



Action FA0803



**Proceedings of
WG2 Workshop
BeeBook Chapter Writing by WG2
Authors
Prevention of Honey Bee COLony LOSSes**



**Hacettepe University
Antalya, Turkey**

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A. Agenda

TIME	PROGRAMME
31st January 2012	
19:00 -	Welcome and social event (cocktail)
1st February 2012	
09:00 – 09:30	Registration
09:30 – 09:40	Welcome and plenary session: organizational matters Aslı Özkırım
9:40 – 10:00	Plenary talk by Vincent Dietemann (BEEBOOK: progress report and publication strategy)
10:00 – 10:30	<i>Coffee break with snacks</i>
10:30 – 12:00	10min talks by Senior authors about their progress and schedules Dirk de Graaf (AFB) Eva Forsgren (EFB) Annette Bruun Jensen (Fungi) Ingemar Fries (Nosema) Vincent Dietemann (Varroa) Geoffrey Williams (hoarding cages) Joachim de Miranda (Viruses) Aslı Özkırım (Alexandre Aebi) (Endosymbionts)
12:30-14:00	<i>Lunch</i>
14:00-15:00	Round table discussion on the development of the <i>BEEBOOK</i>
15:00 – 15:30	<i>Coffee break with snacks</i>
15:30-18:30	group work on the <i>BEEBOOK</i> chapters, Video-conference with the co-authors by internet (skype, msn etc.)
20:00-	<i>Social dinner</i>
2nd February 2012	
09:00-11:00	group work on the <i>BEEBOOK</i> chapters, Video-conference with the co-authors by internet (skype, msn etc.)
11:00 - 11.30	<i>Coffee break with snacks</i>
11:30 – 12:30	Plenary session – final discussions, planning of the next steps
12:30-14:00	<i>Lunch</i>
14:00-	<i>Free time around Antalya</i>
19:00-	<i>Social dinner</i>

Registration on site is required:

Registration fee: 25 €

Conference Location:

Paloma Renaissance Beach Resort & Spa Hotel, Antalya-TURKIYE

CONTACTS FOR FURTHER INFORMATIONS	
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B. List of Abstracts

The writing of the BeeBook chapter devoted to American foulbrood

*Dirk C. de Graaf**

*Ghent University, Laboratory of Zoophysiology, Ghent Belgium

In this lecture the author will give an overview of the progress made so far in the writing of the BeeBook chapter on American foulbrood (AFB). Eighteen authors will cover a broad range of techniques related to the diagnosis and research of this bacterial pathogen of bees. Also bio-safety measurements that must guarantee that no germs can escape during these manipulations are included. Each author is responsible for one or two paragraphs that are compiled thereafter into a full chapter. We hope to get at this workshop the detailed information on the format that should be followed. The following topics have been retained for this AFB chapter:

- Sampling for AFB monitoring or diagnosis
- Biosafety measurements for AFB research and diagnosis
- Cultivation of *Paenibacillus* larvae
- Identification of *Paenibacillus* larvae
- Microscopy of *Paenibacillus* larvae
- Genotyping of *Paenibacillus* larvae
- Measuring susceptibility/resistance to antibiotics of *Paenibacillus* larvae
- Measuring colony resistance to AFB
- In vitro sporulation of *Paenibacillus* larvae
- Long term conservation of *Paenibacillus* larvae vegetative cells
- Artificial infection of in vitro reared honey bee larvae by AFB
- Artificial infection of honey bee colonies by AFB
- Comparing the virulence of *Paenibacillus* larvae strains
- Selection of *Paenibacillus* larvae reference genes
- Quantifying gene expression of *Paenibacillus* larvae
- Transformation of *Paenibacillus* larvae
- Knocking down gene expression of *Paenibacillus* larvae

BEEBOOK: progress report and publication strategy

*Vincent Dietemann**

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An editorial meeting taking place in Bern on the 25-26th of January will determine the publication strategy of the BEEBOOK. On this occasion, a state of advancement of the BEEBOOK will be established and the way forward will be mapped. I will report, in my presentation, on the progress of BEEBOOK (focusing on the chapters which are not represented by the senior authors participating to this meeting) and on the various points discussed and decisions taken during the editorial meeting.

BEEBOOK: progress report on the Varroa chapter

*Vincent Dietemann**, *Stephen Martin*¹, *Francesco Nazzi*²

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1. Department of Animal and Plant Sciences, Sheffield University

2. Dipartimento di Scienze Agrarie e Ambientali, Università di Udine

During the presentation, we will present the progress achieved to date on the Varroa chapter. During the group sessions we will revise the contributions already available and work on further method standardization necessary to complete the chapter's outline. A schooling on the online platform on which the contributions will be uploaded will also be provided.

European foulbrood

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The BeeBook chapter on European foulbrood covers diagnostic of the disease in the field and in the laboratory. Suggestions for sampling and different methodologies to identify the causative agent *Melissococcus plutonius* are presented, such as protocols for cultivation, microscopy, immunology and PCR-based methods. The chapter also includes a section on exposure bio-assays using in vitro rearing of honey bee larvae.

NOSEMA

Ingemar Fries^{*1}, *Marie-Pierre Chauzad*², *Elke Genersch*³, *Mariano Higes*⁴,

*Robert Paxton*⁵, *Geoffrey Williams*⁶

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⁴Centro Apícola de Castilla-La Mancha, Laboratorio de Patología, Marchamalo, Spain

⁵Institute for Biology/General Zoology, Martin-Luther-Universität Halle-Wittenberg, Germany

⁶Swiss Bee Research Centre, Liebefeld, Switzerland

The Bee Book *Nosema* spp. chapter covers methods used for working on *Nosema apis* and *Nosema ceranae* in the field and in the laboratory. The field methods section concerns colony level infections and includes suggestions for standardized procedures regarding sampling, field trials and infection control studies. The lab method section concerns both colony level and individual bee infections and includes procedures for describing infection levels, *Nosema* spp. identification, standardization of cage trials, inoculation methods, incubation conditions and *in vitro* rearing of *Nosema* spp. This section also includes suggested procedures for different types of experiments

The BeeBook and the contribution by WG2 authors

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The BeeBook as one of the most important deliverables of the COST action COLOSS will feature several contributions by members of WG2 “Pests and Pathogens”. This workshop will bring together many of these contributors and allow them to work frantically on finalizing their chapters. Our group will i.a. contribute chapters on ‘Nosema in cell culture’ (Gisder & Genersch), ‘Genetic manipulation of *P. larvae*’ (Poppinga & Genersch), ‘-omics approaches for the study of *P. larvae* virulence’ (Fünfhaus & Genersch), and ‘Strand-specific RT-PCR for the analysis of viral replication in bees’ (Genersch).

Methods of fungal brood diseases – chalkbrood caused by *Ascosphaera apis* and stonebrood caused by *Aspergillus spp.*

*Annette Bruun Jensen**

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In honeybees two fungal brood diseases are known, chalkbrood and stonebrood. The fungus causing chalkbrood shows many specific host adaptations and thus requires many specialized methods, whereas a lot can be drawn upon standard insect pathological methods when working with stonebrood fungi. In the BeeBook chapter we try to compile and discuss these methods and provide detailed protocols. The chapter will include a short introduction and then guidelines and protocols for the following subjects: qualitative detection and diagnostics, quantitative detection, production and quality of inoculum, bioassay (in vitro reared larvae and in colonies), hygienic behaviour and disease control measures.

The tri-continental author team consists of six; Jensen AB, Flores JM, Spivak M, Vojvodic S, Palacio A, Aronstien K, all leading scientists within the field.

Standard Virus Protocols

Joachim de Miranda^{*1}, *Elke Genersch*², *Nor Chejanovsky*³, *Sjef van der Steen*⁴,
*Giles Budge*⁵, *Dirk de Graaf*⁶, *Lina de Smet*⁶

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⁶University of Gent

The virus chapter has three sections. It starts with an INTRODUCTION that explains the unique characteristics of viruses that are fundamental to the design, analysis and interpretation of virus experiments and assays. This section is largely finished. This is followed by a section on PROPAGATION, PURIFICATION & BIOASSAYS, which is a critical area for standardization in experimentation and for which there are very few clear guidelines in the literature. Oral and injection bioassays/propagation in larvae, pupae and adults are covered, with recommendations for all different viruses, plus purification methods, storage recommendations and confirmation of the propagations. This section is about halfway done: the main missing items concern tables for recommended propagation-purification methods for the different viruses. The final section covers HONEYBEE VIRUS SURVEYS & DETECTION, which deals with surveying strategies, statistical considerations, sampling-transport-storage methods, primary processing, a number of different types of assays and their corresponding design, optimization and data analyses and a sub-section on quality control, including how to manage false positives/negatives and criteria for reporting data. This section has very little work done. Also here a large amount of work will be required to design tables with different assays for the different viruses.

BeeBook Endosymbionts Chapter Contribution

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We will present the progress achieved to date on the Endosymbionts chapter. During the group sessions we will revise the contributions already available and work on further method standardization necessary to complete the chapter's outline.

Laboratory cages: a protocols chapter in the COLOSS BEEBOOK

Geoff Williams^{1*}, *Cedric Alaux*², *Cecilia Costa*³, *Tamás Csáki*⁴, *Piotr Medrzycki*³,
*Randy Oliver*⁵, *Dave Shutler*⁶, *Robert Brodschneider*⁷

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Many aspects of honey bee science – parasitology, toxicology, physiology, for example – can be studied by maintaining honey bees within cages maintained under a controlled laboratory setting. However, when designing cage experiments, researchers must control for a number of variables, ranging from selection of study subjects (e.g., parasite and host strains) to experimental environment (e.g., growth chamber conditions, food quality and quantity). Although decisions typically do not jeopardize the scientific rigour of a study, they may profoundly affect results, and may make comparisons with similar, independent studies difficult. Here we will give an update on the progress of this *BEEBOOK* chapter, discussing some important factors that researchers must consider when studying honey bee system using laboratory cage experiments.

C. List of participants

Participants' list

	Name-Surname	Country
1.	Dirk de Graaf	Belgium
2.	Elke Genersch	Germany
3.	Joachim de Miranda	Sweden
4.	Annette Bruun Jensen	Denmark
5.	Geoffrey Williams	Switzerland
6.	Eva Forsgren	Netherlands
7.	Vincent Dietemann	Switzerland
8.	Ingemar Fries	Sweden
9.	Francesco Nazzi	Italy
10.	Stephen Martin	UK
11.	Aslı Özkırım	Turkey