EIP-AGRI Focus Group

Bee health and sustainable beekeeping

MINIPAPER 02: Disease control and emergency situations

September 2020

Authors

Fani Hatjina (Coordinator), Marc Bock, Pilar De la Rua, Constantin Dobrescu, Aleš Gregorc, Zeid Nabulsi, Ana Paula Sançana

funded by European Commission

Table of contents

1. Introduction

This minipaper aims to summarize and highlight briefly the best beekeeping practices and the innovative methodologies available in order to improve disease prevention, diagnosis and control under the pressure of conflicts such as migratory beekeeping, resistance to chemicals and climate change. At the same time, we intend to highlight that any control measures should also maintain the quality and safety of hive products given as they are used not only as human food but also as cosmetics and medicines.

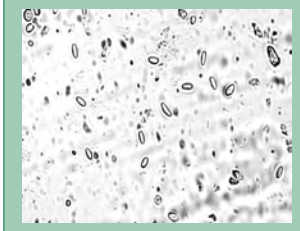
The need for this paper has come about because the beekeeping sector is facing many challenges in the 21st century. One of the main challenges is how to ensure high efficacy of methods to control diseases, a) without any adverse effects of the chemicals used, b) with the lowest costs, and c) ensuring the highest quantity and quality of all hive products. The main problem in dealing with disease control arises from the intensive use of veterinary medicines, the resistance of varroa mite on the chemicals used, the accumulation of residues in the honeybee products and the synergy with the agricultural products used in the environment of the colonies. At the same time, the income of the beekeepers can only be assured through the improvement of their products' quality and the continuous monitoring of colony health and status.

Therefore, there is a great need for innovative and cost-effective methodologies to be used and anticipated in education, research and extension, at national and international level. In addition, the increase in information transfer within the beekeeping community as well as among all stakeholders (scientists, beekeepers, farmers, consumers, policy makers), will ensure that all steps needed in research and extension programmes are taken to tackle the specific problems, their solutions and their dissemination.

funded by European Commission

2. Dissertation

The honeybees are afflicted by several pests and pathogens, which are considered to play a key role in the global decline of honeybee populations. A comprehensive summary of the main pests/ pathogens is given in Table 1. At the same table there is information on monitoring methods and links to the recent research or surveillance projects. Apart from pests and diseases, environmental variables, nutrition issues and hive management practices are adding stress to the honeybee colonies.

	Description	**Monitoring / detection / diagnosis**	**Recent projects ***
Varroa destructor (Varroa mite, a, b)  	• The female feeds on the fat body and haemolymph • It reproduces in the capped brood cells • Is known to be a vector and activator for several bee viruses, the most profound being the Deformed Wing Virus (DWV) • It damages the immune system, shortens the life span and diminishes the productivity of the colony	- Naturally fallen varroa mites among debris found on the bottom board - Adult varroa mites on adult bees (using icing sugar, ether, alcohol, CO_2) - Varroa reproduction rates in capped brood cells - Varroa infestation in capped brood cells - Molecular identification of varroa biodiversity- - Digital scanning of mites on bodies of bees (beescanning)	**SMART BEES** http://www.smartbees-fp7.eu/ **EurBeST** https://eurbest.eu/ **BeeScanning** https://beescanning.com/
Nosema apis/ Nosema ceranae (Nosema desease, c) 	• It infects the epithelial cells of the midgut of adult bees • It causes digestive disorders which leads to a shorter life span • It also leads to energetic stress • *N. ceranae* is more destructive • *N. ceranae* spores are smaller and they do not germinate after been subjected to cold temperatures • *N. ceranae* affects the colonies all year round • North European countries face lesser problem by Nosema disease	- Combs are soiled with faeces only in the case of *N. apis* infection - Lack of apparent symptoms connected to *N. ceranae* infection besides the poor colony fitness and depopulation (Martín-Hernández *et al.* 2018) - Samples of live old workers for microscopic examination in both cases (Cantwell 1970) - PCR-methods are used for species identification (Martín-Hernández *et al.* 2007; Bourgeois *et al.* 2012)	**BPRACTICES** http://www.izslt.it/bpractices/the-project/ **NOLESSBEES** https://www.eurostars-eureka.eu/project/id/5928

funded by European Commission

Melissococcus plutonius (European Foulbrood, EFB, d) 	• Non-spore forming bacteria • EFB is not notifiable in all countries • Cappings of pupae are higher than cell walls and irregular • Larvae have abnormal colour and position in the cell • Smell not as AFB, but as dead larvae • Capped and uncapped cells being found scattered irregularly over the brood frame	- Symptoms of EFB may easily be confused with other diseases or abnormalities in the brood, making diagnosis difficult - Capped and uncapped cells being found scattered irregularly over the brood frame - Use of the EFB diagnostics kit	**Bee Aware** https://beeaware.org.au/archive-pest/nosema/#ad-image-0 **POSHBEE** http://poshbee.eu/
Paenibacillus larvae (American Foulbrood, AFB, e) 	• Spore forming bacteria • Affects larval stages from 12 hours to 13 days post hatching • Capped brood have the dark brown, glue-like larval remains of infected larvae • Smell very strong like a glue • Capped and uncapped cells being found scattered irregularly over the brood frame • AFB scales cannot be removed from the cells	- PCR methods for identification and genotyping of the pathogen from comb samples have now been extensively developed, also for honey (De Graaf *et al.*, 2013) - There is also a great need to develop early diagnosis tools that might prevent the spread of the disease - Use of the AFB diagnostics kit	**EPILOBEE** https://www.anses.fr/en/content/european-epilobee-programme **DeBiMo** https://ag-biene.uni-hohenheim.de/en/debimo
Aethina tumida (Small hive beetle, f, g)  	• A pest of the sub-Saharan African honeybees, where it is a minor pest • Very harmful to European subspecies • Recently discovered in Calabria (Italy) • An invasive and a very rapidly established species	- Different types of commercial traps, homemade traps and many different materials have been employed in order to monitor - Knowledge about beetle appearance is needed - DNA analysis of the hive debris can also be used as a detection tool - As it prefers the dark, it would be easy to spot under the roof, or at the bottom of the hive	**APENET/ BeeNET** https://www.izsvenezie.com/bee-health-in-italy-national-monitoring-results/
Vespa velutina (Yellow legged hornet or Asiatic wasp)	• First recorded in southwestern France in	- Measure to control the entrance at the ports	**STOP VESPA**

funded by European Commission

	2004. Latest distribution is shown in Fig. 2 • Predates on all insects, preferably honeybees • Mainly stands at the entrance of the hive, snatching and killing returning bee foragers, the colony responds by closing foraging efforts so that is weakened by predation levels and may starve to death by lack of food • Limited scientific assessment of the overall impact of *V. velutina* (Monceau *et al.*, 2014). • Protein supplementation and slight energy supplementation of the colonies might be necessary	and unintentional human transport - Pest preventive programmes (e.g. surveillance and quarantine programmes) at the European level - Detection methods for nests radio-telemetry (Kennedy *et al.* 2018) or drones (unmanned aircraft system or UAS) - - Establish traps filled with different substances (mainly sugar, but also fish or proteins) in the surrounds of the bee yards	https://www.vespavelutina.eu/en-us/ **BeeBase** http://www.nationalbeeunit.com/index.cfm?pageid=206 **COLOSS** https://coloss.org/

* The above-mentioned projects, do not necessarily refer to only one type of pathogen

Table 1: Short description of the main bee pests and pathogens

(a) The Varroa mite females feeding on a honeybee pupa. (b) A bee with deformed wings and shortened abdomen due to the DWV infection. (c) *Nosema ceranae* spores seen in 400x magnification. (d) A brood comb with twisted, diseased larvae attacked by *Melissococcus plutonius*. (e) a brood comb with brood cappings clearly punctured and scattered brood pattern attached by *Paenibacillus larvae*. (f) The small hive beetle images. (g) The small hive beetle larva. (h) The yellow-legged hornet (Photos a, b, c, d, e, f, g: Anna Gajda, Photo h: Per Kryger) (Hatjina *et al.*, 2019).

funded by European Commission

Fact No 1.

VARROA MITE IS THE ONLY PEST OF THE HONEYBEE FOR WHICH CHEMICAL TREATMENTS ARE ALLOWED!

Varroa control through beekeeping practice

- For over 25 years, a number of "hard" chemicals has been used to fight varroa, with their success diminishing as resistance of varroa is increasing. This type of varroa control is also leading to residues in beeswax, honey, pollen, propolis, royal jelly and bees larvae. As these are also consumer products for humans, the residues will finally end up in human organism. To limit these effects, or even prevent them, the management of varroa control is one of the keys for sustainable food products from honeybees.

- Several 'soft' chemicals or organic substances such as organic acids and essential oils, namely formic acid, oxalic acid, lactic acid and thymol have also been used with increasing frequency, mainly aiming at controlling varroa resistance to chemicals and reducing chemical residues in wax and honey. However, with the use of all the above substances (although at not at the same level) one cannot avoid the partly destruction of the flora and fauna in the beehive.

- Extension for varroa control also calls for synchronised control in terms of period of the year, and type of application, which can minimise the risk of reinfestation in permanent/non migratory apiaries. Training is also very important in varroa monitoring or control schemes, as good beekeeping practices also include measurements of infestations level and then control of varroa if infestation is above a certain threshold. Especially young beekeepers should be trained to use all sampling methods available and recognise early symptoms of apparent viruses.

funded by European Commission

Alternative Varroa control

- Alternative ways to control varroa have also been developed such as trapping of mites in worker or drone brood, making artificial swarms, use of wire netting bottom boards, heat and powder sugar (Rosenkranz *et al*, 2010; Gregorc and Sampson, 2019), as well as complete brood removal or caging of the queens (Gregorc *et al*, 2017; Buchler *et al*, 2020).

- Breeding for resistance: *Varroa* resistant colonies are however thought to be the best solution in eliminating the problem of colony losses due to this parasite, but it seems that global beekeeping and varroa management need to be controlled and advised in a way, that will allow for the resistant bees to thrive. Recent European projects address this issue in detail (SMART BEES, EurBeST).

Example of practical experience from Finland!

Use of cell size of 5.1mm instead of 5.4. This management practice together with the removal of capped drone brood keeps varroa levels very low. "It is important that varroa has always open brood at her disposal". Results are yet to be confirmed!

THE GOOD BEEKEEPING PRACTICE SUGGESTS SAMPLING VARROA INFESTATION BEFORE ANY TREATMENT.

AS AN EXAMPLE, WE PRESENT HERE THE ILLUSTRATION OF THE ICING SUGAR METHOD (FIG. 1).

Figure 1 Icing sugar sampling method for varroa infestation on adult bees

funded by European Commission

Fact No 2.

NOSEMA CERANAE SPORES DO NOT GERMINATE AFTER THEY HAVE BEEN SUBJECTED TO COLD!

Fight against Nosema!

- Negative effects on queen survival and egg-laying of newly emerged queens in queen-breeding apiaries should be prevented to assure good quality of queens.
- Nosema shortens bee honeybee lifespan and survival may be dependent on the level of infection.
- Selective breeding for Nosema resistant bees could also become an important tool in reducing the incidence of nosema infections in honeybee colonies.
- It is crucial for the beekeeper to minimize the negative effects of potential nosema infestation on colonies development and also their survival.
- The vast negative effects of nosemosis on individual honeybees and whole colonies call for effective and accurate diagnosis, preventive methods and therapy without the use of antibiotics.
- Extension/training including 'good beekeeping practice' and colonies management need to be carried out.
- Fresh running water is essential and the colony density in an apiary needs to be controlled. Training of beekeepers in good beekeeping practice, nutritional aspects and early diagnosis is needed, especially for young beekeepers.

Fact No 3.

THERE ARE SEVERAL GENES IN THE HONEYBEE RESPONSIBLE FOR RESISTANCE AGAINST AFB AND EFB!

Eradication of the two bacterial diseases

- **EFB**

✓ As there is no treatment for bacterial diseases, burning of infected combs, is the best solution so far. Furthermore, all that apply to AFB can be regarded as important also for EFB.

- **AFB**

✓ There is no easy method to control AFB, apart from burning the infected beehives. In some countries, the antibiotic oxytetracycline (OTC) has been used for decades but there are several studies now showing resistance to it. In any case antibiotics do remain in the honey for years and do not kill the spores or destroy the AFB scales. However, the best method for controlling it is prevention by testing and keeping resistant populations, using the hygienic behaviour test (Spivak and Reuter, 2001). Buying second-hand material or colonies must be done after careful examination. Old hives should be thoroughly disinfected prior to reuse.

- **Extension / training**

✓ There is still a great need for intensive educational courses and training sessions for young beekeepers in this infectious disease. All beekeepers should be able to recognise the symptoms immediately, and most importantly they should know how to look and recognise the scales in empty frames.

funded by European Commission

Fact No 4.

IF *AETHINA TUMIDA* ESTABLISHES IN AN AREA IT IS IMPOSSIBLE TO GET RID OF IT!

Measures against Small hive beetle

- Avoid over-supering hives, which increases the area that the honeybees must patrol.
- Maintain a clean apiary and honey house to reduce attraction to beetles.
- Avoid tossing burr comb onto the ground around hives, which may attract pests. Adult beetles tend to prefer shady locations.
- Good beekeeping management practices in the bee yard and in the honey house are sufficient to contain hive beetle problems in most cases.
- Making splits from heavily infested hives can cause a serious outbreak.
- The use of grease patties for tracheal mite control, or the addition of protein supplement patties for spring build-up, may attract more the SHB.
- Adult beetles tend to prefer shady locations.
- Wax cappings are an attractive food for beetles. Cracked and rotten hive bodies provide beetles with many places to hide.
- Unnecessary complicated hive systems might also offer an ideal habitat for SHB in the honeybee colonies.

Fact No 5.

THE ASIAN HORNET IS HERE AND ITS TERRITORY IS EXPANDING!

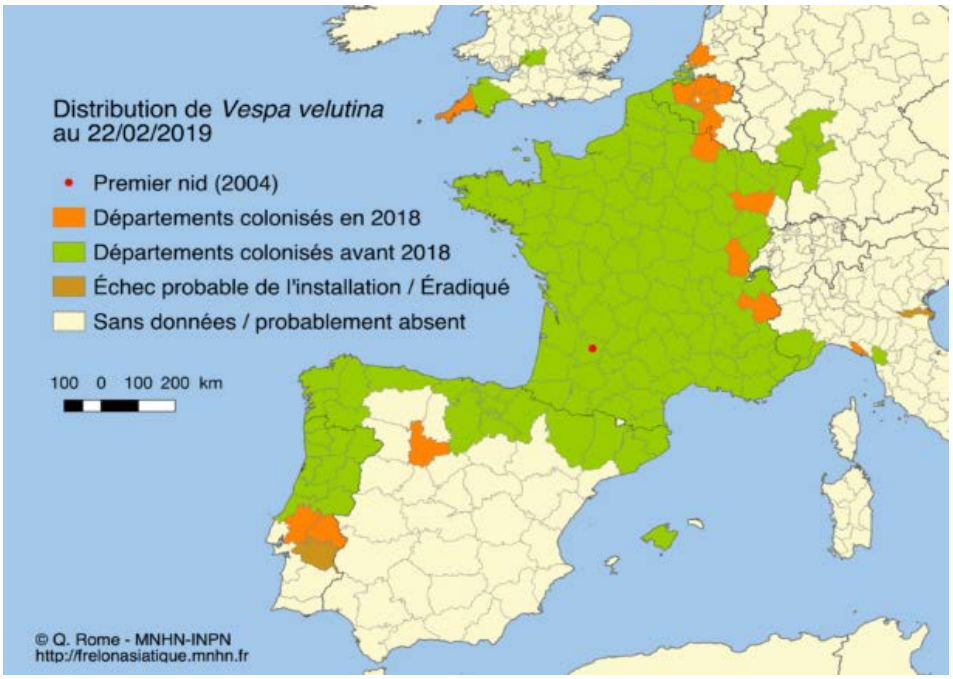


Figure 2 Map of distribution of the Asian hornet (*Vespa velutina*) in Europe, date 22/02/2019: In green, areas colonised before 2018. In orange, those colonised during 2018

Source http://frelonasiatique.mnhn.fr/

funded by European Commission

Asian hornet: Management of apiaries

--- Maintain strong colonies and the viability of the colonies with focussed and regular breeding programmes. Here, the importance of good-quality queens arises. It is important to ensure the balance of the population/reserves, so that there is no longer a stress factor, as well as ensuring an adequate relation space and volume of the hive/population.
--- Nest destruction (mechanically or chemically in using insecticides or biocide gas like sulphur dioxide injected in the nest). Hornets can be trapped using food baits (carbohydrates or proteins). Those traps can be used for monitoring or for reducing the predation pressure (mass trapping or traps baited with insecticides).
--- After chemical destruction (if any) the nests should be removed.
--- Traps decrease predatory pressure in the bee's apiary and 'defensive' behaviour. The placement of traps should be the target of adapted scheduling in each country, depending on the phenological stages of the wasp, bees and other pollinating insects. They should be as selective as possible and should be monitored.

Alternative control aspects

-- Some colonies tend to adopt strategies to fight against *Vespa velutina*. A behaviour of adaptation? For example, they form a kind of mat of honeybees covering the entrance board in order to disturb the hornet from knowing from which point the forager would fly. This behaviour is known as “honeybee carpet”.
--- Other honeybees adopt the “shimmering” behaviour: they are moving their abdomens thus creating specific patterns said to frighten the hornet.
--- Still others do the “heat balling”: to trap the hornet in order to heat up its body temperature up to lethal limit.

Emergency situations

The honeybees are in need!

A honeybee health emergency occurs when a beekeeper is confronted with direct events related to the health of their bees that need urgent intervention, otherwise risking massive losses of their colonies and/or endangering neighbouring ones. In such a situation the beekeeper needs urgent advice from bee health experts. Coping with an emergency should follow the following steps:

1. The beekeeper makes a correct, comprehensive and relevant description of the situation and relays it to the expert;
2. The expert attempts to understand the emergency and advises the beekeeper;
3. The beekeeper acts according to the advice of the expert;
4. The expert monitors the situation after the action of the beekeeper and, if needed, advises him on further action.

The most common bee health emergencies are the following:

a. Intoxications
b. AFB
c. *Vespa velutina* or SHB (invasive species entry)

funded by European Commission

d. Unidentified severe and generalized situation of poor health of bee colonies whose causes must be discovered

Intoxication incidents

Pesticide toxicity is a complex issue, with new debates emerging regularly. Intoxication incidents occur mainly by the use of synthetic phytosanitary products due to the bees behaviour to collect nectar and pollen from flowers. Exposure to pesticides can impact foraging honeybees, nurses and larvae as well as reproductive individuals. Intoxication incidents have also been documented due to some substances of botanical origin used in organic farming. However, several chemicals used by the beekeepers against varroa can also cause intoxication.

- Symptoms - direct effects and behavioral changes - indirect effects

The usual symptoms of the acute intoxication are death of foragers, reduced thermoregulation, loss of nurses, reduced larvae survival, reduced worker longevity, decreased queen weight and colony survival. However, sublethal effects caused by various pesticides can lead to physiological modifications, changes in individual honeybee behavior, and alterations in cellular physiology consistent with chemically induced stress. Since honeybees can be exposed to multiple chemical agents at once, synergistic or antagonistic interactions among these pesticides could also play a role in bee and colony health. Honeybees are very sensitive to most of the pest control chemicals and the reasons and mechanisms for this sensitivity are mostly unclear. Specifically, for the veterinary medicines used against varroa, tau-fluvalinate, coumaphos, amitraz, thymol, even oxalic acid and formic acid (under some circumstances).
Interestingly, intoxication can occur through residues accumulated in wax, therefore all substances (pesticides, acaricides, miticides) could be blamed for this.

- Remedies, recommendations

It is difficult to identify methods to remedy the intoxication. However, simple management practices can always help, such as the removal of the contaminated food, the reinforcement of the colony with workers and the movement of the colonies away from the problem, when the cause is a phytosanitary product. When the cause is a veterinary product, the problem is caused from inside the colony and it is not easy to be remedied. However, the recovery of an affected colony depends always on the dose as well as on the duration of the effect.

The EFSA approach

Risk Assessment of Bee Pest Entry into the EU

The European Food Safety Authority (EFSA) and its Animal Health and Welfare (AHAW) Panel have published a report on methodologies and recommendations for risk assessment of the exotic pests such as small hive beetle and *Tropilaelaps* mite. Further information can be found in this link: **http://www.efsa.europa.eu/en/press/news/130314**

funded by European Commission

3. Research needs

Research needs from practice identified by the Focus Group experts

- Implementing Health Status Index (HSI) in order to predict colony losses.
- Pilot testing for varroa control strategies in different countries using local honeybee subspecies and populations.
- Testing good apiculture practices (feeding regimes, hygiene practices) and promoted through experts to beekeepers.
- Need of surveillance projects against invasive species.
- Need to engage epidemiological studies in research projects.
- Need for continuously develop and improve diagnostic and control methods: applied research and extension.
- Identifying the sub-lethal effects of the veterinary medicines used in each country.
- Research of effectiveness of synchronized varroa control treatments (a social approach inside the beekeepers communities for controlling varroa based on a simple synchronization of treatments at the level of the whole neighborhood in a certain locality (like a village).

Directions for further research proposed by the experts

- Holistic approach on the effects of veterinary medicines and the buffer capacity of the colony to recover.
- Use of standardization data and tools (such as HSI) for identifying the fate of the colonies.
- Good beekeeping practices for different beekeeping topics (honeybee health, honey production, hygiene, preventive, etc) need to be further tested in field conditions. Special attention should be given to the testing and development of simple and accurate varroa diagnosis and controls as well as their application into practice in the different European countries.
- Establish the biological and economical thresholds of varroa infestations in several countries, according to the climatic conditions.
- Test and determine the size of the cells in combination with the capped drone brood removal in maintaining colonies without therapy for varroa.
- Studies of the understanding of the role of all the potential environmental pollutants and their synergy with pathogens.

4. Ideas for innovations

The "Bee Ambulance"

A Smart phone application could provide a valuable help in emergency situations.

1. Description by the beekeeper of the situation.
   1.1 The App must provide the beekeeper with basic information on the symptoms specific to several bee diseases. In this way they can provide the most relevant information to the expert in the attempt to identify or confirm the bee health issue that is suspected. In this stage the beekeeper should be able to see a list of symptoms and check the ones that they recognise. According to their response, the App will take them to the required information they have to provide to the expert.
   1.2 Information automatically sent by the App: the GPS location and the phone number of the beekeeper.

funded by European Commission

1.3 Information sent by the beekeeper via the App:
- taking photos of honeybees, hives, frames, surrounding vegetation, etc;
- recording short videos or at least sounds;
- checking menus provided by the App;
- providing written text.

2. After receiving information from the beekeeper, the expert may have a positive diagnosis or need more information, in which case they can contact the beekeeper via the App. In the end the expert identifies the nature of the emergency and gives proper advice to the beekeeper.
3. The beekeeper performs the actions recommended by the expert.
4. According to the nature of the emergency, the App reminds the expert at the adequate interval of time to contact the beekeeper in order to monitor the development of the situation. If needed, the expert may inform the competent authorities via the same App.

The name of this App can be 'Bee Ambulance', which has the potential of turning into a powerful honeybee health surveillance tool in the future. An example of an existing smartphone App of a somehow similar kind is 'Bee Health', available both for iOS and Android.

Bee Connected

As most beekeepers have a smart phone nowadays and the cost for at least one intelligent monitoring device for GPS location is not high, ALL beekeeper members of an organization could ask for a subsidy to be connected in a global network/platform by just registering their location (e.g. APIMONDIA could manage the platform). In case of an emergency (a disease breakout or a disaster such as a fire, flood or AFB) all surrounding apiaries/beekeepers are immediately notified to move their colonies. The electronic registry of the beekeepers and their apiaries (permanent or temporary) is a must in each country.

Artificial Intelligence and varroa monitoring

Counting on bottom boards is a quite simple method, which can be even faster with an automatic counting device, and it will be very helpful to beekeepers in order to monitor varroa (Dupleix *et al.* 2019).

Bio-monitoring Stations

Permanent monitoring stations as a basic and preventive strategy for epidemiological and environmental studies. Biomonitoring stations can be of great importance in providing permanent information on different parameters of environmental quality (extrinsic factors) and the health of the colonies (intrinsic factors). Over time, we would be able to have data banks of great interest. From the epidemiological point of view, they can prevent the appearance of diseases and pests, know their prevalence, facilitate their control, study their evolution and avoid their extension. Beekeepers, Technicians or Advisers and Researchers (BAR) can participate and collaborate in them.

5. Conclusions

- Honeybees are very sensitive to be affected by several pests and pathogens as well as to the use of chemicals outside and inside the colony. Apart from varroa for which veterinary medicines exist for control, all other pathogens and pests need to be controlled mainly by using a combination of

funded by European Commission

management practices. Therefore, good beekeeping practices are essential to be communicated to beekeepers and mainly to young professionals.

- It is also apparent that although many research projects have been undertaken, a lot of knowledge has not yet been communicated to beekeepers. One of the essential parameters for improving the health of bees is organic beekeeping and to use less medicines in the hive, which still needs to be developed further. The adverse effects of several veterinary medicines needs to be determined with accuracy.

- Monitoring for the health parameters of bees might be a solution for disease prevention. There are several monitoring tools for each disease, but still there is no one simple monitoring tool for all diseases, as well as environmental factors. It is possible that the Health Status Index and data standardization, if established, could be a monitoring tool for predicting the fate of a colony, under specific circumstances.

- Many attempts have been made recently in order to consider the epidemiological cycle when it comes to quantify the disease, to determine the risk factors or to evaluate the methods used to reduce disease occurrence and their efficiency in controlling the disease. In the future, epidemiological approach could and should be broadly used to study and prevent honeybee diseases. Long-term solutions require long-term projects and international cooperation.

- Intensification of breeding efforts towards resistance to varroa might be the solution for the future (**IHBBN, reducing colony losses by breeding locally adapted honeybees: https://ihbbn.org/**) .

- Finally, tools need to be developed for dealing with emergency situations.

Further research needs coming from practice, ideas for EIP AGRI operational groups and other proposals for innovation can be found at the final report of the focus group, available at the FG webpage **https://ec.europa.eu/eip/agriculture/en/focus-groups/bee-health-and-sustainable-beekeeping**

6. References

Arca, M., Papachristoforou, A., Mougel, F., Rortais, A., Monceau, K., Bonnard, O., ... & Arnold, G. (2014). Defensive behaviour of Apis mellifera against Vespa velutina in France: testing whether European honeybees can develop an effective collective defense against a new predator. Behavioural processes, 106, 122-129.

Bourgeois, L., Beaman, L., Holloway, B., & Rinderer, T. E. (2012). External and internal detection of *Nosema ceranae* on honeybees using real-time PCR. Journal of invertebrate pathology, 109 (3), 323-325.

Büchler R., Uzunov A., Kovačić M, Prešern J, Pietropaoli M, Hatjina F., Pavlov B., Charistos L., Formato G., Galarza E., Gerula D., Gregorc A., Malagnini V., Meixner M., Nedić N., Puškadija Z., Rivera-Gomis J., Rogelj Jenko M., Smodiš Škerl M., Vallon J., Vojt D., Wilde J., Nanetti A.(2020). Summer brood interruption as an integrated management strategy for effective Varroa Control in Europe. Journal of Apicultural Research, in press.

funded by European Commission

Cantwell G.E. (1970). Standard methods for counting Nosema spores. American Bee Journal 110, 222–223.

De Graaf, D. C., Alippi, A. M., Antúnez, K., Aronstein, K. A., Budge, G., De Koker, D., ... & Fünfhaus, A. (2013). Standard methods for American foulbrood research. Journal of Apicultural Research, 52 (1), 1-28.

Dietemann, V., Nazzi, F., Martin, S. J., Anderson, D. L., Locke, B., Delaplane, K. S., ... & Rosenkranz, P. (2013). Standard methods for varroa research. Journal of Apicultural Research, 52(1), 1-54.

Dupleix A., Jullien D., Reutenauer V., Pfister F. (2019) Automated Varroa counting system on bottom board based on image recognition. Apimondia Conference, Montreal, 8-12 September 2019.

Gregorc A., & Sampson B. (2019). Diagnosis of Varroa Mite (*Varroa destructor*) and Sustainable Control in Honey Bee (*Apis mellifera*) Colonies—A Review. Diversity, 11(12), 243.

Gregorc A., Alburaki M., Werle C., Knight, P. R., & Adamczyk, J. (2017). Brood removal or queen caging combined with oxalic acid treatment to control varroa mites (Varroa destructor) in honey bee colonies (Apis mellifera). Apidologie, 48 (6), 821-832.

Hatjina, F., Gajda, A. & Dar, S.A. (2019). Current Drivers of Taxonomic Biodiversity Loss in Asian and European Bees, In The Phylogenetics of the Bees. Ilyasov, R.A., Kwon, H.W. (E

Fries, I., Chauzat, M. P., Chen, Y. P., Doublet, V., Genersch, E., Gisder, S., ... & Paxton, R. J. (2013). Standard methods for Nosema research. Journal of Apicultural Research, 52(1), 1-28.

Kennedy, P. J., Ford, S. M., Poidatz, J., Thiéry, D., & Osborne, J. L. (2018). Searching for nests of the invasive Asian hornet (Vespa velutina) using radio-telemetry. Communications biology, 1 (1), 88.

Martín-Hernández, R., Meana, A., Prieto, L., Salvador, A. M., Garrido-Bailón, E., & Higes, M. (2007). Outcome of colonization of *Apis mellifera* by *Nosema ceranae*. Appl. Environ. Microbiol., 73 (20), 6331-6338.

Martín-Hernández, R., Bartolomé, C., Chejanovsky, N., Le Conte, Y., Dalmon, A., Dussaubat, C., ... & Higes, M. (2018). *Nosema ceranae* in *Apis mellifera*: a 12 year post detection perspective. Environmental Microbiology, 20(4), 1302-1329.

Monceau, K., Bonnard, O. & Thiery, D. (2014). *Vespa velutina*: a new invasive predator of honeybees in Europe. Journal of Pest Science, 87, 1–16.

Forsgren, E., Budge, G. E., Charrière, J. D., & Hornitzky, M. A. (2013). Standard methods for European foulbrood research. Journal of Apicultural Research, *52* (1), 1-14.

Rosenkranz, P., Aumeier, P., & Ziegelmann, B. (2010). Biology and control of Varroa destructor. Journal of invertebrate pathology, 103, S96-S119.

Spivak, M., & Reuter, G. S. (2001). Resistance to American foulbrood disease by honeybee colonies Apis mellifera bred for hygienic behavior. Apidologie, 32(6), 555-565.

Van Engelsdorp, D., Lengerich, E., Spleen, A., Dainat, B., Cresswell, J., Baylis, K., ... & Le Conte, Y. (2013). Standard epidemiological methods to understand and improve Apis mellifera health. Journal of Apicultural Research, 52(4), 1-16.

Thiery, D., Monceau, K. (2015). Frelon à pattes jaunes: un prédateur d'abeilles introduit en Europe par l'homme. BIOFUTUR-PARIS-, 365(1), 48-48.

funded by European Commission