Minutes of the 5th COLOSS Small Hive Beetle Taskforce Meeting Lamezia terme, 25 - 26 February 2020

Editors: Bram Cornelissen & Anna Pappach

Meeting Minutes

(by Anna Pappach edited by Bram)

Figure 1. Group picture of the participating TF members, left to right: Michela Mosca, Roberto Bava, Anna Granato, Akinwande Lawrence, Giovanni Formato, Franco Mutinelli, Audrey Sheridan, Marc Schäfer, Peter Neumann, Bram Cornelissen, Marco Pietropaoli, Anna Pappach, Orlando Yañez, Alexandra Ribatis, Lorella Barca, Giovanni Federico (Picture by Christian Doe).

Task A Ring test for molecular diagnostic tools (Coordinator Peter)

Orlando performed comparison of several methods – found gold standard method for SHB molecular detection (Li et al., 2018). In particular in regard to false negatives this method outperformed the others. However, it is pricey. Bram suggests to try the cheaper LNA version which Marga (van Gent) developed.

Orlando will check how much DNA is available to determine how many labs can participate, will be one plate per laboratory, also determine how many repetitions. Orlando also shares the correct DNA concentrations, define different concentrations to set up limits of detection for this method. Exact same samples will be used for the ring test – from both clade A and clade B, check those samples that were negative for other methods and maybe include them? Estimated costs for the ring test – 100-300 euros, depending on probe. A detailed budget will be defined at a later stage. DNA analysis of SHB faeces – for now Bram has a method that works. We have been working on this. Looks promising

The timeline for the ring test is approx. as follows:

- >April: test LNA and decide on method

- April/May: announce Ring test + conditions (protocol draft)
- June / July: registration
- August – November: perform Ring test
- December: Results deadline

- **Action:** Bram will ask to Marga to send Orlando the LNA version to test as a possible gold standard option
- **Action:** Ring test Orlando and Bram will draft a protocol for ring test, once the LNA version is tested for the gold standard
- **Action:** an expert group should be initiated to discuss specific techniques on molecular analysis. For instance, to discuss extraction options and molecular detection of SHB eggs and larvae, or how to deal with positive internal controls and knock off CT-values in Real time PCR results etc. The focus for now will be on SHB, but since the principles are the same for other targets, it could function COLOSS wide (publish guidelines for molecular techniques in honey bee pathology by 2022??).
  Currently, Orlando, Marga, Anna Granato and Alexandra, will be in this group. **Marga van Gent (@: Marga.vanGent@wur.nl) ** will coordinate the BDEG. For future reference, we will call this the **Beetle DNA Expert Group (abbrev. BDEG).** An invitation to participate will be sent around in March 2020 by Marga.

Task B Improving tools for SHB management (Coordinated by Mary Reed)

Actions: Handbook on SHB IPM management – COLOSS publishing, also one page note in Bee World Mary Reed prepared 14 pages on SHB IPM, which will be reviewed by Marc, Bram, Jamie, Giovanni and Peter). Then contributors will be invited to add to the document (in exchange for Co-authorship). Peter remarked that the normal procedure is to make a framework and then assign contributors to write paragraphs. Bram agreed, but we can't be critical here since Mary has done a tremendous job. All agreed.

Timeline:

- February / March: identify missing info
- April: Invite contributors
- 1 June: Deadline for contributions
- June - August: Review contributions and edit document
- 1 September: Submit to Bee World / COLOSS publishing? + one page note to in Bee World

Task C Better Traps for Control and Diagnostics (Coordination Giovanni and Marc)

This was again discussed at length. We still seem to lack understanding of SHB distribution in colonies. Bram argues that any effort to increase knowledge will help. Peter suggests that only instantly killing and dissecting colonies on a large scale will contribute to the understanding of such knowledge. Since we as a TF have no funds for this, we will have to hold our horses until we do.

Giovanni suggest to move forward with the question formulated in Montreal (2019, what is the relation between number beetles on the lid and in the colony as a diagnostic tool for beetle

infestation) and combine this with the use of a divider as done in Calabria in a citizen science setting. In the end we agree to proceed with making a protocol to answer some basic questions.

Giovanni and Marc (with the help of Bram and Peter) will write a protocol for a CS project to

- Verify the efficacy of some traps used in the same hives;
- Verify the amount of SHBs under the inner cover, respect to those present in the hive or behind the mobile divider. Peter suggested installing data loggers in the hives to test for possible environmental factors governing the number of adult SHBs underneath the lids (i.e. any correlations between temperature and beetle numbers) as well as sacrificing some colonies (e.g. 10 to have a baseline). Timeline for the protocol: end of April.

Final remarks: Marco feels there is lack of monitoring tools for SHB and that has to be addressed. He will contact a company that wants to make a video recognition system for the hives.

Task D Survey on the Impact of SHB (Coordinated by Audrey, with Peter, Anna P. and Arrigo Moro)

Main goal is to give monetary value to the SHB damage, see the real impact it has on beekeeping – that will help to raise awareness among stakeholders and get estimate of a SHB problem that can be used for future funding application. Survey to be distributed via COLOSS website and regional contact points.

Questionnaire - 10 questions max, easy to answer, send around

Possible questions discussed:

- Ask to provide country and zip code
- Do you have SHBs?
- Do you take any measures against SHB?
- Do you have SHB larvae?
- What type of hives you have? (Africa)
- SHB seasonality presence?
- Nature of beekeeping: pollination, queen production, honey production, mating nucs?
- Total economic cost of SHB damage? (GDP can be used later to compare costs between countries)
- How many years ago you noticed SHBs in your apiary?
- What type of damage you have: honey, colony loss, etc
- Are you a professional beekeeper, semi-professional, backyard kind?
- Specific question on the impact on mating nucs (see below)

The survey from Australia can be used as a guideline for our survey – Bram will share it with us: LINK: https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0005/167378/Small-Hive-Beetle-Aethina-tumida-in-New-South-Wales-Apiaries-2002-6-Survey-Results-2006.pdf

Timeline:

- >May: Develop questionnaire
- May/July: send out questionnaire
- August/September: Compile results and report
- Make a scientific note of the results

Task E Understanding Mass Reproduction

(no coordinator yet, to be appointed when task is initiated)

Again, we discussed the issue of mass reproduction and the lack of general understanding. When do colony collapse due to SHB? Bram suggest to make a most wanted factor list. Which factors do we suspect most of being involved.
One thing agreed was that there seem to be many issues in mating nucs. Possibly due to colony build up (of lack thereof). Audrey suggested that the worker population is mostly young nurse bees. Bram argued that maybe DOL is an issue. If mating nucs are more susceptible, they might be a good platform to study the problem of mass reproduction. Idea for future: check different bee castes and their aggression towards SHBs, their eggs and larvae. Use of mating nucs for testing triggers of mass reproduction is therefore suggested but – first quantify the problem with mating nucs.

Compare mating nucs with full-sized colonies – is there a higher chance to have mass reproduction in mating nucs compare to full-sized colonies? Also ask whether beekeepers control SHBs in mating nucs?

Action: Make a separate survey targeting queen breeders only: Audrey will lead as part of Task D

Survey: USA (ideally Florida, Hawaii but also temperate regions), Australia, Italy. **Audrey** will contact Denis vanEngelsdorp for queen rearers in Hawaii. **Bram** will try to get in touch with people in Australia. **Giovanni** in Italy?

Also discussed was the interaction between SHB and Varroa as both peak in Fall.

Further actions on studying mass reproduction are pending the outcome of the survey and funds for future research.

Task F. SHB Seasonality (coordinated by Audrey and Bram)

Bram and Audrey noticed particularly small SHBs in the fall of reasons yet unknown. Due to food availability? Also the consequences are unclear. Are the small SHBs able to reproduce? What is the longevity of these beetles? First question to answer is if in fact there is seasonal variation in SHB size? Also, there might be a difference between beetles in the natural and invasive range.

Action: make a simple protocol to measure 50 beetles 4 times a year to be performed by TF members in exchange for Co-authorship of an anticipated manuscript. (**Bram and Audrey make a protocol)**

Time line

- April: make protocol
- May: Invite TF members to participate
- July: initiate research
- July 2021: finish sampling

Other objectives and remarks

Update Beebook (All)– first make a screening of the existing chapter and then make and add on with molecular diagnostics, improvement of individual rearing. Wait for the Golden Standard first (Task A). **Options to be discussed next TF meeting in 2021.**

Update OIE manual (coordinated by Marc and Jamie) – molecular diagnostics, honey treatment for export – Brazil sieving honey through mesh, and include also wax trade as possible way of SHB spread. Same as beebook: **options to be discussed in 2021.**

Policy leaflet (coordinated by Marc) based on 2018 paper (Schäfer et al): Marc and Peter are writing a concept note. TF members will be invited to comment on the note. To be published as an update (short communication). Contributors will be co-authors.

COST action: an effort was made in 2018 to get a COST action granted. This was not approved, but also could be done better. Peter and Bram will make a new effort with the focus on invasive honey bee pests and parasites and climate change. Due end 2020.

Poster presentation for EURBEE: Bram and Peter will make a poster of the TF for the EURBEE conference.

A picture database of SHBs – no actions or decision decided

Webpage update (Bram): Action for **all Task Coordinators (Aurdey, Bram, Mary, Peter, Giovanni)** to write a short paragraph on their task. **Bram** will then collect the contributions and send them to Jan for a webpage update.

Social media for SHB – at the moment no volunteers/actions etc

Remarks

Audrey wishes to remind us that anyone with a graduate student who wants to work on Small Hive Beetle in an unrestricted area (ie, Mississippi State University) should have them apply to the Fullbright Scholarship program. Applications for the next academic year are being accepted now until October. Audrey can provide details upon request.

Brilliant idea that need some attention

Coffee break discussion: Review examples of successful containment of invasive SHB populations, examples from Italy, Canada, Egypt and Australia – look for beekeeping practices, weather, official actions?

Next COLOSS meeting – just ahead of EurBee, 15th-17th of September, Belgrade, Serbia

Next Task Force Meeting – May-June 2021, Mississippi State University in Starkville, USA.

Program

Tu 25th February 2020

09:00 – 09:30	Registration and coffee
09:30 – 09:40	*Giovanni Formato* – a word of welcome
09:40 – 09:50	*Bram Cornelissen* - State of the Task Force - Objectives / program updates
09:50 – 11:00	Progress report – SHB tasks **TASK A *Ring test for molecular diagnostic tools*** **Presented by Orlando Yanez** **TASK B: *Improving tools for SHB management*** **Presented by Bram Cornelissen** **TASK C: *Determine in-hive trap efficacy against SHB*** **Presented by Bram Cornelissen**
11:00 – 11:30	*Coffee Break*
11:30 – 12:30	Progress report – SHB tasks / discussion
12:30 – 13:30	*Lunch Break*
13:30 – 13:50	**Akinwande Lawrence - *Survival and Reproductive development of Small hive beetle in the soil***
13:50 – 14:10	**Audrey Sheridan - *Small hive beetles model effects of thiamethoxam accumulation in agricultural soil***
14:10 – 14:30	**Peter Neumann - *Starvation resistance of small hive beetles***
14:30 – 14:50	**Anna Papach - *Journey on small hive beetle mating***
14:50 – 15:10	**Lorella Barca – *Rearing Small Hive Beetles in Calabria***
15:10 – 15:30	*Coffee Break*
15:30 – 15:50	**Bram Cornelissen – *Mark, Release and some Recaptures of Small Hive Beetles***
15:50 – 16:10	**Alexandra Ribartis - *Monitoring for the presence of the small hive beetle (SHB) in Austria***
16:10 – 16:30	**Giovanni Formato – *Hive inspection in the SHB era: the use of the movable divider***
16:30 – 16:50	**Marc Schäfer - *Alternative ideas for treatment of infested soil***
16:50 - 17:10	**Marco Pietropaoli – *Procedure for SHB post-mortem investigation***
17:10 – 18:00	Setting controls for the heart of the taskforce (warm up for the Task Force action plan discussion tomorrow)
19:00 – late	*Social Dinner*

wed 26th February 2020

08:30 – 10:00	Task Force Action Plan
10:00 – 10:15	*Coffee break*
10:15 – 11:00	Task Force Action Plan

11:00 – 11:30	Wrap up (next meeting?)
11:30 – 13:00	*Lunch*
13:00 – 16:00	Group excursion to apiary in Gioia Tauro

Abstracts

Peter Neumann - *Starvation resistance of small hive beetles*

The time an individual can survive under complete food deprivation (= starvation resistance) can govern invasion success of a given species and may differ between endemic and invasive populations. However, comparative data between indigenous African and invasive US populations are currently lacking for small hive beetles (SHBs). Here, we show that adult SHBs from Africa can survive up to two months without any food, which may foster their invasion success.

Audrey Sheridan - *Small hive beetles model effects of thiamethoxam accumulation in agricultural soil*

Non-target soil-dwelling organisms are at a higher risk of damage or death from insecticides due to the accumulation of toxins in agricultural soils. Pollinators and other beneficial insects are particularly of concern. Insecticide contaminated soil has been shown to negatively affect lethal and sublethal endpoints (e.g. pupation success and reproductive capacities, respectively) in bees, but only a handful of other organisms have been investigated. Here, we used small hive beetles (SHBs), Aethina tumida Murray, as a surrogate model organism to investigate the possible effects of neonicotinoid contaminated soil on pupation success and reproductive capacity. We chose this organism because SHBs can be reared under standardized laboratory conditions, are not endangered and pupation occurs in the upper 10 cm of the soil column, where exposure to insecticide is most likely to occur . We hypothesize that chronic exposure to field-realistic levels of Thiamethoxam (TMX) (25 ng/g, 100 ng/g, and 400 ng/g) will negatively interfere with SHB physiology, thus decreasing pupation success and affecting reproductive parameters. Laboratory-reared last instar larvae (N=1200) were randomly allocated to one of four treatment groups: Control, TMX-25, TMX-100 or TMX-400. Each treatment consisted of three individual pupation containers filled with a soil mixture and 100 larvae. All containers were incubated under standardized rearing conditions (30 Â° C, 60% RH) until adult emergence. On a daily basis, the containers were visually inspected for newly emerged individuals, which were then weighed and sexed. A subset of 50 males and females from each treatment were used to perform mating experiments to measure oviposition. TMX contaminated soil revealed significant negative lethal (i.e. pupation success) and sublethal (i.e. emergence mass and oviposition) effects. Our findings may be due to the insecticides altering genes associated with metabolism and detoxification during metamorphosis, as well as negatively affecting the sperm quantity and quality in exposed males. It remains uncertain whether these results can be extrapolated to bees. However, the data emphasize the importance of considering and evaluating the effects of soil-contaminations on non-target ground-dwelling insects, ultimately to improve our conservation efforts.

Bram Cornelissen – *Mark, Release and some Recaptures of Small Hive Beetles*

The dispersal by flight of the Small hive beetle (SHB) is a bit of an enigma. We know it flies and we know it can fly far. However, it is still unclear what the dispersal capacity of the beetle is, when it takes flight. In 2017, we performed mark, release, recapture (MRR) experiments to investigate how far SHBs fly and what environmental factors are involved. Beetles were reared and en masse fed with a food dye. They were then released from in a centred release point, around which honey bee nucleus colonies were placed at regular spatial intervals up to 3.2km. We then recaptured the beetles after 1,2,4 and 7 days and checked them for the presence of dye. This was repeated 6 times. We had three additional locations at up to 32 km from the release point, where we applied the concept of a needle in a haystack approach. With any luck we might be able to capture a lone beetle that had taken to the air. Luck and other aspects of the experiment are discussed.

Giovanni Formato – *Hive inspection in the SHB era: the use of the movable divider*

Due to its dark body colour and fast movements, SHB are not easily identified within a colony, particularly when at low population densities. Moreover, SHBs avoid light, hide in crevices or cavities of the hives or fly away from combs. After six years of observing SHB colonization behaviour since its appearance in Calabria (2014–2020), we are now able to share our practical experience. In order to improve hive inspections, these practical suggestions should be followed:

1. A divider made of wood, felt, cardboard or a similar material should be placed laterally between the hive wall and the external comb, to act as a refuge for SHB. The inspection of the nest should be started on the opposite side from the divider, transferring combs one by one into an empty hive or nuc box. When three combs and the divider are left, the combs are moved to the opposite side of the hive. Removing the combs causes SHBs to move progressively towards the divider, where there will be a higher probability of finding them.
2. Areas with higher probability of finding SHB's (e.g., corners and inner side of the walls of the hive, behind dividers, combs containing pollen and honey, etc.) should be inspected more thoroughly, while brood combs should only be quickly scanned due to the lower probability of finding SHBs there.

3. While inspecting combs, avoid removing the bees by shaking the comb as SHB could be dislodged with the bees; observe the frame at a distance further than normal, with arms fully extended, to guarantee a vision of the entire comb surface and facilitate identification of SHB movements across the comb; SHB is much easier to detect on the lighter wax of newly built combs, so more care should be used when inspecting older, darker combs.
4. Once SHB infestation is suspected, sampling is needed for ID confirmation. The body shield is small and hard, making them difficult to capture. While standard leather beekeeper's gloves are not useful for SHB sampling, tight fitting latex gloves are more convenient for examination, handling and sampling of beetles.

Marco Pietropaoli – *Procedure for SHB post-mortem investigation*

Procedure for SHB inspection in frozen colonies will be described according with experiences given by monitoring and research activities in Calabria region.

Lorella Barca - *Rearing Small Hive Beetles in Calabria*

The Italian Ministry of Health has authorized a breeding of SHBs in Calabria for use in research. The breeding was carried out by IZSM in the provincial section of Reggio Calabria, using adults from outbreaks. The protocol that allowed SHB to complete its biological cycle in the laboratory will be described.

Practical information

Registration

The registration fee of € 50 (payable on site, cash only) will cover all coffee breaks, 2 lunches and the social dinner. Participants will not be reimbursed for travel and accommodation.
Note that membership of COLOSS and the Task Force is required for participation.
Email Bram.cornelissen@wur.nl to join the SHB Task Force.

Venue

Masseria I Risi – Azienda Agrituristica
Via Jan Palach 1
88046 Lamezia Terme
Province of Catanzaro, Italy

Getting Around Lamezia

Local shops and venues are in walking distance. Downtown Lamezia can be reached by train. Transport between the Lamezia airport and the Workshop venue will be arranged with the shuttle service of the "I Risi" (Workshop venue, €5 one way). Rental car options and Taxis are available at the airport and train station.

For questions regarding the meeting and task force: Bram.Cornelissen@wur.nl
For questions regarding travel, stay and local organisation: giovanni.formato70@gmail.com

Participants list

Name	EMAIL	Affiliation	Country
Lorella Barca	lorella.barca@cert.izsmportici.it	Istituto Zooprofilattico Sperimentale del Mezzogiorno	Italy
Roberto Bava	bavaroberto23@gmail.com	Università degli Studi Magna Graecia di Catanzaro	Italy
Bram Cornelissen	Bram.cornelissen@wur.nl	Wageningen University & research	Netherlands
Giovanni Federico	giovanni.federico@izsmportici.it	Istituto Zooprofilattico Sperimentale del Mezzogiorno	Italy
Giovanni Formato	giovanni.formato@izslt.it	Istituto Zooprofilattico Sperimentale del Lazio e della Toscana	Italy
Anna Granato	agranato@izsvenezie.it	Istituto Zooprofilattico Sperimentale delle Venezie	Italy
Hemma Koeglberger (not present)	hemma.koeglberger@ages.at	AGES Institute for seed and propagating material	Austria
Akinwande Kayode Lawrence	akinkay20032001@yahoo.com	Federal University of Technology, Akure, Nigeria	Nigeria
Michela Mosca	michelamoscabologna@gmail.com	DVM, practitioner	Italy
Franco Mutinelli	fmutinelli@izsvenezie.it	Istituto Zooprofilattico Sperimentale delle Venezie	Italy
Peter Neumann	peter.neumann@vetsuisse.unibe.ch	Institute of Bee Health, Vetsuisse Faculty, University of Bern	Switzerland
Anna Pappach	anna.papach@vetsuisse.unibe.ch	Institute of Bee Health, Vetsuisse Faculty, University of Bern	
Marco Pietropaoli	marco.pietropaoli@izslt.it	Istituto Zooprofilattico Sperimentale del Lazio e della Toscana	Italy
Alexandra Ribarits	alexandra.ribarits@ages.at	Institute for Seed and Propagating Material, Phytosanitary Service and Apiculture	Austria
Marc Schäfer	marc.schaefer@fli.de	Friedrich-Loeffler-Institut	Germany
Audrey Sheridan	as526@msstate.edu	Mississippi State University	USA
Orlando Yanez	orlando.yanez@vetsuisse.unibe.ch	Institute of Bee Health, Vetsuisse Faculty, University of Bern	Switzerland

IMPORTANT NOTIFICATIONS

- make sure to bring a bee suit if you will be going on the group excursion