DE3) using the pET 100/D-TOPO expression vector. In order to discover their function, the purified recombinant venom proteins will be used in different bioassays.

Abstract P-4:

DISTRIBUTION OF NOSEMA APIS AND NOSEMA CERANAE IN BULGARIA

Kalinka Gurgulova[1], Rumen Valchovski[2], Plamen Petrov[3], Evgeniya Ivanova[4]

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Nosema ceranae was found as a parasite of Apis mellifera in many countries in Europe. In 2009 the investigation by molecular techniques (PCR) proved that N. ceranae exists in Bulgaria. To determine its distribution in Bulgaria was made a screening of 396 honey bees samples from 94 apiaries in different regions of the country. The samples were investigated for microsporidian spores presence using light microscope and results were conformed by molecular diagnosis. PCR specific primers for a region of the 16S rRNA gene of Microsporidia were developed.

The results showed that 100 samples from 42 apiaries were positive for Nosema spores by microscopic analysis. The investigation by molecular techniques (PCR) identified Nosema apis only in 2 samples from one apiary and Nosema ceranae in 139 samples from 47 apiaries. N. ceranae was found to be more prevalence than N. apis and the PCR method was more sensitive and gave the possibility to identify two types of Nosema.

Abstract P-5:

Molecular cloning reveals the existence of multiple alternative splice variants of hyaluronidase and icarapin in honeybee venom glands

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Honeybee stings have the potential to elicit a systemic allergic reaction, which can result in life-threatening anaphylaxis. Nowadays 10 honeybee venom allergens have been discovered. However, protein heterogeneity might broaden this allergen spectrum extensively. Here, we report on an analysis of transcript heterogeneity of the venom allergens hyaluronidase (Api m 2) and icarapin (Api m 10) using an RT-PCR-based cloning approach. The results indicate that icarapin contains at least 11 transcripts derived from the same genomic locus by complicated alternative splicing. Also for hyaluronidase 11 alternative splice variants were identified. Availability of these recombinant expressed variants will allow us to do a comparative analysis of their allergenic potential.

Abstract P-6:

The use of formic acid against *Varroa destrcutor* in the Belgian climate

Laget Dries (Dries.Laget@UGent.be); Nguyen Kim B.; Mignon Jacques; de Graaf Dirk C.; Haubruge Eric.; Jacobs, Frans J.

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Varroa destructor is an ectoparasite of the honeybee and causes severe losses of colonies worldwide. Formic acid and oxalic acid have proven to be effective in the treatment against varroa. In this study we investigated the effectiveness of both products in the Belgian climate. 96 hives on four sites with different environment (urbanized, agricultural, orchards and nature reserve) were monitored before, during and after treatment. Comparing the number of dead mites on the hive bottom before and after treatment shows an augmentation in the control group (no treatment) of 17,83 ± 6,06 times the initial amount. Hives treated with formic acid give a better result (group 1: 7,34 ± 5,56 and group 2: 8,44 ± 0,99). However hives treated with thymol (Thymovar®) have the smallest increase: 1,48 ± 0,81. During the formic acid-treatment we observed a significant reduction of the closed brood and the number of larvae. The number of eggs remained the same.

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