2

Internal stress factors

KEYNOTES

Evolution of each bee stadiums and their amount inside hive could be used as marker of stress, which is the hive exposed.

Environment inside hive, availability of food and used medicaments against bee diseases and parasites represent combination of factors which determine vitality of hive.

In some matrices could occur the accumulation of harmful substances, according to their physical-chemical and toxicological properties.

Beside external stress factors *(e. g. climate, hive location, humidity, presence of hazardous substances in environment or pressure of infection diseases)*, the hive microbiome is mostly affected by beekeeper practice and affinity of bee development stages to diseases. The forager's state and number appear to be as a response to colony stress, and foragers are also the behavioural caste exposed to the greatest stress. *(vanEngelsdorp and Meixner 2010, Even, Devaud et al. 2012)*

The strong hive could consist approximately of 60.000 of forager bees at top of the season, but when the monitoring of stress factors as availability of quality food all seasons *(continuous supply of pollen and nectar)*, as monitoring of mites, pathogens

WORKER BEES

~ 60 000

IN ONE STRONG HIVE

and signs of diseases, is ignored, then the generation of winter bees cold be significantly damaged and could not keep over winter. To ensure the overwintering of bees, the sufficient feeding and the treatments against *Varroa* mite by use of chemicals or zootechnical methods are recommended.

However, this could fail in the case when the chemicals are overused, improperly applied or combined. Together with hazardous substances incoming from outside of the hive, the vitality of winter bees significantly could decrease.

Apparently unimportant could be the environment inside hives, which consist of bee wax, wood *(natural)* or plastic *(synthetic)* materials used at construction of hives, as painting and protections, as chemicals used at disinfection of hives.

All these materials are in contact with development stages of bees, as stored pollen and nectar. Between the microbiome and construction materials occur the exchange of various substances. According to substance character *(lipophilic of hydrophylic)*, then the diffusion into bees, bee wax or water containing compounds *(e. g. nectar, royal jelly)* could arise, and the bioaccumulation of hazardous substances increases. *(Sattler, De-Melo et al. 2016, Benuszak, Laurent et al. 2017)* From that point, the omission of this fact causes an indirect stress at development stages of bees, also the increased bioaccumulation of lipophilic hazardous substances leads to higher levels of wax and honey contamination.

The formic acid

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2·1

Hive management mistakes

BEEKEEPING STATISTICS IN EU

~ 15 000 000

HIVES

~ 900 000

BEEKEEPERS

KEYNOTES

Lot of beekeepers has beekeeping as free time activity and many novices use classical zootechnic techniques of keeping bees, which crossing over the border of sustainable beekeeping. Main task for experienced bee-keeper is the ensurance of healthy honey bees and respect of natural development of hive, however the absent education programme lead novices to make some mistakes (non-presence of queen, later addition of food or insufficient amount of food for overwintering), what leads to decrease of honey bee vitality or death of the colony.

Beekeeper should take care about honey bees such way, that no harming or decrease of honey bee health arise. In this part we mention some common mistakes at beekeeping, which directly influence whole bee hive. The task for any beekeeper should be securing of health of bees according to beekeeper's knowledge and best beekeeping practice.

The role of beekeeper is to ensure health of bees by its knowledge and proper management practice. *(Phillips 2014, Rortais, Arnold et al. 2017)*

HEALTHY HONEYBEE COLONY

The colony has an adequate size and demographic structure.

The colony has an adequate production of bee products.

The colony provides pollination services.

BEEKEEPERS IN THE EU

However, in the EU where is over 900.000 of beekeepers with ca. 15 million of hives and nearly 97 % are non-professionals holding ca. 67 % of hives, is beekeeping mostly taken as hobby or free time activity. In the last decade, the popular connection between beekeeping and environment protection lead to increasing amount of beekeeping novices. Many of novices learn the classical beekeeping zoo-technique, which go beyond natural development of hive and beekeeper's action hit border of sustainability *(the large amount of hives at one place, collection of all honey, chemical control of Varroa mite, etc.)* without respect to natural development of bees. This can often have adverse effects on bees, leave residues in honey, decrease of bee vitality and it can result in high cost for beekeeper.

The common mistakes are connected with absence of queen in the hive, insufficient winter reserves, disassembling of whole hive, too early spring visit, insufficient carry of pollen and nectar or taking of too much honey away, wrong time of hive space increase or decrease, nectar and pollen flow gaps, late serving of feeding *(late brood rearing)*, wrong use of chemicals against *Varroa* mite. Too late treatment against *Varroa* mite and late winter feeding affect significantly the winter generation of bees, which develops after last honey harvest. If this mistake occurs, then the vitality of winter bees rapidly decreases in late autumn and bees mostly did not survive over winter.

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2·2

Effect of chemicals inside beehive

KEYNOTES

Substances brought by bees into hive could react between each other or start their toxic effect. When harmful substances accumulate, bees or parasites might become resistant. In many cases the biotransformation of harmful substances inside the hive is not fully studied.

Many chemical substances can be found inside the hive. They are transported from the environment directly to the hive, or they occur after mutual interaction or transformations. In this part we will focus on acute and sublethal impacts to the hive as well as on occurring resistances against some chemicals.

In the last two decade, the increased amount of reports about colony losses has increased in several countries. The causes of this decline are various, ranging from diseases, poisoning and lack of food, to complex relationships between global presence of varroosis and climate change. However, the microbiome of the hive reacts to the materials coming from surrounding environment, inside of the hive, interaction between the stored materials and the bees takes place.

Materials from the surrounding environment *(pollen, nectar, water or resins)* are not pure in nature; they are exposed to various chemicals and influences, which could cause contamination. For example bee collected pollen could contain pesticides which were sprayed in the field. If the pollen that is stored inside the hive is used for feeding the larvae, their health is influenced. However, the direct relation between pesticide residues in stored pollen samples and colony losses is not evident, even when fluvalinate, chlorfenvinphos or fipronil are steadily detected. Nevertheless, it is necessary to mention, that the synergism among acaricides with other pathogens or chemicals coming inside microbiome, it is not well studied. *(Bernal, Garrido-Bailón et al. 2010, Mullin, Frazier et al. 2010)*

Another problem refers to substances, which do not react with the microbiome, like microplastics, dust particles or organosilicones. These substances mostly pass through the microbiome without any change, or could accumulate inside without any observed effects. For example organosilicone surfactants increase the foliar uptake and therefore increase the field performance of agrochemicals. They are possibly not metabolised by bees and go out without any change. However, if there are both pesticides and organosilicones present inside the microbiome, then the mass flow of the pesticide could be increased, leading to a rapid enhancement of uptake and an increase of toxic effect to bees. *(Knoche 1994, Chen and Mullin 2013, Chen, Fine et al. 2018)*

Another effect to bees' behaviour it is connected with low dosages of some neonicotinoid insecticide *(e.g. imidacloprid)*. By measuring the time interval between two visits at the same feeding site, it was found that the normal foraging interval of honey bee workers is within 300 s. When pesticides in the feeding are present, the honey bee workers delayed their return visit for >300 s. This delayed return time depends on the concentration, and the lowest effective concentration was found to be 50 µg/liter. If the concentration was higher than 1.200 µg/liter, bees showed abnormalities in revisiting the feeding site. Some of them went missing, and some were present again at the feeding site the next day, however, with delays. This demonstrates that sublethal dosages of pesticides from the outside affect foraging behaviour of honey bees, and which can lead to the starving of larvae; therefore, pesticides and chemicals from the outside do have an indirect effect on the hive. *(Yang, Chuang et al. 2008)*

Beside this, substances in the environment could change or degrade according to local conditions. For example the insecticide coumaphos degrades into coumaphos oxon *(the oxidative metabolite)*, and the related degradate, chlorferone was often detected in comb wax, whereas the concentrations in pollen or bees was relatively lower. These metabolites are equally or more toxic than their parent compounds, what present a high risk when applying acarides.

Another problem concerning the use of acaricides refers to the resistance of the Varroa mite against some acaricides. It may develop rapidly as a result of constant exposure varroosis to miticide-impregnated wax comb. The removal of these residues in the wax may help to ensure the efficiency of miticides at proper level against the Varroa. It is generally agreed that the mite *(Varroa destructor)*, is playing a key role in the demise of honey bee health, and that intensive use of miticides has led to the evolution of wide-spread mite resistance among European strains of honey bees. Fluvalinate and coumaphos, but not amitraz, are highly persistent in the hive with an estimated half-life in beeswax of 5 years. A broad sampling of U.S. honey showed frequent but very low levels of coumaphos and fluvalinate up to 12 ppb, and only a few detections of lesser amounts of four other pesticides. *(Mullin, Frazier et al. 2010)*

At least, the "natural" compounds, such as thymol, can lead to problems inside the developing stages of honey bees. It is known that thymol accumulates in bee products and if the contamination of food by thymol occurs, there is notable risk for the early-developing larvae. *(Gael, Cyril et al. 2014)*

The complications regarding the transformation inside the microbiome are not predictible, because there is a time gap between when the contaminated pollen is collected and when it is actually consumed by the bees and the brood. The potential biotransformations of pesticides in beebread is completely unknown. The interactions between substances among various formulations with other stressors including Varroa and Nosema, viruses, as well as with beneficial hive microbes and with the bee immune systems all require further study. *(Mullin, Chen et al. 2015)*

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2·2·1

Chemicals used against bee diseases

KEYNOTES

In conventional beekeeping it is mostly common to use chemical methods for the control and elimination of pathogens and parasites. Chemical substances have broad-spectrum effects too they affect the health of bees in all development stages, and they accumulate in each bee matrices. The combination of medicaments against the Varroa mite and externally brought pesticides in sublethal dosages can affect health of workers.

The vitality and survival of managed honey bee colonies mostly depends on interaction between viral pathogens and infestations of the ectoparasitic mite *Varroa destructor*. Nowadays, the common strategy for reducing viral infections is the chemical control of mite populations. Combating the Varroa mite by applying chemicals means that also the honeybees are exposed to acaricides, carrying several side effects for the honeybee health.

The exposure to an acaricide may have a negative impact on the ability of honey bees to tolerate viral infection, as well as the microflora inside digestive tract. *(O'Neal, Brewster et al. 2017)* found **that amitraz and its metabolite significantly alter honey bee cardiac function, most likely through interaction with octopamine receptors. The results suggest a potential drawback to the in-hive use of amitraz** and raise intriguing questions about the relationship between insect cardiac function and disease tolerance. **Acaricides might also affect the neurological system of honeybees by having a negative impact on their olfactory ability, their antennae, their cognition, their learning ability as well as their memory.**

However, the primary target of veterinary treatment is elimination of *Varroa* parasite inside the hive. **Used chemicals—acaricides** *(some of them are insecticides)* **should kill these without influencing the bees' vitality. Acaricide medications for *Varroa* treatment often contain tau-fluvalinate, coumaphos and/or fenpyroximate, and these have synergism with fungicide or pesticide residues. Also, it seems that coumaphos, thymol and formic acid are able to alter some metabolic responses** *(e.g. detoxification)* **that could interfere with the health of individual honey bees or even entire colonies.** Synthetic acaricides such as fluvalinate, flumetrine, amitraz, coumaphos, and cymiazole can leave residues in the wax and honey. **Since the half-life of tau-fluvalinate and coumaphos is 5 years in wax, these pesticides can easily accumulate in colonies to reach unsafe levels. In last decade, the intensive utilization of many chemicals against *Varroa* has resulted in the development of mite resistance to acaricides.** *(Sánchez-Bayo, Goulson et al. 2016)*

Besides, recent studies have shown that coumaphos can alter some immune and detoxification gene expression pathways, affect queen and drone reproductive quality, and diminish lifespan. The pyrethroid tau-fluvalinate, an isomer of fluvalinate, targets the sodium channels of mites and insects altering neuronal electrical activity. Tau-fluvalinate has already been reported as impacting queen and drone performance and competitiveness.

The pyrethroid has direct effects on honeybees by increasing their susceptibility to Deformed Wing Virus (DWV) infection. Some antennal olfactory receptor neurons also seem to be strongly sensitive to this pyrethroid. Both acaricides are applied by beekeepers through pesticide-impregnated plastic strips and are subsequently distributed throughout a colony by nestmate interaction and trophallaxis.

Another known acaricide, amitraz has impact on learning and cognition of honey bees. Amitraz was reported targeting receptors in either the nervous or neuromuscular systems. Now amitraz is reregistered in some states of the USA and EU, and frequently it is found in beeswax. **It has been found, that pre-exposure to amitraz can increase the toxicity of other acaricides.** Evidently, these complex combinations of pesticides may produce synergistic effects on the insect nervous system, especially when they affect the same physiological targets.

The double exposure of chemicals due to Varroa control treatment as well as contaminated wax can produce higher doses than LD_{50}, that have a significant effect on the survival of worker honey bees. If the dose is ten times higher LD_{50} then 100 % of the worker bees die within 72 hrs. When the sublethal dose is applied *(0.5 times LD_{50})*, the acaricide treatments resulted in death rates of 20 %, which is still significantly high. *(Giacobino, Molineri et al. 2015, de Mattos, Soares et al. 2017, Gracia, Moreno et al. 2017)*

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2·2·2

Contaminants in nectar, pollen, propolis and wax

KEYNOTES

For years, residues of chemicals have been found in bee matrices as they can pass from one to another matrice and transform in each matrice. All matrices in the hive are connected and can exchange pollutants, some compartments being more persistent than others. In cells made of beeswax, honey and pollen are stored and larvae are bread.

The effects of a number of active substances and formulations on Apis mellifera cognition and mortality are well known. Recently much attention has been paid to storage of insecticide residua in materials collected by bees. *(Benuszak, Laurent et al. 2017)* Secondary, beekeepers are required to use *Varroa* mite control management to avoid colony death. Control methods, however, can often have adverse effects on bees, leave residues in honey and can be expensive for beekeeper. *(Silvina, Florencia et al. 2017)*

The contact between beeswax and other matrices enable the transfer of diffusing contaminants. Studies on contaminated matrices therefore need to take into account that the sources of contamination are two-fold, as contaminants can be brought from the outside into the hive, but they can also be mutually transferred within the hive through the matrices. **The exchange between beeswax pollen would be an interesting because of their high level of pesticide contamination. The contact between beeswax and honey and the chemical transfer between these two matrices is of concern for human consumption and for honeybee health. The miticide coumaphos, for example, can migrate from beeswax to honey.** The insecticide transfer often depends on the lowest distribution coefficient between octane and water ($K_{o/w}$): thiamethoxam = 0.741; imidacloprid = 3.72; acetamiprid = 6.31. The relationship between the physicochemical property $K_{o/w}$ and the pesticide transference into bee products allows to predict the behaviour of other compounds *(according to lipophilic and hydrophilic properties)*.

Honey kept within the brood chamber is stored longer and can be contaminated by other matrices or be repeatedly exposed to pesticides and veterinary treatments. Not only for honey, but for all honey bee matrices, comparative studies are needed to better know what kind of samples *(number and location)* is representative of a colony exposure. On the same issue, it is still unknown how many colonies should be sampled to be representative of an apiary when assessing pesticide exposure. **In the case of ecological honey, the presence of any residue over the regulatory limit is not allowed.** *(Chiesa, Labella et al. 2016)*

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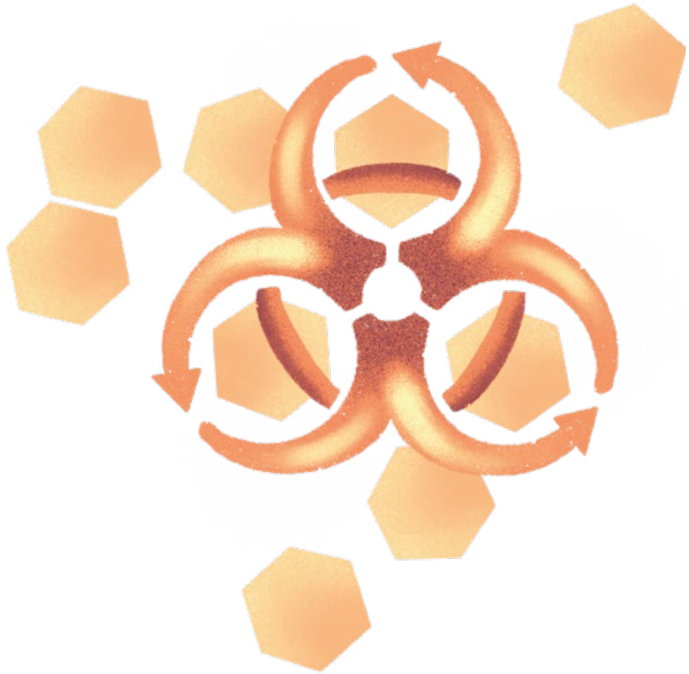
2·2·3

Contaminants in wax circle



KEYNOTES

Beeswax is the comb architecture element manufactured by honey bees themselves that is the walls, home, nursery, pharmacy, storage pantry and dance floor for the numerous inhabitants of the colony. When visiting flowers, honey bees collect nectar rich in carbohydrates (i.e. the honey sugars fructose, glucose and sucrose) These are required for the high energy demanding process of wax formation by means of their specialized wax-secreting epidermal glands found on the ventral side of the worker bees' abdomen.

(Lopez, Lozano et al. 2016, Calatayud-verná, Calatayud et al. 2017, Garcia, Duque et al. 2017)

Beeswax is a very complex mixture of lipophilic compounds. Its major components are hydrocarbons and lipids, which make up to 80 %. Of all beehive products, beeswax has the lowest replacement rate and can remain in the hive for many years, thus leading to a greater accumulation of different non-polar xenobiotics applied in beekeeping and agriculture. **Due to that, beeswax is the most contaminated beehive product and has already been used as a bioindicator of environmental pollution.**

Since the worldwide spread of the parasite *Varroa destructor*, beekeepers started to use acaricides to control the mite population, and to avoid damage thresholds. The use of veterinary agricultural treatments in beehives and their environment involves a risk of contamination of honey bees and related apicultural matrices *(wax, honey, pollen, royal jelly and propolis)*. Their analysis has also shown a widespread contamination. **In addition to the recycled beeswax used in beekeeping, beeswax is found in myriad products: lipsticks, facial creams, pill coatings, salves, chewing gum, candles, floor and furniture polishes, and waterproofing materials.** As being used as ingredient in cosmetics and pharmaceuticals it should contain minimal amounts of contaminants. Therefore, studying residues in beeswax is relevant not only to beekeeping issues but also to economic, environment and to public health purposes. *(Bonvehi and Orantes-Bermejo 2017, Calatayud-Vernich, Calatayud et al. 2017, Garcia, Duque et al. 2017)* described analyses of various wax types.

DIFFERENT TYPES OF WAX

VIRGIN BEESWAX

was determined as the least contaminated by pesticides — residues of coumaphos were found at mean concentration of 550 $ng.g^{-1}$; DMF and chlorfenvinphos were also found;

CAPPINGS BEESWAX

average load of 9 pesticides, higher concentration of Chlorpyrifos, Chlorfenvinphos and Coumaphos, other DMF, fluvalinate and acrinathrin;

FOUNDATION BEESWAX

average load of 7 pesticides, identified malathion, azinphos-methyl and fenthion-sulfoxide. The most frequent residues were coumaphos, chlorfenvinphos and fluvalinate. Detection of pyrethroids acrinathrin and flumethrin, and amitraz degradation product DMF. Dichlofenthion and chlorpyrifos;

OLD COMBS BEESWAX

residues of 11 pesticides were found acaricides — coumaphos, fluvalinate and chlorfenvinphos, detection of pyrethroids acrinathrin and flumethrin dichlofenthion, chlorpyrifos and DMF.

Today, beewax contains high levels of miticides, insecticides and fungicides residues. These residues come from veterinary treatments or pesticides used in the surrounding environment in region. In some cases, the use of illegal acaricide against varroosis — chlorfenvinphos causes a problem for bee brood. All foundation and recycled old combs beeswax can contain fluvalinate residues and it could be also found in beeswax from virgin combs and cappings.

Lipophilic beeswax can store non-polar pesticides , pesticides from the wax to other hive products *(propolis, royal jelly, pollen, honey)*. Key figures dictating the maximum limit of pesticides (MRLs) therefore indicate the quality of beeswax. **Most of pesticides found in beeswax are very stable once absorbed in this matrix. Many of the pesticides resist the process of comb recycling and some are concentrated by these treatments** *(e.g. coumaphos content does not decrease after 2 h at 140 °C)*.

High half-life times *(e.g. coumaphos, $t_{1/2}$ = 115–346 days)*, and elevated partition coefficients *(Log $K_{O/W}$)*, between 5 and 7.6 for some compounds, are the main factors involved in their stability in beeswax. Such persistence in this matrix lead to long-term simultaneous accumulation of many pesticides. **Long term accumulation of miticides in beeswax creates a propitious environment for acaricide resistant *Varroa*.** *(Kochansky, Wilzer et al. 2001)*

Manufacturers reusing and recycling the beeswax by treating foundation sheets involves other problems. **Wax from capping and recycled old combs are the only 2 beeswax sources used by manufacturers. High demand on wax foundations leads to increase of additions of cheap paraffin and stearine into beeswax** *(a.o. the concentration of stearine over 15 % causes brood damage)*. The closed beeswax market, where pesticide residues are maintained and introduced beeswax is being replaced by cheap and bee harming substitutes, offer cheap wax foundations for candle making, not for beekeeping. Therefore, foundation wax has to derive from capping or from virgin combs where less harming substances are present. *(Li, Kelley et al. 2015, Calatayud-Vernich, Calatayud et al. 2017)*

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2·2·4

Quality of supplementary feeding

KEYNOTES

Honey bees have evolved many strategies to fight with parasites and pathogens, but if they are nutritionally stressed, they face a major battle. Therefore, it is necessary to take into account the interaction of possible nutrition-related effects with other factors, such as the influence of nutrition on susceptibility or tolerance of honey bees to parasites, pathogens and pesticides, the energetic stress of bees caused by parasites and the role of nutrition in building up the honey bee's immune system.

The adult bees of a colony obtain their dietary protein from the pollen which is collected by workers from the flowers and brought back into the hive, or provided by the beekeeper. The proteins of some pollen are deficient in certain amino acids required by bees. Some of these amino acids are essential for bees and cannot be synthesized by them; therefore, the pollens or protein supplement diet of emerging bees and nurse bees should contain protein with an amount and variety of amino acids that will satisfy their nutritional need. Young bee larvae and the queen obtain their protein from the food *(royal jelly)* they are fed by nurse worker bees. It is required that the proteins have a precise quality and definite amino acid composition to ensure the development of brood and the optimum growth of young adult bees.

Another required foodstaff that larvae and adults need for their growth and development are carbohydrates. Carbohydrates in the bees' diet are mainly needed to generate energy for muscular activity, body heat, and vital functions of certain organs and glands, such as wax production. **Nectar and honey are the chief sources of carbohydrates in the honey bee's natural diet.** *(Brodschneider and Crailsheim 2010, Pohorecka, Szczesna et al. 2017)* described, that adequate nutrition supports the development of healthy honey bee colonies. **When the food source is contaminated or when larvae are starving,the bees are disturbed in their development stages, leading to weakened colonies.**

REASONS FOR SUPPLY FEEDING

① To ensure continued colony development in places and times of shortage of natural pollen and nectar. ② To develop colonies with optimum populations in time for nectar flows. ③ To develop colonies with optimum populations for pollination of crops. ④ To build up colony populations for autumn and spring. ⑤ To sustain brood rearing and colony development during inclement weather. ⑥ To build colonies to high populations for queen and package-bee production. ⑦ To maintain colonies and extend the season for high drone populations for queen matings. ⑧ To maintain colonies in feeding conditions. ⑨ To build up colonies after pesticide losses. ⑩ To provide adequate food reserves for overwintering colonies.

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2·2·5

Effect of stress factors on bee queen

KEYNOTES

Increased rates of honey bee queen failure have been reported in recent years, and it is mostly ascribed to widespread use of pesticides in agriculture and at rural environment.

Poor queen bee health is considered an important cause of honey bee colony mortality in North America and Europe, but few data can explain these observations over such broad regions. The literature describes lethal and sub-lethal effects of pesticides on social bees in the field and under laboratory conditions. Workers are more affected than the bee queens as they forage for nectar and pollen and therefore are more in contact with harming pesticides. **Queens exposed to pesticides showed altered reproductive anatomy** *(ovaries)* **and physiology** *(spermathecal-stored sperm quality and quantity)*, **which are likely to determine the success of the queen** *(production of alive and producing worker offspring)*.

The role of queens is indispensable for the survival of social bee colonies, and it relies heavily on the successful development and successful mating flights that trigger profound molecular, physiological, and behavioural changes. Honey bee queens are highly polyandrous, and normally embark on a series of mating flights within 14 days of emerging from their cells during which they should be fertilised with a sufficient number of spermatozoa that last their lifetime; they rarely leave the colony once they start ovipositing.

Therefore, the longevity of honey bee queens depends less on environmental conditions outside of the hive, than on proper development of sexual maturity and appropriate behavioural, anatomical, and physiological changes that occur following successful mating. Negative effects of pesticides on delicate queen reproductive systems result in abnormal physiology or anatomy, and impair the storage of spermatozoa or oviposition, and it can cause the replacement of the queen by the colony or by the beekeeper.

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2·3

Chemicals and materials used at hive construction

KEYNOTES

Hives for ecological beekeeping have to be mostly fabricated from natural materials.

In conventional beekeeping, the use of synthetic polymer materials and metal foils for protecting honeybees against electromagnetic smog is an observable trend.

Disinfection, recycling and disposal have to be considered for any material to minimize environmental impacts.

Ecological beekeeping allows only wood as material for hive construction, whereas in conventional beekeeping, various materials can be found. In the following parts we focus on some materials, that are used for the construction of hives and that comes in touch with honeybees and bee products.

In modern beekeeping, removable frames in hives, top bars and bottoms are used. Before their invention, bee colonies were found inside tree holes, later log hives, clay pots, or coiled straw skep. From log hives to the construction of the movable hive, developed since 1650, was mostly wood as the common used material. *(Crane 1992, Wilson 2006, Francis 2012).* In the case of organic beekeeping nowadays only wood could be used.

Recently, hive equipment has been made of materials other than wood.

Nowadays, the plastic materials are used nearly for all hive parts, including molded combs. *(Detroy 1980, William 1963)* The development and wide use of plastic materials in the last two decades lead to polyurethane hives, whose physical properties impede the attack of vermin *(Glasscock and Pearson 1980)*. Currently, the market for construction materials offers PVC board *(Rittberger, 2014)*, polystyrene and other plastic materials. Also, the combination of plastic and metalic materials is possible. For example *(Shtatnov 2010)* used thin metallic foils as shielding material on beehive walls and plastic insulation in order to protect the bees from harmful electromagnetic waves, to create a warm environment, and to properly ventilate the hive. Also, the use of thin antimicrobial silver foil on plastic walls was tested, for hive construction as well as the use of vermiculite with cement. Despite the benefits of plastic materials, **the release of risk compounds from plastic, their degradation and problematic disinfection, give good reasons to think if these materials are "environmental friendly".**

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2·3·1

Natural or synthetic materials

KEYNOTES

From the view of beekeepers, the lightweight materials are suitable for handling, however these materials could pose a risk at disposal. New trends include the use of biodegradable materials for hive construction, which are not certified for ecological beekeeping.

The following chapter describes the advantages and disadvantages of natural or synthetic materials as well as their recycling.

What type of hive should I chose, what material is suitable for myself and honeybees, and what frame size should I use? These are questions that nearly every beekeeper has at the beginning. In the wild, bees colonies were found around tree branches and holes in trees. Trees are made of wood, and so this material was the first choice of beekeepers. The requirements related to the construction material remained unchanged – the material should keep the hive completely waterproof, allow the rain to shed on the outside, allow condensation in the inside to help keep the bees under significantly drier conditions, and allow an easy hive cleaning. Wooden hive have some drawbacks: they can saturate from the outside in heavy rain, absorb condensation into the walls or draw up moisture from the ground promoting a damp internal atmosphere.

The use of plastic materials was firstly mentioned in mainland Europe around 30 years ago. Since then their use has spread and they now represent a significant proportion of the hives sold on the continent. The use of plastic was promoted; plastic should be a sustainable material, environmentally friendly and easy to clean. It is 100 % recyclable, using less resources than recycling paper. On the other side, the use of synthetic materials could cause problems because of the recycling process and additives which they contain. *(Dyter, 2011)*

(Richards 1987) describe that other materials besides wood can be used for beekeeping; e.g. from laminate, cocoon fibres and compressed pine wood, till to synthetic blends of plastics used for civil construction – such as polystyrene, polyurethane, etc. These materials have good insulation properties, are mass produced, and are lightweight. It was determined that to give comparable strength, polystyrene hives have significantly thinner walls than their wooden equivalents *(over 60 mm)* with a thick insulated roof and deep floor. Polystyrene helps to keep the hives warmer in winter and cooler in summer, meaning the bees need to consume less energy maintaining the correct brood nest temperature. This makes polystyrene hives ideally suited to the damp, rainy and variable temperatures of the maritime climate.

(Dodologlu, Dülger et al. 2004) studied the effects of two Langstroth hive designs *(wood or polystyrene)* and two feeding regimens *(sugar syrup or "bee pasture")* on selected performance characters of honey bee colonies. Colonies housed in wooden hives achieved superior performance over polystyrene hives as measured by overwintering colony survival, winter population loss, brood area, number of frames of bees and low defensiveness. Across both hive designs, brood area was significantly increased in colonies that received supplementary feeding regardless of feeding method. In polystyrene hives, colony weight gain during the nectar flow was significantly higher in colonies receiving supplementary feeding regardless of feeding method. Generally, feeding colonies with sugar syrup in autumn and with bee pasture and syrup in early spring provided optimum colony build-up for the production season.

The use of biodegradable materials for hive construction is a new material trend. This was firstly described by *Kueneman, et al. (1996)*. The hive was constructed from wax coated cardboard, with ventilation holes and an attached float feeder. In any case, the hives for ecological beekeeping have to be fabricated from natural materials.

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2·3·2

Painting and wood protection

KEYNOTES

The hives made of untreated wood start to decompose within two years if exposed to a certain level of moisture.

The lifetime of wooden hives is extended by using chemical substances that might affect the bees' health or contaminate bees products.

In ecological beekeeping, wood treatments can comprise methods and technologies that use heat, ozone or plasma.

Wood is the basic material, that has been commonly used by beekeepers for a long time. In this chapter, the influence of its expiration period and influences on the honeybee health are described. Every dangerous chemical compound that enters in contact with the wood — whether intentionally or unintentionally — has to be registered, assessed and classified, so that health risks are avoided.

The average life of wooden beehives and frames is maximally about 10 years. Wood is subject to decay when its moisture content is above the fibre saturation point, which averages about 30 percent water by weight. This level is often reached when wood rests on the ground because it readily absorbs water from the soil. In some countries, insects *(termites, wood-worm, carpenter ants, etc.)* and fungi pose an additional problem for beekeepers. Moreover, the lifespan of wooden hive parts varies according to the care given the hives. The bottom boards of untreated wooden hives that are set directly on a moist soil start to decay within 2 years. Therefore , it should be the beekeepers' interest to extend the useful life of their hives *(Kalnins and Detroy 1984, Bogdanov, Imdorf et al. 2003)*. Some wood preservatives or paintings are unsuitable for treating wooden hives and should be avoided, or they use is forbidden by law. Moreover, it is not recommended to store wooden bee equipment or wax combs in buildings where these materials have been used or treated.

TABLE

Technical details on different wood preservative type

TYPE OF PRESERVATIVE	GEOGRAPHICAL DISTRIBUTION OF USAGE		ADVANTAGES	DEMERITS/RISKS
	POPULAR IN	RESTRICTED IN		
Water based preservatives — MAINLY USED IN DIPPING AND PRESSURE TREATMENT, SAME ARE POSSIBLE TO BE BRUSH APPLIED				
Chromated copper arsenates (CCA)	Used in some developing countries without restriction	Strictly controlled in USA, Europe and Australia	Low cost, anticorosive	Contains Arsenic, Chromium and Copper; As and Cr are known to be toxic and carciogenic
Alkeline copper quatemarey	USA	–	Accepted as health & environment friendly	Highly increases the corrosion of the metal accessories in contact with wood
Copper azole	USA, Europe	–	Accepted as health & environment friendly. Effective in smaller quantities.	Moderately increases the corrosion of the metal accessories in contact with wood
Micronized copper	USA, Europe	–	Accepted as health & environment friendly	–
Borate preservatives	Throughout the world	Some countries discourage use	Low cost	Borate is leachable after application and may contaminate water and soil. Copper Chrome Boron (CCB) leaches less, but more toxic.
Sodium silicate based preservatives	Traditional technology practiced around the world	–	–	Easily washed away. Low penetrability. Not used in large commercial applications.
Bifenthrin spray preservatives	Australia	–	–	Low penetrability
Organic solvent based preservatives — OR OIL BASED PRESERVATIVES. MAINLY BRUSH APPLIED, OCCASIONALLY USED IN DIPPING AND PRESSURE TREATMENT				
Cole-tar creosote	Throughout the world	–	Useful in large rough application such as rail-road sleepers	Not suitable for valuable woods or internal applications. Highly toxic as a pigment.
Linseed Oil	New Zealand and Australia (Similar natural oils used in Europe)	–	Natural product	Mainly effective as a water repellant than actual biological action.
Light organic solvent preservatives (LOSP)	New Zealand and Australia	Europe	Clear non-viscous liquid that leaves no stains or shine on wood	Contains Volatile organic Compounds
Pentachloropenol	–	–	–	Can be highly toxic, normally used in pressure treatment

Several chemical wood preservatives *(copper naphthenate, copper-8-quinolinolate, and acid copper chromate)* have been reported to be harmless to bees or bee products.

Others *(creosote, chromated copper arsenate (CCA), tributyl tin azide (TBTO), and pentachlorophenol)* are reported to contaminate hive products or harm bees. Preservatives may be applied by brush, dip treatment, hot and cold baths, and commercial pressure-treating processes. Painting oil-based paint on all exterior surfaces of brood chamber and honey supers, including the top and bottom edges, is still the best control for wood-destroying organisms.

Treating wood with copper naphthalate will prevent against wood-destroying fungi, but the copper naphthalate is potentially harmful to bees. Similar the use of Creosote *(a distillate of coal tar)* can affect the flavour of honey and is harm bees.

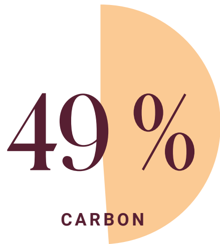
Use of pesticide like pentachlorophenol leads to contamination of beewax and and bees. It was also detected in honey from hive bodies treated with pentachlorophenol *(to a level of 6.4 kg/m^3)*. The beeswax averaged 39.8 parts per million of pentachlorophenol; 5.2 and 0.14 parts per million of the preservative were found in the bees and the honey, respectively.

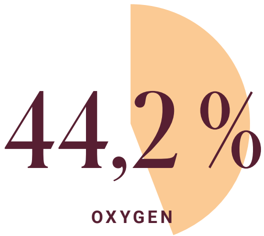
Beside this, some chemicals used in the woodworking industry at hive construction are classified as hazardous and have specific safety requirements for treatment, preservation, and painting and its removing, etc. Chemicals used in woodworking can cause a number of health problems. Each hazardous chemical therefore needs to be identified, assessed and controlled to minimise health risks to bees, beekeepers and the environment.

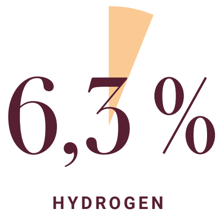
Some glues, resins and isocyanate based paints and varnishes can diffuse from hive wooden parts into the colony and affect the health of colony. Unpropper wooden materials like Medium Density Fibreboard *(MDF)*, or oriented strand board *(OSB)* contain glues based on formaldehyde resins, which could release bee harming volatile substances.

Another way to preserve wood is to use heat *(flame)* treatment method where the internal structure of a timber is altered by heat without using chemicals, making it less susceptible to pest attack. Generally heat treatment is expensive and only used in large industrial applications.

WOOD COMPOSITION







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NITROGEN

CONTENT OF WATER

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2·3·3

Chemicals used for disinfection and maintenance

KEYNOTES

In ecological beekeeping, physical methods (steam or direct fire) for disinfection are allowed; however, this methods cannot fully withstand possible reinfections due to limited effectiveness. Some earlier used ecological compounds can still be found in surface water, soil, sediments and other parts of the environment.

The process of disinfection requires following the safety rules to protect the health of bees and beekeepers.

Hygiene is the essential condition for sustainable health of honey bees and quality honey bee products. The following chapter deals with the possibilities for disinfecting beekeeping material and equipment and with the negative effects of disinfection on the environment, where honeybees are present.

Maintaining a good hygiene plays an important key role for preventing the spread of infection. Hygiene is ensured by a proper beekeeping management, including proper disinfection. Disinfection could be done by physical or chemical methods, and it is targeted to decrease or eliminate insects, mites, fungi, viruses, and bacteria, such as microbes that cause American or European foulbrood *(AFB and EFB) (Paenibacillus larvae and Melissococcus plutonius)*. The eradication of these diseases is realized only by incineration of all beekeeper's wooden materials and disinfection of of all used metal/plastic equipment.

Spores of AFB/EFB were found deep inside wooden equipment, and they are highly resistant to physical and chemical disinfection of materials. Commonly used chemicals for disinfection are: the caustic soda, sodium hydroxide, or sodium hypochlorite. Other approved chemicals used at disinfection are: Lactic acid, Oxalic acid, Acetic acid, Formic acid, Sulphur, Etheric oils, Pyrethrins, Potassium bi-carbonate, iodine, sulphuric acid, phosphate acid, alcohols, quaternary ammonium compounds, phenols or tensides. The killing efficiency of chemical methods is moderate and even scorching the wood by flame treatment is not able to reliably remove spores to prevent re-infection.

CHEMICAL SOLUTIONS FOR DISINFECTION

CAUSTIC SODA

SODIUM HYDROXIDE

SODIUM HYPOCHLORITE

LACTIC ACID

OXALIC ACID

ACETIC ACID

FORMIC ACID

SULFUR

ESSENTIAL OILS

PYRETHRINS

POTASSIUM BICARBONATE

IODINE

SULFURIC ACID

PHOSPHORIC ACID

ALCOHOL

QUATERNARY AMMONIUM COMPOUNDS

PHENOLS

SURFACTANTS

Recently, new methods were developed, based on plasma treatment which was able to effectively remove spores from wax, which, under protocols currently established in veterinary practice, normally is destroyed by ignition or autoclaved for sterilization. The second methoduses ozone to achieve the elimination of spores and degradation of pesticide residues. *(Priehn, Denis et al. 2016)*

The use of oxidative substances at disinfection it is known for a long time. The strong oxidizers generate toxic gaseous products if they react with other ecological substances or chemicals. To avoid respiratory problems it is necessary to use them in good ventilated areas. Besides disinfection, the reaction *(by-)* products can irritate the skin, eyes, or highly corrosive for metal equipment. Chlorine can occur, for instance, when wooden material containing remains of acids is dipped into sodium hypochlorite solution. Also, allergic reactions can occur, for example when iodine solution is used for disinfection.

The use of chlorinated organic compounds, like pentachlorophenol *(PCP) is well described in the literature*. PCP has wide spectrum of applications, from pesticides, as well as disinfectant and antifouling paint. However, PCP has been detected in surface waters and sediments, rainwater, drinking water, aquatic organisms, soil, and food, as well as in honey and bees. Even if it is banned, PCP is still found in surface waters due to rain and the soil by run off and leaching as well as from manufacturing and processing facilities.

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2·4

Chemically induced changes in beehive microbiome

KEYNOTES

When exposed to different concentrations of chemical substances, changes occur on microscopic scale (cells, tissues, organs and on macroscopic scale (individuals, population).

In ecological beekeeping, no synthetic medicaments are allowed. Some allowed medicaments, however, provoked changes in the metabolic responses and detoxification mechanisms, eventually influencing the development of bees, active substances (e.g. neonicotinoides) in formulations can influence the insects' neurocognitive functions.

In every development state, honeybees are in contact with many substances, and some of them can harm their health. In this chapter, it is discussed how compounds could significantly affect detoxification or methamorphosis during larval state. Bees are exposed to various substances and stress factors, which leads to activation of detoxification pathways, decrease hemocyte density, encapsulation response, and increase of antimicrobial activity. An overload of detoxification mechanism, which might occur when bees are exposed to a large quantity of pesticides, for example by the application of several miticides, potentially harms bee colonies by decreasing their ability to detoxify other natural or synthetic compounds.

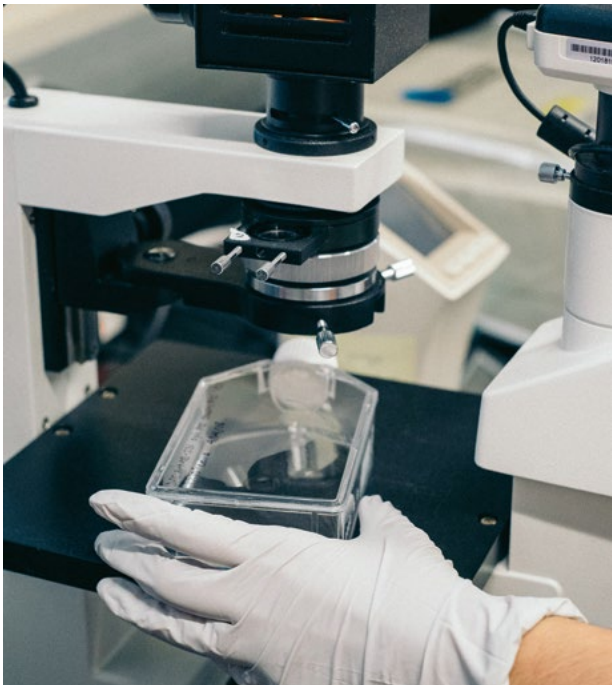
Once a harmful substance is introduced into an organism, interaction takes place and the substance releases its toxic effect on the targeted organism *(toxicodynamics)*. The effects might occur on a molecular level but have observable "macroscopic" impacts, for example changes of behavioural and cognitive functions as well as in cells, tissue, organs on an individual and on population level.

Beekeepers have been using acaricides and other chemicals to control *Varroa* mites for more than three decades. In a study conducted by *Boncristiani, Underwood et al. (2012)* bee hives were treated with five different acaricides: Apiguard *(thymol)*, Apistan *(tau-fluvalinate)*, Checkmite *(coumaphos)*, Miteaway *(formic acid)* and ApiVar *(amitraz)*. The results indicate that thymol, coumaphos and formic acid are able to alter some metabolic responses. These include detoxification gene expression pathways, components of the immune system responsible for cellular response and the c-Jun amino-terminal kinase *(JNK)* pathway, and developmental genes. These could potentially interfere with health of individual honey bees and entire colonies. It is to assume, that in-hive acaricide applications can potentially interfere with honey bee health.

The laboratory experiments of another study showed similar results; the challenge with thiacloprid, imidacloprid, or clothianidin *(which all three are insecticides of the neonicotinoid class)* significantly reduced the antimicrobial activity of the hemolymph, along with a decrease of the encapsulation response. These findings may be interpreted as impairment of disease resistance capacities of honeybees resulting from the exposure to neonicotinoids *(Brandt, Gorenflo et al. 2016)*.

The results from experiments conducted by Christen, Mittner et. al *(2016)* on the influence of environmental realistic levels of neonicotinoids on honeybees showed severe molecular changes in the brain of worker bees. However, not only the brain can be affected; the effects also involve influences on the learning ability and cognitive functions.

Uncontrolled alteration of ecdysone production could be very influential to hive sustainability, culminating in unpredictable consequences.



The detoxification mechanism of honey bee immune system could be damaged as well. *Boncristiani, Underwood et al. (2012)* described, that expression of P450 family monooxygenase genes *(cyp6A, cyp306A)*, which are associated with environmental responses, including resistance to pesticides, could be altered by Thymol and Coumaphos treatment of beehives. These gene are associated with synthesis of one of the most important insect hormones, 20-hydroxyecdysone *(20E)*. 20E and juvenile hormone are key regulators of insect development, including the differentiation of the alternative caste phenotypes of social insects. 20E triggers the key regulatory cascades controlling the synchronized changes in developmental pathways during molting and metamorphosis.

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