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Blesabee

FROM HEALTHY BEES TO HEALTHY HUMANS

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PRLOGUE

Worldwide, high honeybee colony losses have been registered. Since 2006, winter mortality has averaged 30 % in many places, and in some regions beekeepers experience massive losses up to 90 %. The significant decline in the number of bee colonies is directly linked to several factors, amongst others the intensification of agriculture, the overuse of hazardous chemicals in beekeeping practices, the loss of availability of pasture for bees, more frequent and extreme weather conditions, as well as the spread of honeybee diseases and pests.

The region of Central Europe was very rich not only in its natural beauty, but also in the multitude of diverse bee grazing of important value. Beekeeping has developed very dynamically in Central European countries due to the intensive cooperation between beekeeping masters during the Austro-Hungarian monarchy. The aim of these cooperations was to make the best use of knowledge and experience, and to intensify beekeeping activities. However, the desired result—simple and profitable beekeeping—is becoming an ever greater challenge as the bees' needs and their natural environment are being neglected. There are many internal and external stress factors for bees, and under these conditions, bees and beekeepers cannot prosper. Therefore, it is essential to take action and to develop cooperations between different stakeholders so that biodiversity is no longer on decline and the living conditions of bees can be significantly improved.

If we want to support the survival of bees, we need to fulfill their needs and improve their health. This involves: ① Minimizing the amount and type of poisons present inside and outside of the hives. ② Stop treating bee colonies infested with Varroa destructor mites by means of chemicals. ③ Ensuring a constant supply of high quality water, nectar and pollen from spring to late autumn.

This handbook discusses both topics, bee-threatening external and internal stress factors as well as key aspects contributing to the well-being of honeybees. The book specifically focusses on chemical-free strategies to keep healthy bees, which we, lovers of bees, are in deep need.

This handbook is a blueprint showing that chemical-free beekeeping is possible and successfully applied since 2010 by over 1000 beekeepers in Slovakia, Austria, Switzerland, Germany, France and the Czech Republic as demonstrated by numerous testimonials included in the book. This handbook brings a new perspective on beekeeping training for schools, associations and single users. The included QR codes make it easy to access training videos and testimonials. We invite you to become part of a growing international network of chemical-free beekeepers using hyperthermia, and to be an inspiration for other beekeepers in your region!

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FACTORS THREATENING THE LIFE OF BEES

BEES ARE EXPOSED TO AN INCREASING PRESSURE RESULTING FROM ENVIRONMENTAL POLLUTION AND LOSS OF THEIR NATURAL HABITAT. WHICH HONEYBEE PRODUCTS ARE THE MOST CONTAMINATED AND HOW DO PESTICIDES ACCUMULATE AND INTERACT WITH EACH OTHER? ARE WAX UNCAPPING FROM FRESH HONEYCOMBS SUITABLE FOR THE PRODUCTION OF WAX FOUNDATIONS? CAN THE CONTACT WITH ACARICIDES HAVE NEGATIVE EFFECT ON THE ABILITY OF HONEYBEES AND BROOD TO RESIST VIRAL INFECTIONS? THESE ARE ONLY FEW FROM MANY QUESTIONS THAT WE ASKED THE RESEARCHER AND BEEKEEPER, DR. ROMAN SLAVÍK.

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General external stress factors

KEYNOTES

For ecological beekeeping it is necessary to have landscape with various food sources through whole beekeeping season. The use of agents, which are not suitable for organic agriculture (compounds for plant protection, fertilizers and soil auxiliaries, organic feeding materials, products for the cleaning and disinfection of buildings and equipment used for crop production) poses a risk to honeybees and the quality of honeybee products.

The flyaway of compounds could pose a significant risk for ecological beekeeping where they could be present in pollen, nectar or water brought by bees to the hive.

Recently the colony losses are one of most discussed topics and they have direct connection with intensified agriculture, diseases, landscape changes, where honey bees are living. In following chapter we describe some of external stress factors, which influences honey bee health.

During the last few years, there have been an impending "pollination crisis" in certain parts of the world due to decline of honeybee colonies, due to attack by pests and diseases combined with a general increase in the area of bee-pollinated crops. *(Abrol 2013, Capri, Higes et al. 2013, Reuber 2015)*. The outbreak of diseases depends mostly on stress factors affecting bee health.

Stressors affecting bees are multiple in nature and origin and these can be grouped into four broad classes: physical, chemical, biological and nutritional. **Physical stressors** are mostly governed by environmental changes *(e.g. climate change, habitat fragmentation, destruction or loss, presence of invasive species, or changes at flowering periods, or electromagnetic and light smog)* while **chemical stressors** mostly include compounds of an anthropogenic nature *(e.g. farming, urban/industrial/ mining activities, air pollution, pesticide overuse by beekeeping or gardening, etc.)* as well as naturally occurring contaminants.

Biological stressors include bee pests and diseases while **nutritional stressors** may be expressed as a change in the bee's nutritional status *(e.g. proteins, lipids, sugars, vitamins and minerals)*. The qualitative and/or quantitative intake of biological, chemical or physical agents via food as well as exposure from other sources depends on quality of environment and biodiversity. Both biological and nutritional stressors may be modulated by environmental changes and/or anthropogenic activities *(e.g. an increase in bee pests and exotic diseases due to climate change and global trade; nutrition of bees related to resource availability in the landscape and beekeeping management practices)*.

Finally, foragers and in-hive bees can be contaminated via consumption of nectar and/or pollen contaminated by spray treatments. Besides the application rate and application frequency, the means and magnitude of contamination are highly dependent on a number of elements, such as the application methodology, type of hazardous substances, weather condition at the time of application, landscape composition, and the treated crop. In addition, in one crop-dominated landscapes, non-cultivated plants represent an important source of pollen and nectar for bees.

As well, the application of hazardous substances to an unattractive crop or to a crop in an unattractive stage *(i.e. after the flowering)*, can also cause an increase of persistent residues in the soil, as they can be taken up by the roots of an attractive crop in the following season. Off-field areas can be indirectly contaminated by a proportion of the spray liquid drifting away and depositing onto vegetated areas at the field margins or onto adjacent crops. Similarly, dust particles from solid formulations, including formulations used for seed treatment can be deposited to off-field areas.

PRESSURES

① PHYSICAL

② CHEMICAL

③ BIOLOGICAL

④ NUTRITIONAL

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Global changes in bee living environment

KEYNOTES

Global climatic change affect habitat for bees, which cannot adapt to this changes sufficiently. Influence of global changes could affect pollination. Without mankind assistance, there is an increasing decline of bee population.

Honeybees have been living on earth since 150 million years, but at last decades the ecosystem has been deeply influenced by mankind, with severe consequences: European honey bees cannot survive without our help. Together we look on some main extern stress factors influencing their life.

Declines in bee colonies date back to the mid 1960s in Europe, and have accelerated since 1998, while in North America the overuse of agrochemicals and medical treatments of honeybees have let to decreasing health of bees. Since 2004, the colony losses have left the continent with fewer managed pollinators than at any time in the past 50 years. Since the winter of 2006–2007, beekeepers across the globe have reported wintertime losses of 30 to 90 percent of their colonies. Foraging bees seem to simply abandon the hive, including a seemingly healthy queen, and leave immature bees and remaining honey. Scientists are still investigating numerous possible causes, including affects of pesticides and diseases. Another factor contributing to the stress of colonies is the climate-related environmental change. A variety of factors are making these managed colonies vulnerable to decline and collapse. *(VanEngelsdorp and Meixner 2010)*

COLONY WINTER LOSSES

30 – 90 %

SINCE 2006/2007

Nowadays, there have been found factors which may be coming together to cause the decline and they include:

FACTORS

Changes in, rural and urban areas, habitat degradation and fragmentation; biological changes and loss of flowering plant species that provide food for bees.

Overuse of plant protection chemicals, including the so-called “systemic” insecticides which can migrate to the entire plant as it grows and be taken in by bees for consumption of nectar and pollen.

Presence of parasites, pests and invasive species, in particular global presence of Varroa mite increased average year temperature and air pollution.

Overuse of conventional chemical substances to control Varroa mites.

Honeybees are not able sufficiently shift their ranges in order to adapt to a mankind driven climate change, and to ride out potential stressors endangering entire bee populations. If the adaptation to the new conditions is not sufficiently fast, pollination could fail.

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Changes in biodiversity of crop and pollen

KEYNOTES

Honeybee colonies have to be kept in an environment with a high biodiversity, where pollen and nectar are available through the whole beekeeping season.

The pollen quality depends on local environmental conditions where plants are growing, and it influence significantly the development of colonies.

Phytosanitary care within ecological agriculture has to be carry out with proper amounts of authorized agents, so that recommended limits will be not exceeded in the environment.

The climate change and/or the planting of new crop types have resulted in an insufficient and changes variability of food sources for honeybees. The following part focuses on some climate changes that influence the relation of plants and honeybees.

Vital and healthy bees live in environment where collect nectar from various flowering plants or honeydew from different coniferous trees which they deposit into honeycomb in the hive. As bee floral food resources have been reduced over past decades in human-dominated landscapes, a carry-over effect between seasonal disruption in floral resource availability and high colony losses was identified. Even if flowering meadows are planted to improve the biodiversity and even many "pollinator-friendly" seed mixes are available, less is known about the effect of climate change on flower phenology *(Richards and Kevan 2002, Gonzalez-Varo, Biesmeijer et al. 2013, Hicks, Ouvrard et al. 2016).*

Pollen quality depends on optimal growth conditions specific for each flower that is visited by honeybees. These phonologic conditions are shifting nowadays, and there are observations about different timing of flowering, its duration and frequency. However, the changes are deeper in the structure of flowers. Plants growing at unsuitable conditions produce less nectar or nectar without sugar compounds, pollen with different amino acids composition or the flowers just remain in the soil in dormant state. Pollinators respond to this change in different ways: from visiting less attractive plants, till to shift of development stages of honeybees. Let's take the comparison between the rural landscape with an extensive agriculture and the urban area with extensive industry as an example:

In rural landscapes, there is no big variety of plants as many crops offer just monofloral food for bees. Also, the rural crop protection uses higher levels of protective compounds than in urban areas. Urban areas give stable amount of various plants growing through whole beekeeping season. And last but not least, the average temperature in the city is mostly about two degrees of Celsius higher than in rural areas.

Arising climate change caused a spatial and temporal mismatch between pollinators and their food plants owing to shifts in the distribution ranges and phenology. To rear a single bee larva, pollen from between 20 and several thousand flowers is required, and 120 kg of nectar and 20 kg of pollen are collected annually by one average European honeybee colony. *(Requier, Odoux et al. 2017)* **investigated that the change of phenology may have been overlooked as a cause of pollen shortage that is associated with bee colony losses.**

ANNUAL HARVEST OF A SINGLE COLONY

120 kg	20 kg
NECTAR	POLLEN

Dwindling pollen reserves could result in early cessation of brood rearing that could triggers the premature development of long-lived winter bees. Colonies with winter bees that were reared early because of pollen scarcity are less likely to survive the winter than those colonies that rear winter bees later in the fall. Several strategies are therefore recommended to avoid high colony losses in intensive farmland systems.

STRATEGIES TO AVOID COLONY LOSSES

Limiting or avoiding honey harvests in spring.

Monitoring colonies for early-warning signals of colony failure.

Increasing the amount of floral resources available through wise land-use management.

Another problem comprise the occurrence of Genetically Modified Organisms *(GMOs)* in environment. The GMO crops with insecticidal properties are likely to have a negative, albeit sub-lethal, effect on bees which however have not been verified yet. For example, recent studies did not confirmed that worker bees and colonies fed pollen from genetically modified corn did have increased rates of mortality. It was also not confirmed that pollen fromsuch a modified corn affect the microflora in bee intestines or the affect hypopharyngeal gland development. *(Boily, Aras et al. 2017)*

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Effects of climate changes to hive

KEYNOTES

Ecological beekeeping strongly emphasizes the environmental condition and welfare as basic principle.

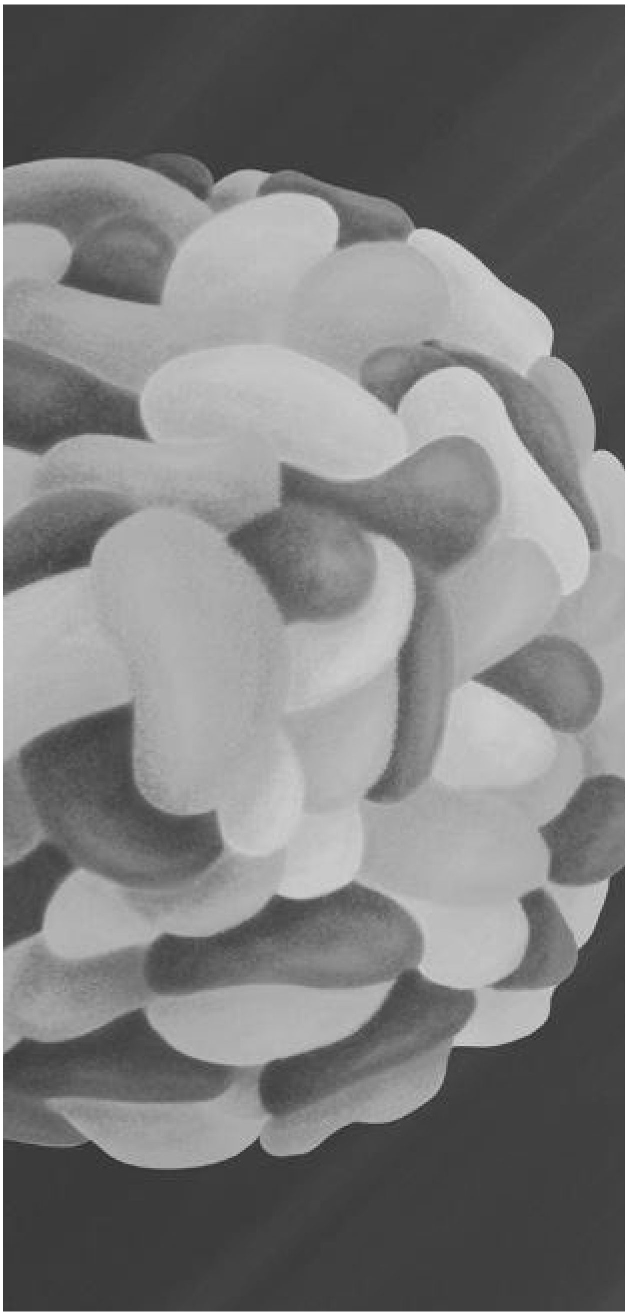
Climatic changes influence the development of bees and their resistance against diseases.

Shift of climatic regions leads to changes in the brood development. In some regions, the colony is breeding throughout the whole year.

Microclimatic conditions around the beehive influence development of colony and of course the food availability. In this chapter we describe how climatic changes influence the microclimate in the hive and consequently the spread of diseases.

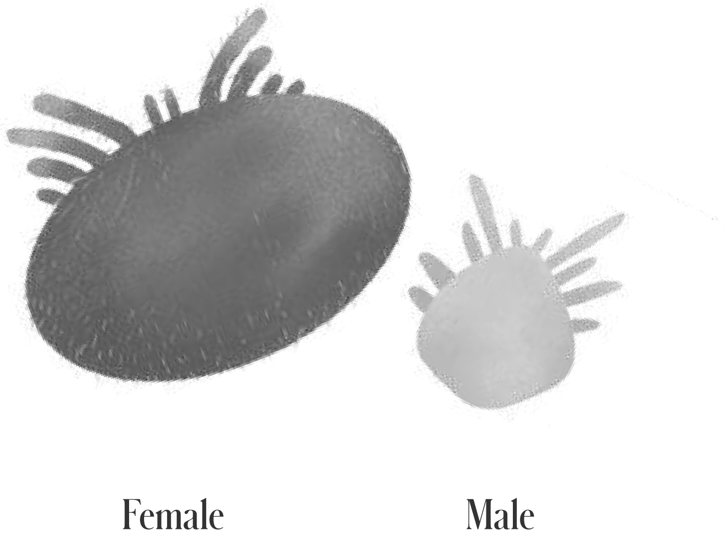
Climate has a very real effect on colony welfare and development. Extended periods of cold, rainy, and hot weather have been blamed on severe, often unexplained, colony mortality in the past. Local weather conditions can have a direct effect on colony productivity. For example, **higher ambient temperatures tend to increase colony productivity because of reduced metabolic demands on foragers, while long periods of rain and cool weather have a detrimental effect on productivity as bees remain in the hive.** Effects of weather on colony productivity could be of positive or negative nature, and their influence is indirect. High temperatures and sufficient precipitation are both correlated to increased nectar production, which in turn translates to an increased colony productivity.

Conversely, insufficient rain or rain at inopportune times can have a negative effect on colony productivity. Both prolonged summer drought and persistent fall rains have been blamed on poor overwintering. Dwindling pollen reserves in the fall result in early cessation of brood rearing that triggers the premature development of long-lived winter bees. Colonies containing winter bees that were reared early because of pollen scarcity are less likely to survive the winter than those colonies that rear winter bees later in the fall *(vanEngelsdorp and Meixner 2010)*.



FUNGUS APIS ASCOSPHAERA

VARROA DESTRUCTOR



Weather can also have an effect on pathogen loads within colonies. For example, temperature and humidity have a direct effect on *Varroa* mite population growth. Conversely, cool weather, especially when a colony’s adult population is small *(which is common in spring)*, can result in chilled brood. While chilling can kill immature bees outright, brood chilling is required for the spread of some pathogens, such as chalkbrood, *Ascosphaera apis*.

Through climatic zone shift and the increase of average year temperature, the condition in beehives leads to all year presence of brood. This is the case in the tropical regions, where higher average temperature and floral resources are available year round resulting in breeding activities all year long. As a consequence, populations of parasites that reproduce on immature bees, like the *Varroa* mite, grow much more quickly than they would if brood rearing was interrupted. Because of the current climatic conditions, there are only few areas in the world, where bees can survive without human assistance.

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Global trade and threats by invasive species and plants

KEYNOTES

Globalization allows widespread of non-native species, whose presence could disturb or impair relationships inside ecosystem.

When invasive plant species produce enough pollen and nectar, they could become important food source for honey bees or they could fill gaps in phenological phases.

There is no guarantee that bees can be sufficiently protected if the spreading of bee-endangering invasive species occurs.

Globalization and shortening of transport distances of goods, animals and plants leads to increasing to an increasing presence of some invasive species. In this chapter we look on risks for bees that result from the presence of invasive plants. However, the spread of invasive species might be beneficial, for example in situations of natural food lacking.

Although only a small proportion of alien species become invasive, a general massive increase of biological invasions has occurred in the last decade due to rapid globalization. Greater problem occur when invasive aliens consume or otherwise physically affect native species, Invasion might affect interactions among native species in the ecosystem, for example via competition for biotic resources, or via alteration of abiotic resource availability. *(Brown and Paxton 2009)*

The change, loss and fragmantation of habitat associated with agricultural intensification are considered as main risks not only to bees. Some introducted plants are most common in nutrient-rich and/or man-made habitats *(including urban and agricultural ones)*. A large proportion of alien plants have been introduced as exotic species, for example in Australia and Europe, and thus many produce showy, eye-catching flowers, making them attractive to various animal. In addition, many invasive alien plants fill a phenological gap of flower resources for bees, allowing an extended foraging season. As a result, invasive alien plants have the potential to impact not only on individual foraging behaviour, but also on colony success of social species, population size and distribution of native bees, bee community structure and entire plant-pollinator networks. From the other side, some alien plant species display flowers specialised for pollination by animals other than bees, and so nectar and/or pollen rewards may not be accessible to bees. *(Kluser, Neumann et al. 2010)*

The quantity and quality of nectar and pollen produced by plants are affected by abiotic resource availability in the habitat, the resource allocation by the plant and the pollination way to which the plant belongs. The nutritional value of pollen varies in protein content among species and this affects the pollen foraging behaviour of honeybees. If invasive alien plants produce nectar and pollen of sufficiently high quantity and quality, they could act as important foraging resources, for example particularly in forage-depleted agri-environments. On the other hand, if invasive alien plant rewards are not beneficial to native bees, but even detrimental to them, they can have the opposite impact. For example, the nectar and pollen of the invasive alien *Rhododendron ponticum* contain grayanotoxins which cause poisoning in humans.

From the view of bees, the invasive alien insects and native bees might compete for resources, if ① they experience a substantial floral resource overlap, ② resources are limiting, and ③ decreases in resource acquisition translate to a reduction of fitness of the less competitive or both competing species *(usually expressed as a reduction in fecundity, survival, or population size)*. One of the biggest threats to honeybees associated with the invasion species is the spread of diseases and parasites.

Some invasive species provide benefits *(including the economic and environmental benefits of increased pollination)*, but others have negative impacts on bees *(e.g. Vespa velutina, Aethina tumida)*. Impacts of invasive alien species on bees may differ and be unpredictable, and probably vary according to landscape context and conditions. *(Mooney and Cleland 2001, Genovesi, Shine et al. 2004, Neumann and Ellis 2008, Stout and Morales 2009)*

RHODODENDRON PONTICUM

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Nutrition and water source availability

KEYNOTES

For ecological beekeeping the hives should be placed at landscape with higher carrying capacity of various pollen and nectar sources which are available through year.

It is recommended to use bioproduced seeds for the ecological production, supporting the system of crop variation.

Exceeding the recommended/prescribed concentration of pesticides might result in pollen contamination, which causes decreased bee health conditions and which therefore has to be avoided

The life and survival of a bee colony depend upon the biodiversity of vegetation in the surrounding environment, specifically on the available pollen and nectar sources. Honey bees need good-quality food to successfully complete their development and to survive through the winter. Proteins from pollen are needed for growth and to carry out vital functions in the body and reproduction. A decline in pollen harvest was often associated with a direct reduction in brood production, leading to a negative effect on the adult population size later in the season, and lower honey reserves before the onset of winter. Furthermore, the decline in pollen harvest negatively impacted health of the colony, resulting in higher *Varroa* mite loads and higher seasonal and winter colony losses.

Nowadays, there may be periods when sufficient suitable pollen or nectar producing plants are not available. In agricultural areas, simplified crop rotation practices limit the diversity of crops or grazing/mowing may occur before flowering. Similarly a drought can reduce flowering and therefore access to sufficient food for bees. Depending on the timing or the provision of alternative food sources the bee colony will be weakened and may not survive through the winter.

Like man, bees live on a varied diet. The more varied the food available, the more resistant bees are to diseases. The intensive use of the cultivated landscape and the increasing development of monocultures reduce habitat diversity. If all nectar flowers provide nectar at the same time and for a short time

INTENSIVELY CULTIVATED LANDSCAPE

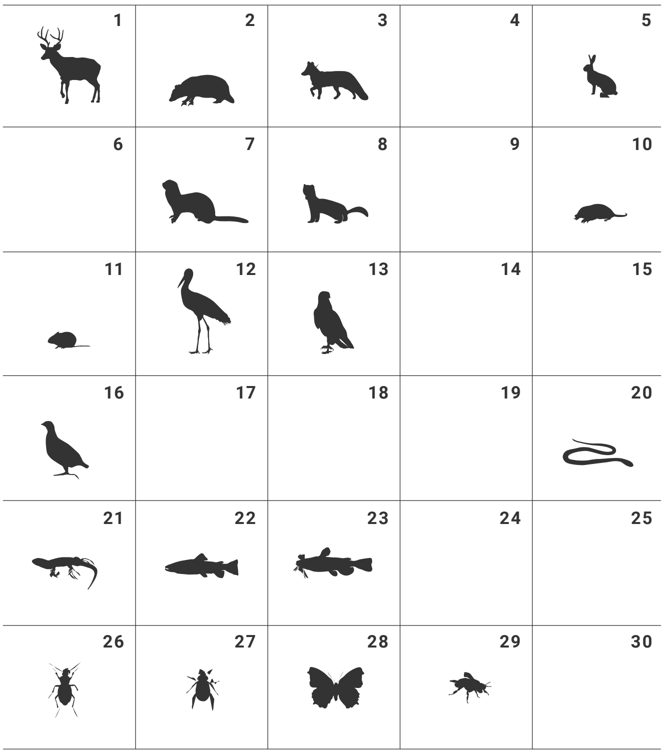
1	2	3	4	5
				
6	7	8	9	10
				
11	12	13	14	15
				
16	17	18	19	20
21	22	23	24	25
26	27	28	29	30

7 species

Legend

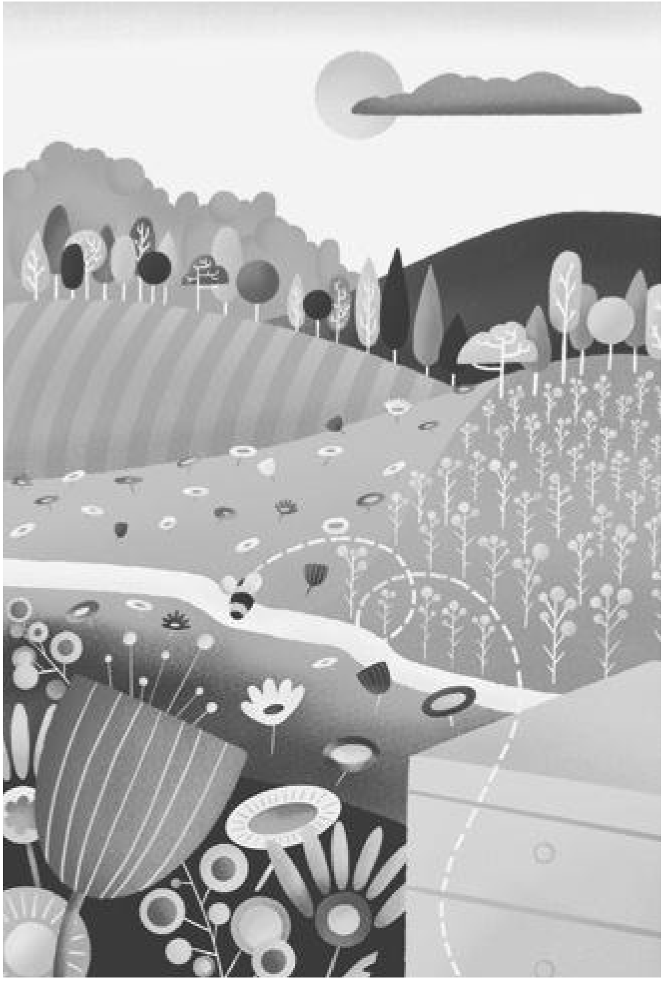
1 • ROE DEER • CAPREOLUS CAPREOLUS
2 • BADGER • MELES MELES
3 • FOX • VULPES VULPES
4 • OTTER • LUTRA LUTRA
5 • RABBIT • LEPUS EUROPEUS
6 • MARTEN • MARTES FOINA
7 • FITCH • MUSTELA PUTORIUS
8 • ERMINE • MUSTELA ERMINEA
9 • HAMSTER • CRICETINAE
10 • MOLE • TALPA EUROPAEA
11 • MOUSE • MUS MUSCULUS
12 • STORK • CICONIA CICONIA
13 • EURASIAN BUZZARD • BUTEO BUTEO
14 • LONG-EARED OWL • ASIO OTUS
15 • WOODPIGEON • COLUMBA PALUMBUS
16 • GREY PARTRIDGE • PERDIX PERDIX
17 • QUAIL • COTURNIX COTURNIX
18 • EDIBLE FROG • PELOPHYLAX KL. ESCULENTUS
19 • COMMON TOAD • BUFO BUFO
20 • GRASS SNAKE • NATRIX NATRIX
21 • SAND LIZARD • LACERTA AGILIS
22 • BROWN TROUT • SALMO TRUTTA FARIO
23 • BULLHEAD • COTTUS GOBIO
24 • BEAUTIFUL DAMOISELLE • CALOPTERYX VIRGO
25 • SILVER WATER BEETLE • HYDROPHILUS PICEUS
26 • GOLDEN BEETLE • CARABUS AURATUS
27 • COMMON COCKCHAFER • MELOLONTHA MELOLONTHA
28 • MOURNING CLOAK • NYMPHALIS ANTIOPA
29 • COMMON CARDER BEE • BOMBUS PASCUORUM
30 • NOBLE CRAYFISH • ASTACUS ASTACUS

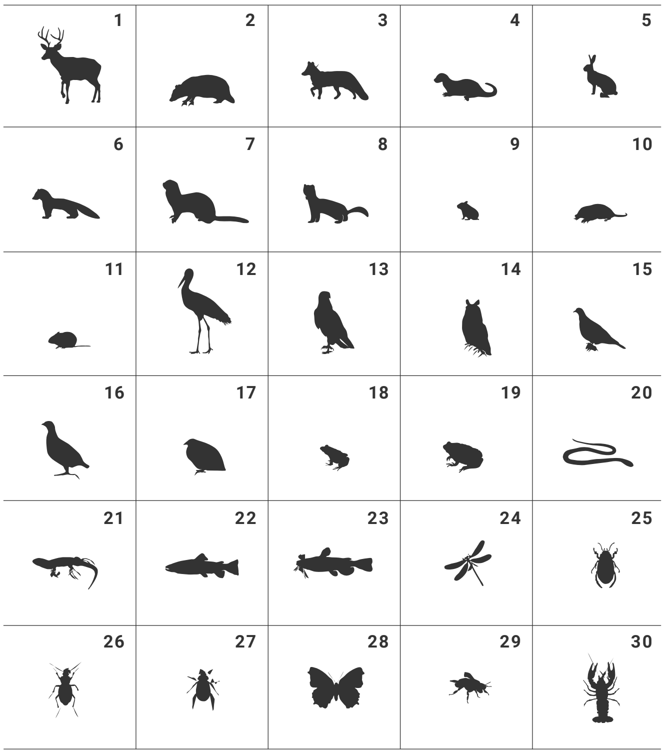
PREDOMINANCE OF MONOCULTURE



19 species

BIODIVERSITY





30 species

only, the remaining months become periods of famine for the animals. In order for bees not to starve, they are dependent on a habitat rich in diversity, with a wide range of flowering plants throughout the year.

Besides pollen and nectar, also bee bread could be contaminated by different types of pesticides associated with crop pest or Varroa control, like residues of amitraz, coumaphos, fluvalinate, chlorpyrifos or thymol. It is likely that the higher chlorpyrifos concentration may have played a role in the decrease of health indicators *(less brood production, wrong temperature regulation) (Meikle, Weiss et al. 2017)*. When the nutrition contains pesticides, long term effect on the bee's health can be expected.*(Baines, Wilton et al. 2017)* describe the extreme toxicity of neonicotinoide *(clothianidin and thiamethoxam)* to winter worker honey bees prior to brood production in spring. This is the most sensitive bee stage identified to date. Chronic exposure to field-realistic levels of neonicotinoids showed a decreased winter survival rate.

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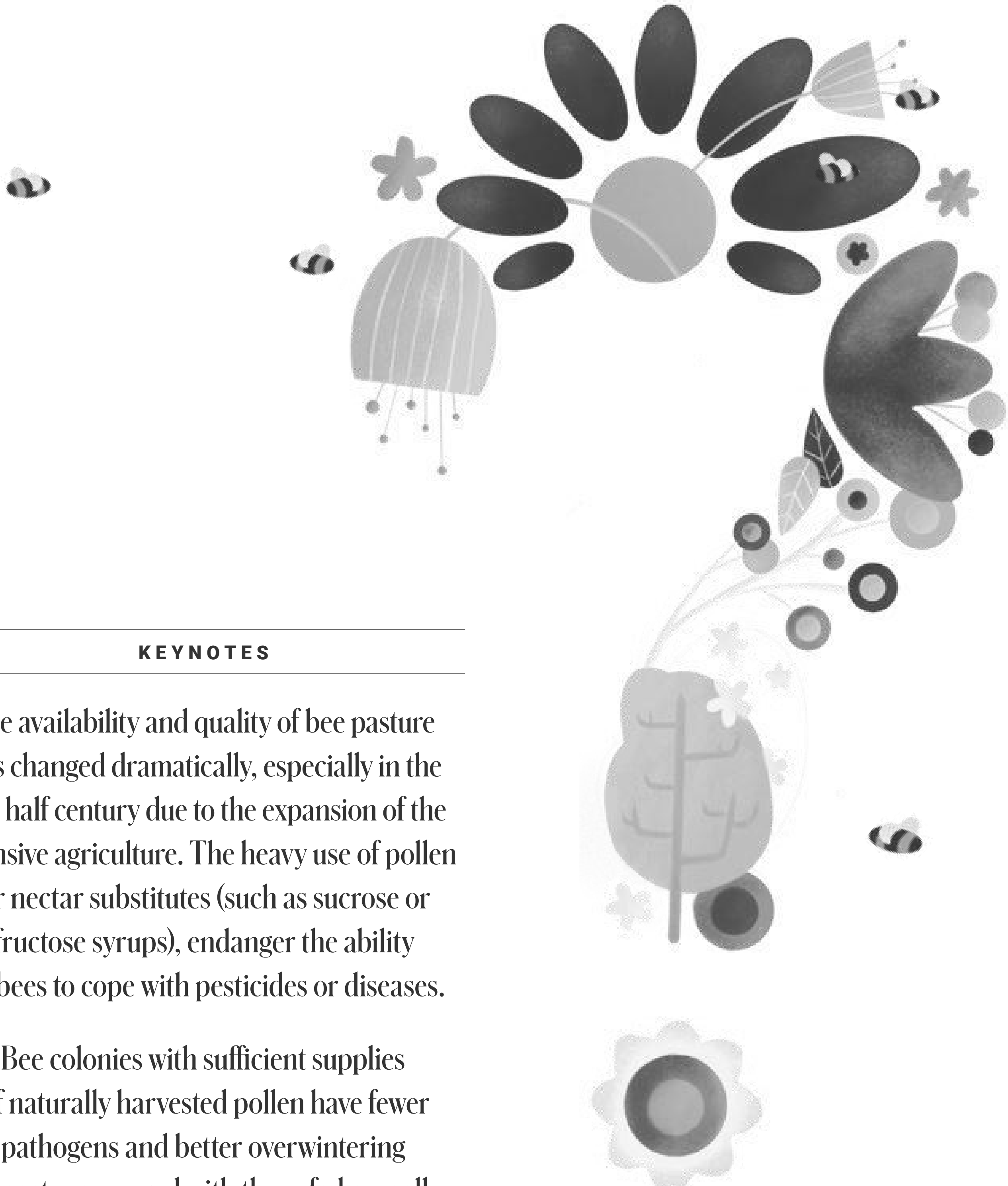
The importance of flowering plants diversity

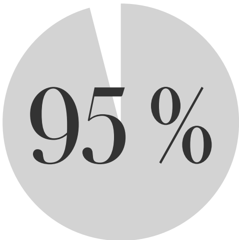


KEYNOTES

The availability and quality of bee pasture has changed dramatically, especially in the last half century due to the expansion of the intensive agriculture. The heavy use of pollen or nectar substitutes (such as sucrose or fructose syrups), endanger the ability of bees to cope with pesticides or diseases.

Bee colonies with sufficient supplies of naturally harvested pollen have fewer pathogens and better overwintering prospects compared with those fed on pollen and nectar substitutes.

FORAGING ACTIVITIES



TAKE PLACE WITHIN 6 KM AROUND THE HIVE

FLYING DISTANCE FROM THE HIVE

MAXIMUM

THE MOST COMMON FLYING DISTANCES

Plant products harvested from the environment and subsequently secretions arising therefrom in bee glands are essential for the self-medication of colonies and the natural antibiotic action of these products against pathogens within a bee colony.

Plant products harvested from the environment and subsequently secretions arising therefrom in bee glands are essential for the self-medication of colonies and the natural antibiotic action of these products against pathogens and pests within a bee colony.

The current fragmentation of the agricultural landscape has reduced the availability and species diversity of flowering plants, causing their lower pollination and fertilization rates.

The intensively used farmland provides bees with a spatially and temporally isolated overproduction of flowers from crops such as oilseed rape or sunflower. The effect of such monodiet on the colony fitness is not entirely clear, and the results may be influenced by pesticide residues in nectar and pollen used in those monocultures.

Depending on the bee pasture availability, single bee colony can cover area of app. 100 km^2, 95 % of the colony's foraging activities take place within 6 km around the colony, although it may be even prolonged under certain conditions. The maximum range is considered to be 12 km from the hive, but most authors report the most common flight distances within the range from 600 m to 2 km from the hive.

When flowering of large monocultures is over, there are usually insufficient food sources within a flying distance of the bee colonies and hives need to be moved. Transhumance can lasts several hours or even days, during which bees are exposed to stressful factors including vibrations, high temperatures and increased carbon dioxide levels. Moving colonies between monocultures exposes colonies to greater risk of contact with pesticides and pathogens, restricts access to assorted pollen sources, force them to re-adapt to the new environment, which can increase its susceptibility to diseases.

Bee forage activities affects not only beekeeping operations profitability, but also bee colonies health. Temporary lack of food sources will reduce or stop queen's oviposition, resulting in offspring decrease, pollen shortage, lack of nursing bees for brood feeding and reduced flight activity. Foraging gaps in our geographic areas are most common after the end of rape and acacia flowering season in June and July, when the strenght of the colony reaches its maximum. If the gaps lasts longer than 15 days, due to the reproduction pause colony will not be able utilize nectar and pollen flows available subsequently.

The availability and quality of bee food sources globally has changed dramatically especially in the last half century, as a result of intensive agriculture methods. As an examples of changes in farming practices can be mentioned massive use of fertilizers eliminating leguminous crops off the crop rotation, the massive use of herbicides preventing the growth of flowering weeds, mowing of forage crops prior their blooming to increase

AMOUNT OF PROTEIN IN POLLEN

2.5 — 62 %

SOME NIGHTSHADES — SOME PRIMROSES

its protein content, applying nitrogen fertilizers on pastures to promote growth of grasses instead of flowering plants, etc.

The protein content of the pollen of different plant species is very diverse, ranging from 2.5 % *(some nightshades - Solanaceae)* to 62 % *(some primroses - Primulaceae)* of dry matter, the same applies to the sugar content of nectar from different plant species.

The substances naturally occurred in pollen, nectar and propolis *(e.g. p-coumaric acid, pinocembrine, pinobanksin 5-methyl ether)* turn on the detoxification genes of bees. In particular, p-coumaric acid appears to be essential in the regulation of bee immune and detoxification processes. Substitute feeds use, such as sucrose or fructose syrups or pollen substitutes threatens the ability of bees to cope with pesticides or diseases. Colonies with sufficient storages of naturally harvested pollen have fewer pathogens and better overwintering prospects compared to bee colonies fed on pollen substitutes. Protein content of beebread is negatively correlated with higher proportion of arable land in the apiary vicinity. On the other hand, pollen protein content as well as the amount of nectar gathered by colonies increase if the colonies are surrounded by flowering meadows and deciduous forests.

Pollen deficiency during larval development has a negative impact on adults. Workers raised in a colony suffering from a lack of pollen are smaller, have a shortened life span, often die within first days of foraging. Bees poorly nourished in the larval stage perform less orientation dances, with less accurate localization of the food source, compared to those raised in colonies with sufficient pollen income.

The expansion of urban and suburban areas has similar negative effects to the environment as the intensification of agriculture. The negative impact of land use and fragmentation trends will be further enhanced by climate changes. The abundance of insect pollinators, as well as the number of flower visits, has a raising gradient from the city to the countryside, but paradoxically gardens and parks in highly urbanized areas offers for pollinators better conditions comparing with rural areas with intensive agriculture.

Improving of foraging capacity of apiary surrounding is possible by planting mixtures of nectar producing plants, when making plant seed mixtures it is important to keep in mind that nectar and pollen production should be balanced throughout the day and season. Bee gardening opportunities exists also in built-up areas, through private, public or community flowering gardens, green roofs, urban parks, wood parks or cemeteries. *(Matteson et al. 2013)*. The positive effect of creating flowering islands and reduced mowing frequency on communities of pollinators in urban areas has been proven.

Long-lasting rainy, hot, or cold weather has a negative impact on colony fitness. Shifts in plant species blooming period as a consequence of climate change induce shorting of nectar and pollen flows followed by forage gaps, influencing subsequent successful development of bee colonies and other pollinators. Since plants and pollinators may not respond equally to climate change, pollination synchronization may be lost. *Switanek et al. (2017)* found that the warmer and drier weather in the previous year increase winter bee colony losses in the Austrian conditions, warming is one of the main feature of climate change.

Plants respond to warming by reduced flower set up as well as reduced pollen and nectar production. Warming reduce insect pollinators flight activity, as well as their weight and lifespan. Climate change is not only linked with warming, increasing of extreme weather phenomena such as storms, floods and droughts is predicted as well.

BEE POLLEN

Contains 5 – 7 times more amino acids than beef, eggs or cheese.

Consits of 3 % – 14 % fatty acids. The most important ones are linoleic acid, linolenic and arachidonic, as the human body cannot produce these acids by itself.

Contains 27 elements.

- SODIUM
- POTASSIUM
- NICKEL
- TITANIUM
- VANADIUM
- CHROME
- PHOSPHORUS
- ZIRCON
- BERYLLIUM
- BORON
- ZINC
- LEAD
- SILVER
- ARSENIC
- TIN
- HELIUM
- STRONTIUM
- BARIUM
- URANIUM
- SILICON
- ALUMINIUM
- MANGANESE
- MAGNESIUM
- MOLYBDENUM
- COPPER
- CALCIUM
- IRON

The impact of climate change will reduce water availability in the Central Europe, especially in the south. More rainfall and snowfall will occur in winter, especially in the north *(20 % or more)*, winter floods will be more frequent. Although the vegetation period will increase by 43 – 84 days, the production potential of the crops should be reduced up to 47 % by 2075. The decline of bee forage will provoke increased need for transhumance, linked with increasing of diseases and pests spreading.

CLIMATE CHANGES TILL 2075

THE PRODUCTION POTENTIAL OF CROPS WILL BE REDUCED BY

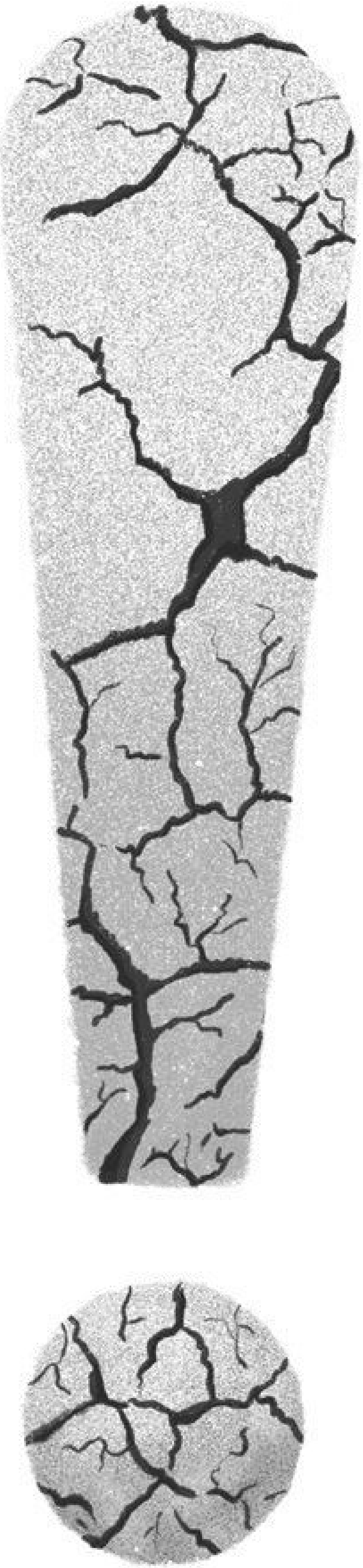
47 %

THE VEGETATION PERIOD WILL INCREASE BY

by 43 – 84 days

PRECIPITATION IN WINTER WILL INCREASE BY

20 %



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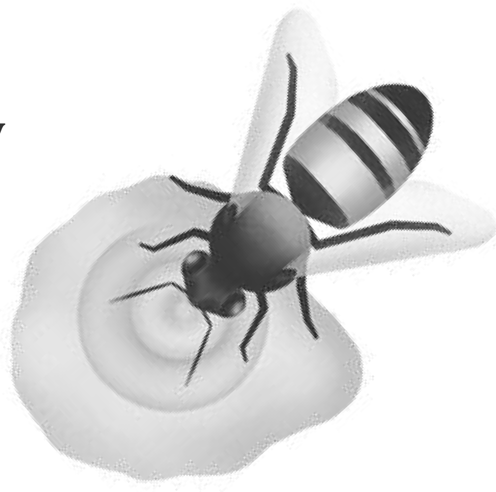
Availability and quality of water source



KEYNOTES

Water is collected from various sources in the landscape and contains wide spectrum of dissolved substances.

For ecological beekeeping it is suitable to use natural water source in the landscape, which usually could contain lower concentration of bee harmful substances.

The water source quality depends on microclimatic conditions and landscape usage.

One of most important substances not only for bees is water, which in this case is collected by bees and transported into hive. Its quality depends from various compounds, which are present in water. Closely we look to flowers gutation fluid, ground and surface water, and how their quality depends on climatic and geologic conditions.

Next to pollen and nectar, foraging bees may collect water from the landscape. There are three different sources of water which can be potentially collected by bees *(namely guttation fluid, puddle water and surface water)*. This water can also be contaminated by various hazardous substances.

The guttation fluid *(droplets)* can be contaminated by pesticide in very high concentrations, but the collection and use of these droplets by bees is highly dependent on a number of biotic and abiotic factors. Different crops shows variability in terms of frequency and intensity of guttation. Guttation fluids may be relevant for both acute *(for foragers when they collect guttation water)* and chronic effects *(when guttation water is used to dilute honey, for example)*.

On the other hand, the collection of water from permanent water bodies is frequent but the concentration of pesticides in this water is usually low. However, the quality of surface water mostly depends on climatic conditions and antropogenic activities near the beehives. During hot summers, the humidity in soil decreases; once it rains, the water is not absorbed by the soil and erosion occurs. By increased erosion the water contains more salts and agrochemicals, which sediment in lakes and rivers, which are permanent water bodies in the landscape visited by foragers. When this water is collected and transported into the hive microbiome, then the increased concentration of hazardous substances causes decrease of hive vitality.

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1·1·5

Environmental contamination by agriculture, forestry and urban gardening activities

KEYNOTES

Pollution from human activities limits the selection of proper place for bee hives, in which surrounding organic crops should grow, or a natural flora or woods should be present.

Crops should be treated by methods with a low environmental impact.

Apiaries should be in proper distance from possible sources, which could cause contamination of bee products or could negatively influence bee health.

Agriculture crop cultivation depends on various supporting activities and materials, so that optimal profit has to be achieved. These materials consists mostly of compounds which cause risks for honey bee health, because they cannot biotransform in the nature into harmless products, and the compounds can be found in water and food circle of honey bees. The following chapter describes which activities and what types of substances could cause risks.

Agriculture, forestry and gardening activity comprise many activities oriented to growth of plants, like soil processing, irrigation, fertilization, plant protection and fruit and vegetables harvesting as well as felling trees. To achieve good yields, it is necessary that the soil has a proper quality and that there is functioning soil ecosystem. It is known how ecosystem health and human health are interconnected *(Schram-Bijkerk, Otte et al. 2018)*.

The potential health hazards in agriculture that have been identified could be summarized as follows: ① contamination of crops grown on polluted soils or irrigated with river water contaminated with industrial and chemical by products; ② microbial and heavy metal contaminants in untreated or improperly treated urban waste and human and animal excreta used in agriculture; ③ zoonotic diseases associated with urban livestock keeping; ④ the use of agrochemicals in confined field; ⑤ encouragement of vector breeding sites. In case of drought the decrease of groundwater level, leads to an increasing level of nitrates in the soil. *(Oren, Yechieli et al. 2004)*. The agriculture in rural areas is intense and oriented to mass production by planting crops with high yields, whereas in urban areas the agriculture *(gardening or urban agriculture)* is oriented to relaxing and selfproduction of food. *(Flynn 1999)*

Urban agriculture encompasses backyard, windowsill, and rooftop gardens. Many urban gardens are located on land that has been vacant or unused because it is otherwise unattractive for urban development. The typical pattern of urban sprawl has created an abundance of empty inner-city lots. The United Nations document on urban agriculture reported that 25 % of urban households in the United States are involved in gardening, including food gardens and landscaping *(Brown and Jameton 2000)*.

However, even rural areas and urban areas are touched by pollution from industry or agriculture. This leads to an increased risk metal content in the soil, as well to an increased bioaccumulation in the topsoil. *(Douay, Pruvot et al. 2008)* studied the contamination of 27 urban topsoils around two lead and zinc smelters. It was shown that Cd, In, Pb, Sb and Zn were major pollutants followed in lesser quantities by Ag, Bi, Cu and Hg. In addition, As, Ni, Se, Sn and Tl were found in slightly higher quantities than in areas with regional agriculture. The other elements *(Co, Cr, Th and U)* were at endogenous levels. The observations have highlighted the strong heterogeneity of the physico-chemical parameters of urban soils and the existence of heavy contamination of the under layers by Cd, Pb and Zn. A potential transfer of metals from the topsoil to the deeper layers and especially of Cd and Zn, cannot be ruled out. Indeed the soil rework is not the only factor explaining contamination level of the deeper layers of the studied soils. The comparison of the studied element concentrations in urban soils with nearby local agricultural values shows that the dust emission originating smelters were not the only source of contamination. Thus a large contamination of the studied urban soils by Sb and In could be explained by domestic combustion of coal for heating.

(Lehmann 2016) provided assessment of water resources contamination by pesticides in a gardening area. Water samples were collected from traditional wells, boreholes and the lake, in which the levels of azadirachtin, chlorpyrifos, imidacloprid and profenofos were determined. Results have shown that in traditional wells, the threshold limit of 0.1 µg/l is exceeded, indicating a potential risk for the consumers. Residues of acetamiprid, atrazine, emamectin benzoate and imidacloprid were found in boreholes but in low concentrations. The levels of contaminants in lakes appeared to vary according to season. When bees collect water from these streams, hazardous substances can accumulate in the hive microbiome, with sublethal effects to the bees.

LEGEND

- Cd • CADMIUM
- In • INDIUM
- Pb • LEAD
- Sb • ANTIMONY
- Zn • ZINC
- Ag • SILVER
- Bi • BISMUTH
- Cu • COPPER
- Hg • QUICKSILVER
- As • ARSENIC
- Ni • NICKEL
- Se • SELENIUM
- Sn • TIN
- Th • THORIUM
- U • URANIUM

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1·2

Apiary location and hive placement

KEYNOTES

The location of ecological apiaries should be well considered — there should be enough sources of nectar and pollen from ecological produced plants and crops with low environmental impacts should within a radius of 3 km.

Although in some areas, the living conditions for honeybees are better in urban areas compared to rural areas, apiaries in the cities could pose a risk of social disputes.

The following subchapter refers to the considerations that should be taken when placing a hive at a new place. The new location should fulfil the bees' life demands and negative influences on health should be avoided. In the following, we will have a look at the consequences of hive density and make observations on the characteristics of a suitable place for honeybees.

Suitable hive placement is important both for promoting healthy colonies and for minimizing or avoiding conflicts with neighbours and public. Considering the location of the hive, it is important not only to have in mind the size of the area under your control, but also natural features and ways of use of the apiaries' surrounding.

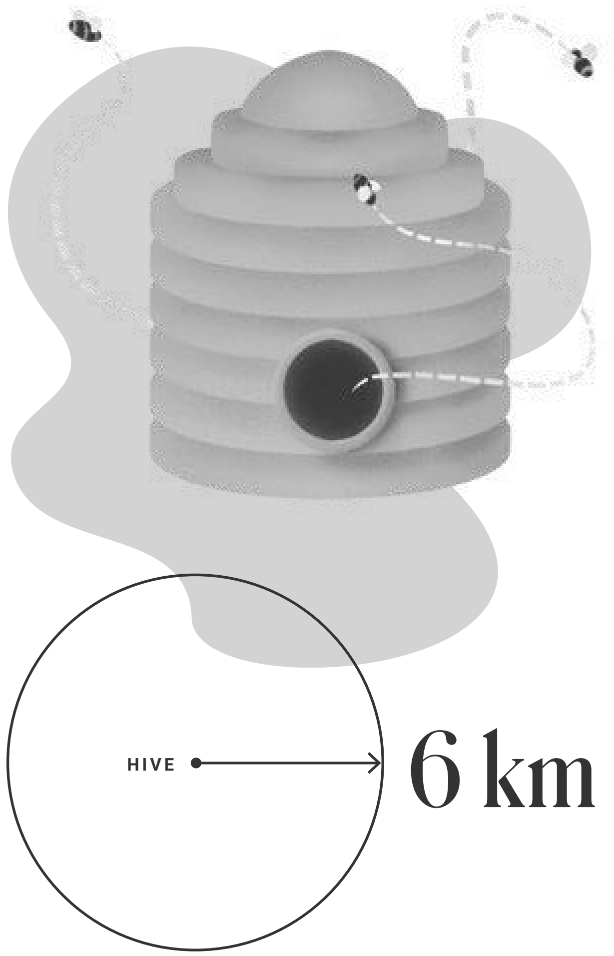
In the past, the common practice of beekeepers was to place hives in rural areas where there was an abundance of nectar and/or pollen. In landscapes with a high biodiversity of flora, the carrying capacity is high and so the saturation of hives will be high. However, it is necessary to avoid an over-saturation of the landscape by placing too many hives within a certain area. Signs of over-saturation include slow colony growth, poor honey production, increased spread of diseases, and excessively defensive behaviour of bees. If the location has an inadequate flowering intensity, it should be considered to reduce the number of hives at an apiary so that the carrying capacity of pollen and nectar is not completely exploited; another option is to move the apiary to a new location.

In rural landscapes monoculture field crops with intensified agriculture are dominant; the placement of hives could cause problems because of application of pesticides, fertilizers and other supporting compounds in this environment. The flowering time of crop is limited, and after that supplementary feeding or the relocation of the hives to provide continuous forage is often required. Naturalized meadow landscapes with surrounding forests with mixed hardwood tree lines, are the most suitable place in rural areas.

When finding an apiary location, it has to be taken into consideration that the bees' foraging radius is about 5 km. Within this radius, enough food and water has to be found from spring to fall *(February to October)*. In rural areas during the hot summer months *(July and August)*, there is often a dearth of food, whereas in *(semi-)*urban areas, there are plants that bloom even during our hottest months.

Honey bees are doing surprisingly well in cities and suburbs, and they often overwinter better in these environments than in rural settings. There is usually abundance and a wide variety of flowering landscape plants and little competition with feral bees for resources in urban communities. When hives are placed on flat-roof, it has to be ensured that the roof below can support the weight of a hive full of honey with cinder blocks on top *(and beekeeper)*; and attempt to reduce the impact of high winds. If the hive will be placed in the backyards or gardens in urban areas, beekeepers must manage the risk of interference with the general public, particularly in those areas used intensively for public access or recreation. When foraging, the bees should consistently fly at least 3 m above public footpaths or recreations areas. To achieve this flight path height, it is necessary to place barriers, such as hedges or shrubs, or instant barriers consisting of shade cloth fixed to a trellis, which may have to be up to 4 m high. Bees will fly over these structures and should not worry neighbours. *(Williams, Corbet et al. 1991, Goulson, Nicholls et al. 2015)*

DISTANCE OF FORAGING ACTIVITIES



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1·2·1

Indirect and direct poisoning by bee harming chemicals

KEYNOTES

Direct and indirect poisoning of honey bees could occur in short period, once the bees gets in contact with the substance.Sublethal doses influence cognitive abilities and health of bees in long term period, so synergic effects cannot be ruled out. Depending on substance's chemical nature bee harming compounds can be found in the honeybee tissues as well as in honeybees products.

Lot of products which for crop protection contain substances classified as harmful for honey bees. This chapter focuses on their presence, and their direct or indirect observed effects on honey bees.

The use of pesticides in agriculture is often discussed as the key factor influencing bee health. Single events of poisonings by spray applications have been reported across the globe, usually due to misuse of products resulting in contamination of food. *(Kasiotis, Anagnostopoulos et al. 2014)* Individual bees leaving the beehive can be in direct contact with pesticides and residues. These compounds could be carried back to the hive by contaminated foragers and contamination could spread to all in-hive bees and brood iwithin only 6 hours.

There are different ways through which hives can be exposed to these substances: through pollen and nectar collected and stored in the hive, through surface water, through air contamination via dust drift of coated seeds during sowing and through extra floral secretions that are produced from some plants such as sunflower and cotton. Foragers can be also contaminated via consumption of guttation droplets from plants and/or by contact with dust drift from sowing treated seeds and/or via inhalation of high vapour pressure compounds during spray treatments. Finally, foragers and in-hive bees can be contaminated via consumption of nectar and/or pollen contaminated by spray treatments. Besides the reduction of the quality of hive products, and the effects on bees' behaviour, the accumulation of insecticides in honeybee tissue can also influence honeybees' health and the colony's population development, as they can act as stressors for the entire colony. Finally, physical and chemical properties of the pesticide also have an influence on the magnitude and the length of the exposure and ultimately on the toxicity. For hazard characterisation, standard test methods investigate acute effects on adult worker bees based on mortality after 48 hrs of a single exposure after oral intake or contact exposure *(expressed as Lethal Dose for 50 % of the individuals (LD_{50}) in µg/bee)*. During the 48 hrs test, observations may be prolonged up to 96 hrs if mortality continues to rise. *(Sanchez-Bayo and Goka 2014)*

In the majority of cases, the concentrations of pesticides detected in bee bodies were much below the LD_{50} values. The toxicity values for bees such as the oral LD_{50} value and the sublethal dose of 0.5 ng/bee were set as a preliminary risk assessment for the main compound detected at high levels in bee pollen. Considering the oral LD_{50} and the lowest observed effect concentration *(LOEC)* value for honeybee larvae to be ≥40 ng a.s./g diet, it cannot be excluded that subjection of bees to polluted pollen at concentration levels more than 1200 ng/g can cause toxicity/mortality in bees. Besides that, one shall keep in mind that there is the potential of cumulative toxicity if bees are exposed to several pesticides. The expressions of risk writen above indicate probabilities of causing serious effects *(e.g. 20 % risk of resulting in 50 % mortality)* within short periods of exposure, i.e. about 2 days. *(Sanchez-Bayo and Goka 2014)*

However, slightly synergistic toxic effects of insecticides *(e.g. thiacloprid)* are described when sprayed in combination with fungicides *(e.g. tebuconazole)*. In this context, a number of studies have identified synergistic interactions, although in some cases, synergist concentrations are often orders of magnitude above concentrations of environmental relevance:
- Prochloraz, an imidazole fungicide and delthamethrin, a pyrethroid insecticide; -Ergosterol-biosynthesis-inhibiting *(EBI)* fungicides, neonicotinoids, pyrethroids and organophosphates;
- Miticides: coumaphos and tau-fluvalinate, coumaphos and tau-fluvalinate and in-hive antibiotics *(oxytetracycline)*; - In-hive antibiotic *(oxytetracycline)* and neonicotinoid insecticides such as imidacloprid, acetamiprid and thiacloprid. *(Rortais, Arnold et al. 2005, Rortais, Arnold et al. 2017)*

Extremely high risks were found for thiamethoxam and lindane residues in honey, which affect primarily nectar foragers and secondarily larvae. Daily consumption of nectar or honey contaminated with these compounds at the average residue levels found worldwide would cause nectar forager mortalities of 50 % or above within 3 days in case of lindane, or a week for thiamethoxam.

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1·2·2

Geologically conditioned occurrence of harming substances

KEYNOTES

It is known that lot of soils is contaminated in such levels with persistent organic pollutants (DDT, HCB, Dieldrin, etc.), which were used in the past. In the EU, these concentrations are nowadays forbidden, and their occurence is tolerated only in concentrations < 0,01 mg/kg for each compound. The compounds can now only have two of such substances in sum.

In rock environment many in substances are present that are part of the honeybees' food circle, and they were also found in bee bodies and bee products.

Soil and its geological composition are one of the key factor influencing growth of plants, which are visited by honey bees. In this part we explain the connection between geology and importance of essential elements for plants and honeybees.

The soil, the environment for the growth of flowers and trees make up a complex matrix which consists of solid materials, air and water. Inside this complex matrix, the exchange of ions and chemical reactions occur. For plants, animals and human health it is necessary to obtain tiny amounts of many inorganic elements *(essential trace elements)*, which are necessary for good health and growth. Most of these elements are taken into the body via food and water in the diet and in the air we breathe. Through physical and chemical weathering processes, rocks break down to form the soils on which the crops and animals that constitute the food supply are raised. Water travels through rocks and soils as part of the hydrological cycle and much of the dust and some of the gases contained in the atmosphere are of geological origin. Hence, through the food chain and through the inhalation of atmospheric dusts and gases, there are direct links between geology and health of animals.

Approximately 25 of the naturally occurring elements are known to be essential to plant and animal life in trace amounts, for example chromium, cobalt, copper, fluorine, iodine, iron, manganese, molybdenum, selenium and zinc. However, elements with probably no recognized biological role are called non-essential elements, often with harmful properties, e.g., cadmium, arsenic, mercury and lead. Several elements are frequently involved in environmental toxicity problems, for example arsenic, boron, chromium, copper, fluorine, molybdenum, nickel and zinc. The links between environment and health are important for subsistence populations who are heavily dependent on the local environment for their food supply.

The presence of toxic elements in soil or rocks, whether due to natural geochemistry or human activities, including pollution, usually influences health of animals living in the area. Bees collecting food in such an environment introduce these elements into the food chain, what could influence their health *(Rashed and Soltan 2004) found such non-essential elements in the bee food (nectar, honey, water, pollen)*. The results showed that in honeys and syrup-feed honey could exhibit higher concentrations of Cd, Cl, Co, Fe, K, Mg Mn, Na and Pb, than in nectar and pollen. This difference could be explained through accumulative effect, while water from stored nectar in honey combs is evaporated by bees and therefore higher concentration of trace elements could be found in honey. *(Bilandzic, Gacic et al. 2014, Aghamirlou, Khadem et al. 2015, Oroian, Prisacaru et al. 2016)*. In the case of honey bees, the content of trace elements in their in bodies depends on metabolism and excretion of food. It was found that cadmium accumulates less than chromium, depending on the toxicological and geological background of the region *(rural, industrial, urban)*.

LEGEND

- **Cd • CADMIUM**
- **Cl • CHLORINE**
- **Co • COBALT**
- **Fe • IRON**
- **K • POTASSIUM**
- **Mg • MAGNESSIUM**
- **Mn • MANGANESE**
- **Pb • LEAD**

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1·2·3

Presence of non-chemical agents and disturbing influences

KEYNOTES

Beside pesticides and other harmful substances, bee health could be influenced indirectly by electromagnetic radiation and electrosmog. Powerful light sources could disturb bees in the night or affect their oriantation at return to the hive.

The exposure of honeybees and other animals, receive electromagnetic and electrostatic field, as well as light and radio has rapidly increased in last decades. This chapter focuses to some influences of electrosmog and another non-chemical influences on honey bees health.

During the last two decades the honeybee colony losses were observed, making them subject of many studies trying to identify the reasons and causes. Many influencing factors with joint impact were found: the global presence of the *Varroa* mite, viral and bacterial infections, single-crop farming, pesticides, mobile apiaries, or genetically modified plants and others. Among these influences electro-, as well as light smog should be included. They do not act directly on honeybees, but they could disturb bees in the hive or cause induced changes in bee biochemistry *(Kimmel, Kuhn et al. 2007, Kumar, Sangwan et al. 2011, Favre 2017).* Insects use several senses to forage, for example detecting visual cues such as colour, shape, etc., but they are also very sensitive to electromagnetic fields. Bumblebees can discriminate floral electric fields, and this sensory mode may facilitate rapid and dynamic communication between flowers and their pollinators. *(Balmori 2014)*

On the other hand, the behaviour of honeybee is altered to some extent by high or low energy fields or electromagnetic radiations has been known for some time. The energy fields could have effects on behaviour and navigation, on distribution or loss of habitats, or adverse influence of radio-background on trees and plants visited by pollinators. Honeybees exposed to EM radiation for short time increase the concentration of carbohydrates in the bees, but when the exposing time increases to 40 min there is a reverse effect and a decline in carbohydrate concentration occurs. Hemolymph glycogen and glucose content also showed the same trend. The concentration of lipids showed similar trend, an initial increase in concentration at the 10 min exposure period, a decrease was observed at 40 min exposure. It was interesting to note that during the exposure time bees became slightly aggressive and started beating their wings in agitation. This mobility of the bees could be responsible for increase utilization of energy sources and consequent decrease in concentration of carbohydrates and lipids in the 40 min exposed sample. *(Kumar, Sangwan et al. 2011)*

Bees are able to see the blue, green and violet spectrum of colour patterns found in the petals of flowers and the ultra violet light to determine the presence of both pollen and nectar. If the ultraviolet light is not present, bees become disinterested in searching for flowers. Honey bees sense the colour of flower differently, which depends on wavelength of light impinging on flowers. On the other hand, foraging honeybees orient according to sun by searching of nectar and pollen sources. However, the amount of lights in bee hive surroundings could lead to negative impacts on colony. Strong sources of yellow light could cause increasing of foraging activity of worker bees at night followed by loss of orientation. Secondary the specific wavelength of light could attract or disturb bees at night, which causes stress to colony.

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1 • 3

Air pollution

KEYNOTES

Pollinators search often blooming flowers according their odour, if the ozone is in the environment present, the odour degrade and pollinators could not find the food source.

Honeybee is electrostatically charged at flight and attract as well solid particles, as pollen, all is then stored inside hive, where could the contamination of each bee matrices occur.

Fragrances are mostly transmited by air, and it is important attractant from flowers to honey bees, which could remember the food source and in the hive could share this information. This part describes the influence between air quality and interaction honey bee — flower.

Pollinators like honeybees rely on scent to find plants from thousands of feet away while foraging for food, but air pollutants break down the scent molecules. Air pollutants interact with and break down plant-emitted scent substances, which insect pollinators use to locate food. The pollution-modified plant odors can confuse bees and, as a result, bees' foraging time increases and pollination efficiency decreases. This happens because the chemical interactions decrease both the scent compounds' life spans and the distances they travel.

Fragrances *(scent compounds)* half-life is shorter when ozone levels increased in polluted air. For example in an ozone-free environment, it took 10 minutes for 20 percent of foragers to find the scent compound beta-caryophyllene. When the ozone level increase, it took three hours for the same amount of bees to find the scent. *(Fuentes, Chamecki et al. 2016)*

A similar problem is caused by diesel fuel exhaust—nitric oxide and nitrogen dioxide, or NOx gases. For example, when NOx gases are mixed with the chemicals in the odor of oil rapeseed flowers, it was described that eight of the floral fragrances altered and two were completely lost.

Because the concentration of scents changes drastically in air polluted environments, this could impact important interactions between plants and insects. As a result, bees spend more time searching for food and less time for pollination.

In the opposite way, honeybees are suitable as an indicator for monitoring air pollution. When bees fly through air, they are electrostatically charged and solid particles *(dust, pollen, etc.)* are attracted to bees' body. Once bees return to the hive, they store collected material inside hive. Within the hive environment, various substances occurring in the hive neighbourhood could be found. *(Despina-Maria, Stef et al. 2004, Bastias, Jambon et al. 2013, Formicki, Gren et al. 2013)*

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1·3·1

Dust and aerosols

KEYNOTES

Small particles stay in the air for long time and could be transported on long distances, if they are contaminated by harmful substances, then honey bees could bring them into hive.

In each honeybee matrice, time-space variations of concentrations of naturally occurring substances were observed.

Dust and aerosols are part of the natural mass circle. However, their concentration in the environment has rapidly increased due to xenobiotic activities and new substances are coming into the mass circle which influence honey bee health. In this part we explain the principle of transfer these materials into the hive and impact on honey bees.

Nearly any anthropogenic activity tprovokes dust and aerosol. Processing of minerals, production of metals, mass movement, building of roads, increased traffic and other activity are closely connected with the industrial development and the growth of cities. These result in increased quantities of waste material and pollutants (technogenic pollution). The involved dust and aerosols present large problems for the air quality. Secondly, the deposition of dust particles affects the quality of soil and water, which are visited by foragers' bees.

According to their size, particles can stay in the air for a long time and be transported over long distances. Under proper conditions, the sand of Sahara *(African dust)* can be transported by air pathways into Europe, where it is deposited. Those pasthways are difficult to detect because aerosols merge and travel together with Asian dust and pollution. A similar but smaller phenomenon occurs when seeding corn in a dry field under windy conditions. If the seeds contain bee harming chemicals, there is a risk, that after the seeding, the chemicals might be released into the environment. When bees fly through the dust cloud, the particles are electrostatically attracted to their body, and after after returning into the hive, these particles are deposited inside bee colony. In case of pesticide contaminated dust, the change of humidity could lead to their activation inside hive and could cause serious damage. *(Habashi 2012)*

Different example is the release of heavy *(risk)* metals or their accumulation in the environment. If is visited by forager bees, they could bring the materials into hive, and the content of heavy metals could be then measured. It is known that content of heavy metals in bees differs significantly according to the season and location. *(Мукминов et al., 2015)* have found that the concentration of cadmium and manganese is higher in summer bees from contaminated locations than in autumn bees. On average, levels of cadmium, lead, manganese in contaminated areas exceeded those in control areas by 2.3 – 4.5 times. There was no discrepancy found in concentration of heavy metals between summer and autumn bees from control apiaries.

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1·3·2

Small sized particles

NANOPARTICLES

Nano

10^{-9} OR 0.000 000 001 METER

KEYNOTES

Nanoparticles are new group of materials, and they are widely used in many fields of mankind activities; however, their release into the environment is not sufficiently controlled.

The impact of nanoparticles on honeybees health is not well studied and because of small dimensions, they could pass through cell membranes.

CONTAMINATED AREAS SHOW

2.3 – 4.5x

HIGHER CONCENTRATIONS OF CADMIUM, LEAD AND MANGANESE

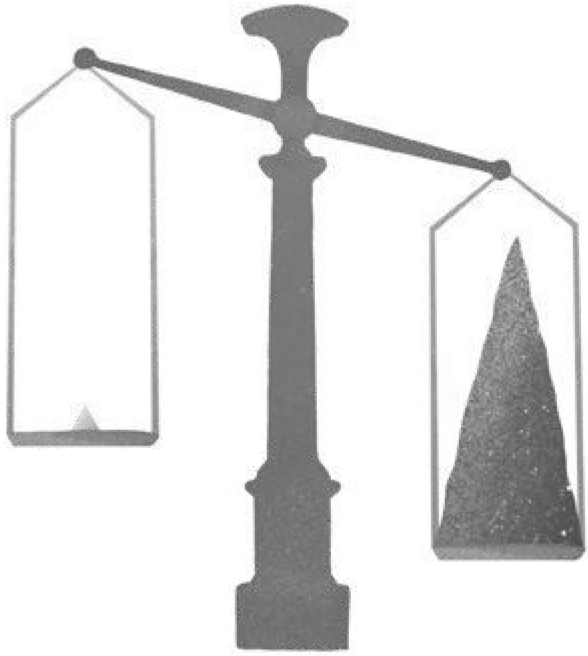
Nanoparticles are a class of materials with properties distinctively different from their bulk and molecular counterparts. The dimension of nanoparticles is 10^{-9} m and they come in different shapes. It is necessary to determine their influence on the environment as well as on animal and human health.

In the last decade, extensive studies have been conducted to understand the chemical and biological processes of nanoparticles and their effects on ecological functions and health. Nanoparticles have high surface area to volume ratio what results in highly reactive and physiochemically dynamic materials in environmental media. Many transformations, e.g. reactions with biomacromolecules, redox reactions, aggregation, and dissolution, may occur in both environmental and biological systems. These transformations and others can alter the extinction, transport, and toxicity of nanomaterials. A range of studies, for example works of *(Biswas and Wu 2005, Bystrzejewska-Piotrowska, Golimowski et al. 2009, Musee 2011, Bakshi, He et al. 2015)* focuses on the nature and properties of natural nanoparticles and their influence on the physical, chemical, and biological processes in plant–soil–water systems. The nanoparticles are involved directly or indirectly in numerous soil processes such as aggregate formation, nutrient retention, microbial activities, and water purification and pollution mitigation and thus affect soil/environment quality and health of animals. *(Lowry, Gregory et al. 2012)*

Over the last two decades, the nanoparticles and nanoproducts have increased in quantity and volume from few kilograms to thousands of tonnes over the last two decades, and their uncontrolled release into the environment is anticipated to grow dramatically in future. However, their potential impacts to the biological systems are unknown. Many nanoparticles contain heavy metals, thus toxicity and bioaccumulation of heavy metals contained in nanoparticles may become important environmental issues.

Although bioavailability of heavy metals contained in nanoparticles can be lower than those present in soluble form, the toxicity resulting from their intrinsic nature *(e.g. their size, shape or density)* may be significant. The applications in agricultural sector are focused to productivity enhancement involving use of nanoporous zeolites for slow release and efficient dosage of water and fertilizer, nanocapsules for herbicide delivery and vector and pest management and nanosensors for pest detection. New way to prepare efficient pesticides is the process of nanoencapsulation with nanoparticles in form of pesticides allows for proper absorption of the chemical into the plants unlike the case of larger particles. Resulting materials release slowly a chemical such as an insecticide but efficiently to a particular host plant for insect pest control. *(Gul, Saeed et al. 2014, Bhattacharyya 2010)*

From this point, the properties of nanoparticles to on bees were not sufficiently studied and there is valid concern about effects on health of bees. Nanoparticles in the small size could migrate through cell walls into the organisms and there could express their toxic effects.



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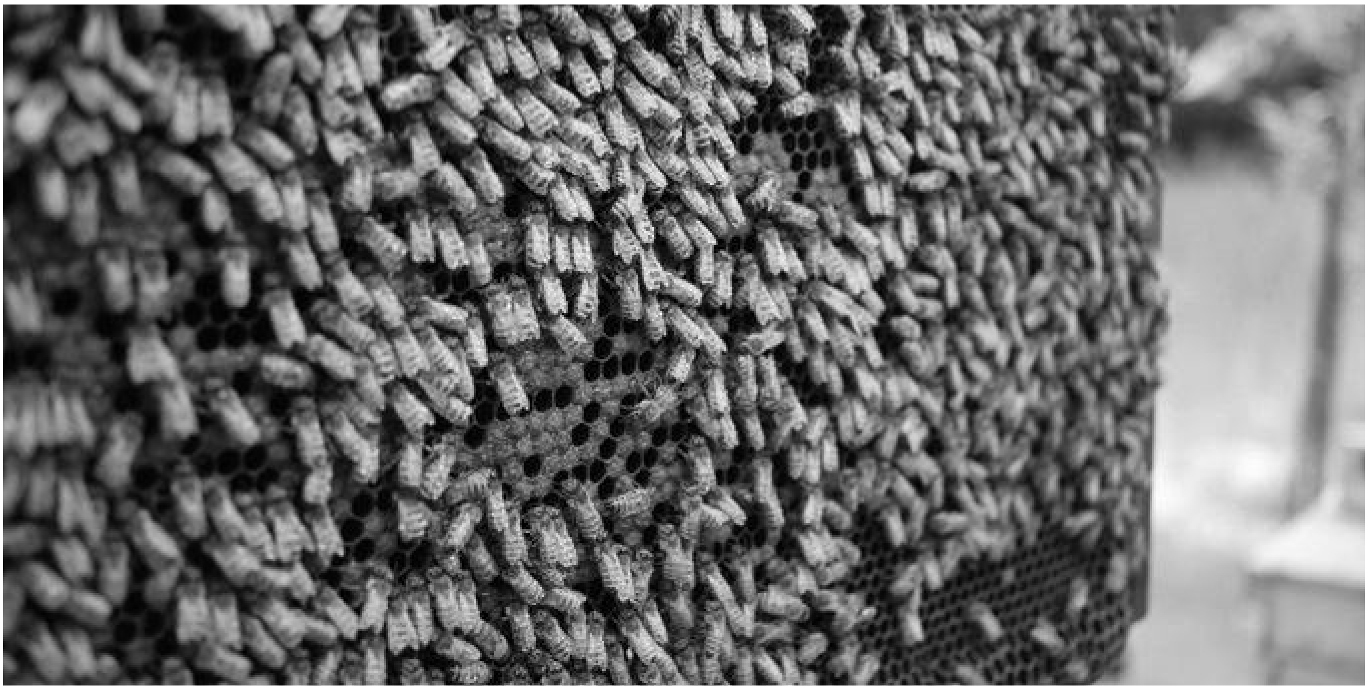
Internal stress factors



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

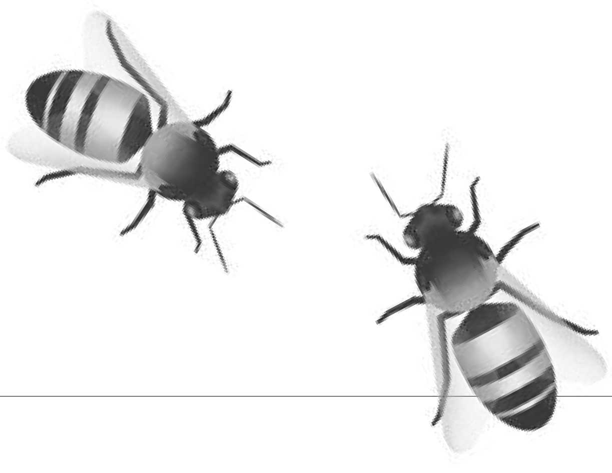
BEEKEEPING STATISTICS IN EU

~ 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 beekeeper 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)*

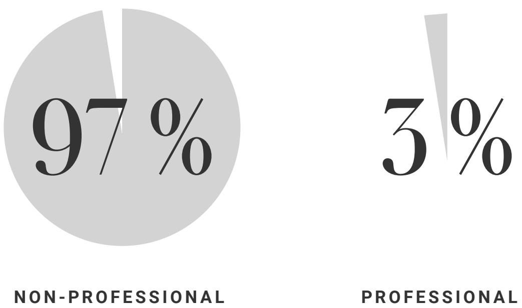
HEALTHY HONEYBEE COLONY

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 physico-chemical 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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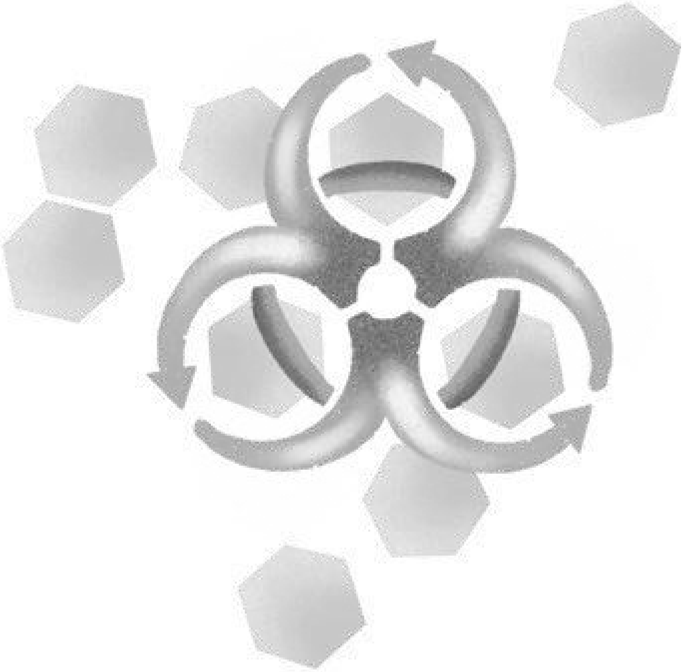
2·2·3

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.

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.

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.**

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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

49 %

CARBON

44,2 %

OXYGEN

6,3 %

HYDROGEN

<1 %

NITROGEN

CONTENT OF WATER

30 %

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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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Priehn, M., B. Denis, P. Aumeier, W. H. Kirchner, P. Awakowicz and L. I. Leichert (2016). "Sterilization of beehive material with a double inductively coupled low pressure plasma." Journal of Physics D: Applied Physics49(37): 374002.

Schmidt, R. H. Basic Elements of Equipment Cleaning and Sanitizing in Food Processing and Handling Operations. University of Florida. FS14. 1997. http://www.sagesanitizingsystems.com/pdfs/Basic%20Elements%20of%20Cleaning%20in%20Foodservice.pdf

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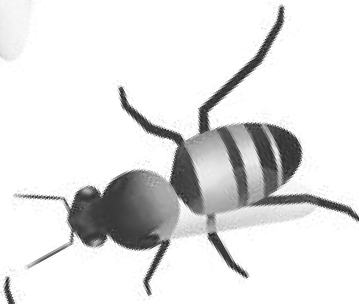
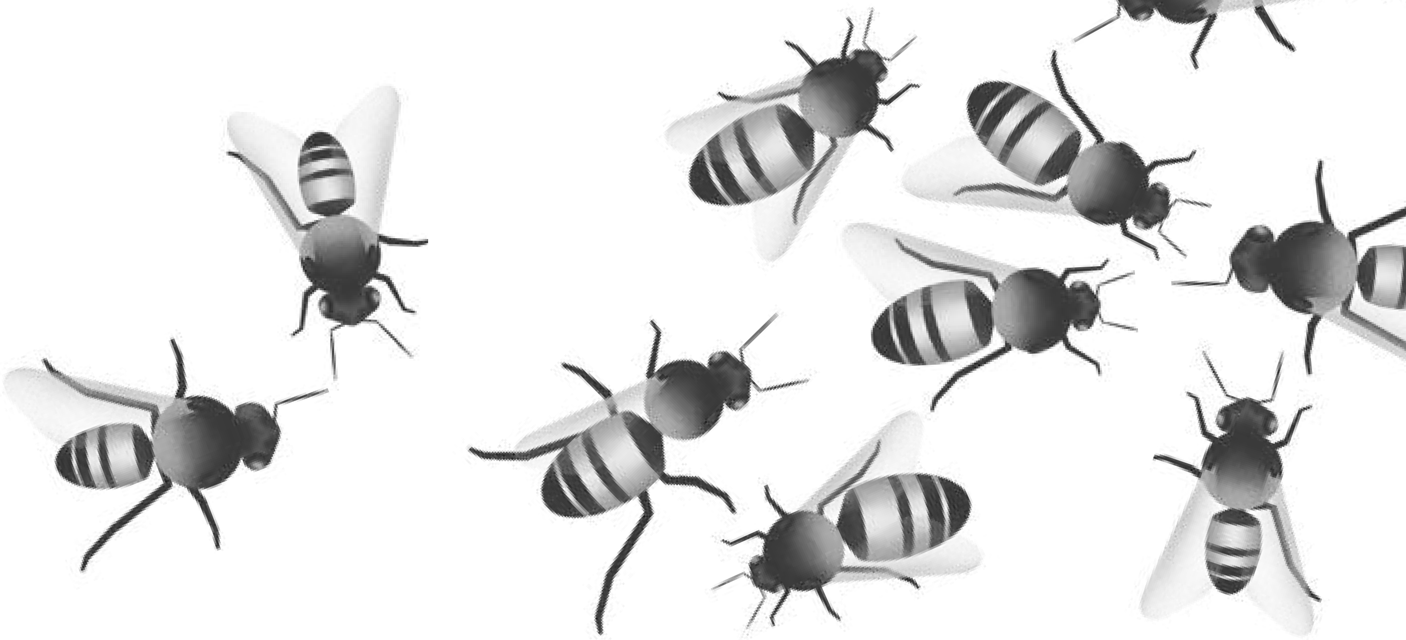
Chemically induced changes in beehive microbiome





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.

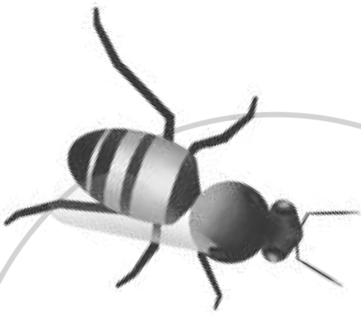
In every development state, honeybees are in contact with many substances, and some of them can harm their health. In this chapter, it is discussed how compounds could significantly affect detoxification or methamorphosis during larval state. Bees are exposed to various substances and stress factors, 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.

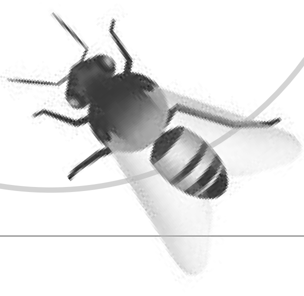
Beekeepers have been using acaricides and other chemicals to control *Varroa* mites for more than three decades. In a study conducted by *Boncristiani, Underwood et al. (2012)* bee hives were treated with five different acaricides: Apiguard *(thymol)*, Apistan *(tau-fluvalinate)*, Checkmite *(coumaphos)*, 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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Christen, V., F. Mittner and K. Fent (2016). "Molecular Effects of Neonicotinoids in Honey Bees (Apis mellifera)." Environmental Science & Technology50(7): 4071-4081.

Questions

1. Have significant colony losses been recorded also in the past?
2. Which factor contributes to the decline in bee colonies?
3. What is the scale of the colony losses in last years?
4. Which factors affect the decline of bees?
5. How does the climate change affect the population of *Varroa* mite?
6. How does the welfare of bees depend on local climate?
7. How does the food availability depend on weather conditions?
8. How depends the pollen quality on development of plants?
9. How does pollen scarcity influence the overwintering of honeybees?
10. What risks are associated with the use of GMO plants or crops?
11. How could invasive plants help to overlap a lack on food resources for bees?
12. What are the disadvantages of the presence of invasive species to pollination specialists?
13. Which mechanism help to spread invasive species among continents?
14. Which substances are important for thermal regulation and brood feeding?
15. How does the decline of pollen harvest or its contamination affect the vitality of hive?
16. Which factors influences the availability of pollen and nectar in nature?
17. Which water sources in the landscape do bees use?
18. How does the guttation water and river water affect the quality of honeybee food in hive microbiome?
19. Explain the influence of hazardous substances in water to bee vitality.
20. What are the health risks driven from agriculture for humans and bees?
21. Where do we find a higher concentration of risk substances—in urban or rural areas? Why?
22. How does the suitability of urban and rural soils differ from the point of plant development?
23. What is the main difference when bee hives are placed in rural or in urban areas?
24. How can we detect that a landscape is over-saturated with bee hives?
25. Under which conditions should supplementary feeding be provided?
26. How are bees outside hive exposed to harming chemicals?
27. What is the value of sublethal dose for bees?
28. In which bee hazardous chemicals synergic toxicity effects were observed?
29. Which trace elements can be found in the soil?
30. Which trace elements are toxic from the nature, and which are essential for the development of honeybees?
31. Which trace elements are concentrated in honey?
32. How do electromagnetic radiation and fields influence the behaviour of honey bees?
33. How could the wavelength of light influence insects?
34. Which spectrum of colours do honeybees see when foraging?
35. How does the ozone concentration influence the availability of fragrances for honeybees?
36. What is the principle of solid particles attraction to honeybees?
37. Why are bees suitable for biomonitoring of air pollution?
38. How is the technogenic pollution produced?
39. How do small particles stay in the air over a long time and can be transported over far distances?
40. How does the atmospheric deposition influence the content of hazardous metals in soil and plants?
41. How small particles are defined *(nanoparticles)*?
42. How nanoparticles can interact with organisms?
43. What is the process of nanoencapsulation?
44. Which development stage of bees is significantly affected by stress?
45. How could the bioaccumulation occur inside the hive?
46. How it is possible to ensure low level of wax contamination by lipophilic compounds?
47. In which conditions is the beekeeping sustainable?
48. Which mistakes beekeepers often do in spring and autumn?
49. When should the treatment against the Varroa mite be done?

50. Which material that is collected in the environment and stored in the hive is the most contaminated?
51. What it is a biotransformation?
52. Is the response to bee harming chemical immediately or with delay?
53. Which methods are used for the detection of contaminants in bee samples, and what is the limit of quantification?
54. What is the half-life time of coumaphos inside hive?
55. How does amitraz interact with other pesticides?
56. Which materials were used for ancient beekeeping?
57. What materials are mostly used at hive construction nowadays?
58. Why plastic cannot be used in ecological beekeeping?
59. What are the drawbacks of wood in hive construction?
60. What are the basic demands for cthe material used for the construction of beehives?
61. What material for hive construction is suitable for ecological beekeeping?
62. How could the average life of wood beehives be prolonged?
63. Which chemicals used for wood protection harm the bees?
64. How is health of foraging bees affected by wood treatments?
65. How does the strong oxidizer react with ecological substances *(e.g. microorganisms)*?
66. Which chemicals are commonly used for hive disinfection?
67. Why does PCP still occur in the environment?
68. Which pathways are mostly affected by using of chemicals?
69. How is the behaviour and cognitive function of bees affected by neonicotinoids and acaricides?
70. How could development of bees be affected by chemical compounds?
71. Which forms of oxalic acid are applied into hive and at which condition?
72. How does oxalic acid influence the beekeeper's health?
73. How could the hive be damaged, if there is a low level of Varroa and the medicine is applied correctly?
74. Which material inside of the hive is the most contaminated by insecticides?
75. How does the distribution of contaminants inside the hive depend on $K_{O/W}$?
76. Which process allows the transfer of contamination inside the hive?
77. Which type of food stuff is used by bees to obtain amino acids, and how susceptible is it to contamination?
78. How does starving, pollen diet or lack of nectar influence the development of bee colony?
79. Can supplementary feeding compensate nutritional-related stress factors?
80. How is the queen bee affected by the application of pesticides in the environment?
81. Is the effect of pesticides larger to bee queen or workers?
82. What are key factors of bee queen longevity?
83. Which substances are in bees wax and which type of compounds contaminate beeswax mostly?
84. At which conditions should be manufactured wax foundation?
85. Which substances withstand high temperatures and are stable in wax?

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2

ANNUAL CHEMICAL-FREE TREATMENT PLAN AGAINST VARROA MITES

THE PRESENCE OF VARROA MITES HAS FUNDAMENTALLY CHANGED BEEKEEPING PRACTICES. AFTER MORE THAN THIRTY YEARS OF CONTROLLING MITES BY MEANS OF PESTICIDES, WE HAVE TO ACKNOWLEDGE THAT TODAY'S SITUATION IS MUCH WORSE THAN WHEN WE STARTED. THE SOLUTION IS TO LEARN BIOTECHNICAL, CHEMICAL-FREE METHODS, WHICH ARE BASED ON DEEP KNOWLEDGE ABOUT THE BIOLOGY OF VARROA MITES AND HONEYBEES.

TABLE OF CONTENTS

1 • INTRODUCTION

Biology of Varroa destructor and the honeybee

Varroa destructor is an external parasitic mite that attacks the honey bees Apis cerana and Apis mellifera. The disease caused by the mites is called varroosis. These mites are fed by the fat body tissue and are reproduced under capped brood cells.

In most parts of the world, bee colonies are managed by various management practices, including methods to control *Varroa* mites. If the colonies are improperly treated or not treated at all against *Varroa* mites, such colonies are most likely to perish within one to two years and become a threat to other bee colonies in the direct range of bee flight activity, which may be as much as 30 km or more from mite outbreaks.

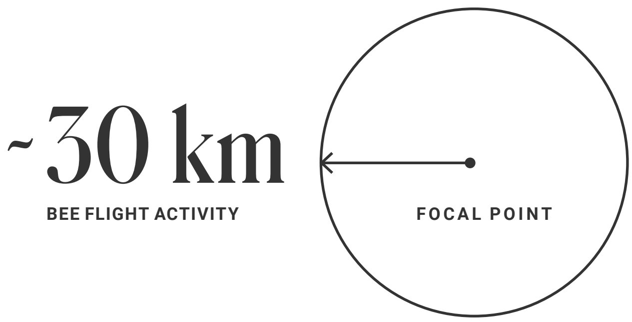
Depending on the developmental cycle of the bee colony, even a relatively small population of *Varroa* mites that parasitizes the colony significantly reduces its overall vitality, weakens the overall immune system, activates and transmits bee viruses, which in turn can lead to complete death of the entire colony.

As a result of the honeybees world trade, the *Varroa* mite has spread to all continents except Antarctica and is considered to be one of the major factors causing excessive bee stress, leading to unprecedented deaths of honeybees worldwide over the past decade.

There is no simple and quick solution to completely remove mites from the colony. The resistance to the chemicals used already exists and worsens the situation. The occurrence of the *Varroa* mite combined with incorrect apiculture treatment practices continues to be a serious problem to the survival of the honey bees. Although the situation has been critical for several years, beekeepers already have effective and sustainable solutions that can prevent colony losses caused by *Varroa* mites.

The development of the mite population can be monitored in each colony, or on a sample of bee colonies in case of large scale bee farms, and controlled using appropriate biotechnical methods and heat treatment, allowing the mite population to stay below the critical levels, so that the colonies can safely survive the winter period, develop healthy in spring, bring honey and naturally continue to live over the years as a healthy and viable organism. From the point of view of apiculture practice, it is necessary to have profound knowledge of the colony biology and *Varroa* mite and, depending on the particular situation in the colony, to intervene correctly and in time using effective non-chemical methods which are explained in the following sections.

VARROA MITES INFESTATION RANGE



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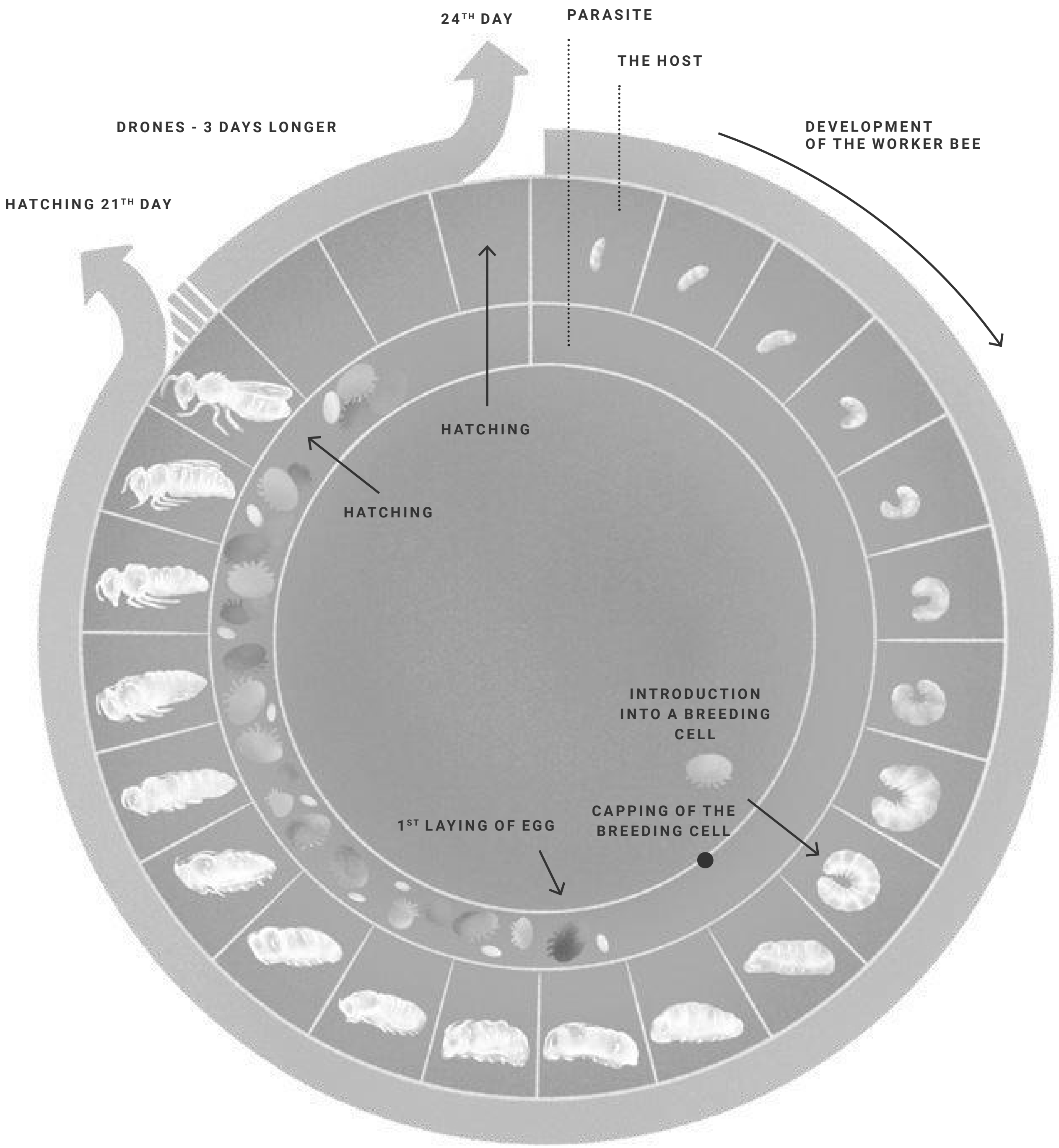
Development of honeybees and the mite Varroa destructor



CHART

Development a Varroa mite and a honeybee

MODIFIED BY SOURCE: BIENENINSTITUT KIRCHHAIN, 2012

This section focuses on relationship that exists between the development of a Varroa mite and a honey bee.

The development of a honeybee from an egg to an adult bee passes through different stages. At the correct temperature of the brood nest at about 36 °C, the queens emerge from their cells in 16 days, worker bees in 21 days, and drones in 24 days.

THE BEE DEVELOPMENT

The egg phase lasts for 3 days — the egg stands, moves and then lies at the bottom of the cell.

The phase of the queen larva lasts for 5 days, bee workers for 6 days and drones for 7 days. The bee worker and drone larva are fed at the beginning with royal jelly, later they are fed with a mixture of honey and pollen.

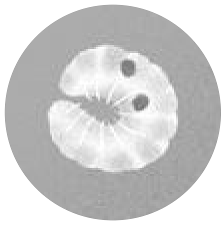
The phase of pupa, from which an adult imago develops, lasts 8 days for the queen, 12 days for the bee workers and 14 days for the drones.

MITE OCCURRENCE IN THE COLONY

They are attached to the abdomen of the bee, primarily of the noursing bees.

They are hiden inside capped cells of drone and worker brood.

PARASITIZING PERIOD



12 – 15 days on brood

5 – 11 days on an adult bee

The development of the honeybee and the mite, *Varroa destructor* are closely related. The *Varroa* mite reproduction and the honeybee methamorphosis occur at the same time during the stage of capped brood.

Wherever the mites are in the colony, they act as real predators, by feeding on the tissue of the fat body of larvae and of the young *(nursing)* bees. The serious damage caused by the *Varroa* mites on the fat body of parasitized colonies often cause their dead in a period of one or two years.

The fat body is responsible for 9 vital functions during development and life of a honeybee. Therefore we will devote more time to this important bee organ in the later sections.

First, the female mite is attached to an adult bee, mainly a noursing bee because their fat body is larger compared to the foraging bees. The female *Varroa* mite needs to feed sufficiently with energy-rich food to be able to produce the eggs. This energy is obtained from the digestion of the fat body tissue of the honeybee. The female mites parasitize on bees approximately 5 to 11 days during the colony development period, though during the winter period, the mites can survive on bees for 5 to 6 months. Soon after the queen begins to lay eggs in spring, the mites also begin to reproduce and multiply in the colony.

Each *Varroa* female mite seeks to have its own offspring and to achieve this, it is necessary that a fertilized female mite penetrates into a brood cell with a worker or a drone larva. In the case of the worker bee brood the mite enters the cell on the 9th day *(after egg laying)*. In the case of a drone cell, it is on the 10th day. In this developmental phase the larva produces specific pheromones, and it occupies the entire bottom of the cell with its fat body. Then it begins to gradually straighten up, so that later on, the complete metamorphosis to an adult bee can occur. The female *Varroa* mite has a well-developed smell organ, which allows it to accurately recognize the the nine and ten day old larvae. The pheromones in the brood are the signal for the female *Varroa* mite to enter into the brood cell just before it is capped by the nursing bees.

About 70 hours after capping, the female *Varroa* mite lays its first egg, out of which a male mite will emerge. Gradually, it lays another 4 eggs in the worker brood cells *(and up to 5 eggs in the case of drone brood cells)*, approximately one new egg every 30 hours.

The male mites develop in 6.6 days, while the female mites need only 5.8 days. The male mite is sexually mature 20 hours before the first sexually mature sister. The male mite couples several times with all sexually mature sisters. When the new born bee hatches from the cell, the male mite dies, like all unfertilized female mites.

The foundress mite and her 1 or 2 fertilized daughters (or even 3 from the drone brood cell) will come out already attached to the emerging bee, which they further parasitize by feeding on the tissue of her fat body.

Coupling of mite siblings takes place in capped brood, and it is important to be aware of this in relation to the spread of bee viruses' transmited by the Varroa mites. Coupling of mite siblings increases the virulency of viruses. We will talk about this aspect later.

9TH day

FEMALE *VARROA D.* ENTERS THE WORKER BEE CELL

~10TH day

FEMALE *VARROA D.* ENTERS THE DRONE CELL

~139 hours

TIME DEVELOPMENT OF FEMALE

~159 hours

TIME EVOLUTION OF MALE

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1 · 2

Seasonal development of the Varroa mites and the honeybee population curve

4

MAIN DEVELOPMENT STAGES OF THE BEE COLONY

This part explains how the population of the Varroa mite changes with the seasonal development of the honeybee population.

The honeybee colony reaches its population peak shortly after summer solstice *(as shown on the graph on page 6)*. Whilst the number of honeybees in the beehive gradually decreases, particularly during the August — September months, the population of *Varroa* mites increases considerably in this same period. The reaction to this situation is that the queen continues laying eggs more intensively. This we call a "deadly spiral" — the more brood in the hive in autumn, the more possibilities for mites to parasitize on winter bees and to reproduce.

Winter bee larvae and the adult winter bees, both with large fat bodies, are ideal hosts for the female *Varroa* mites. When there is less brood available, it is common to have multiple infestations by *Varroa* mites on one single larva, causing death or severe body deformation to developing bees.

①

Population increase phase

MARCH – APRIL

②

Peak phase

MAY – JULY

③

Population decrease phase

AUGUST – SEPTEMBER

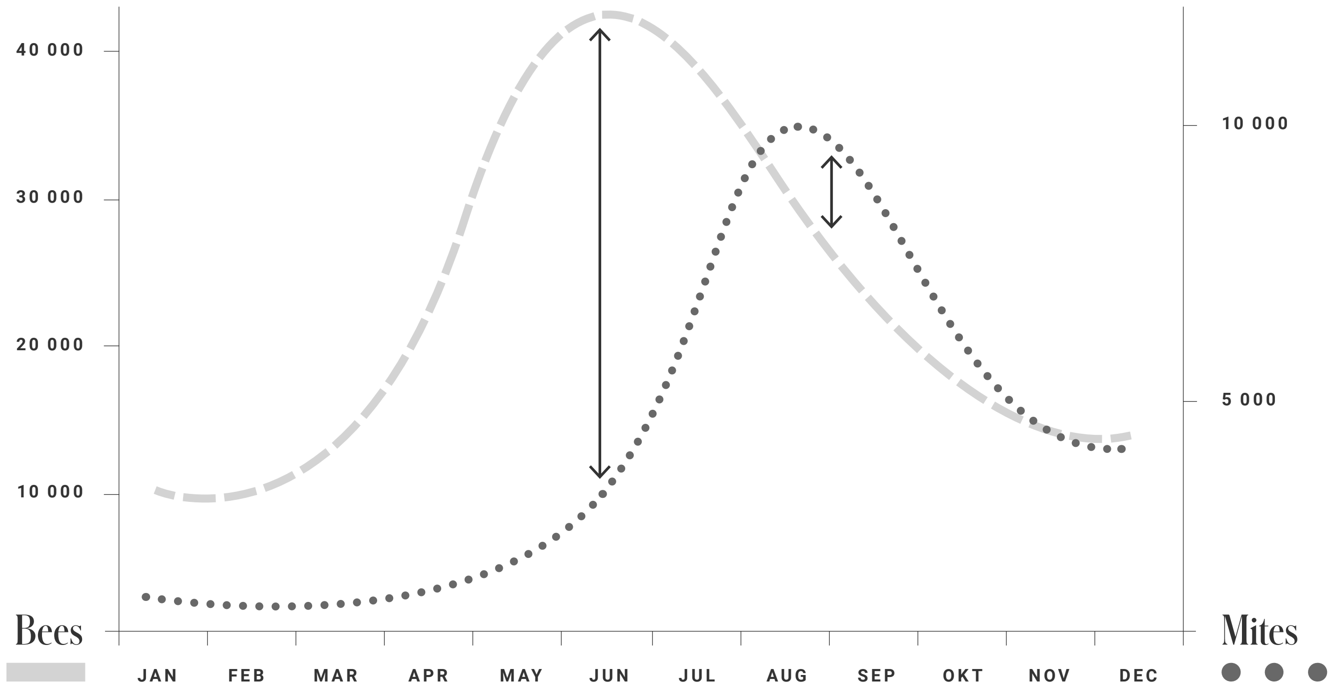
④

Dormant phase (winter rest)

OCTOBER – FEBRUARY

CHART

Honeybee population and the Varrroa mite population during the season



The female mites can survive the winter period attached to the adult bees. As soon as the queen starts laying eggs in spring the female *Varroa* mites start parasitizing the larva of the worker bees. Later on, when drone brood is present, the female *Varroa* mites infest the drone larvae 8 to 10 times more often compared to worker bee larvae. The reason might be that the fat body of drone larvae is bigger than the fat body of the worker bee larvae. In addition the drone brood takes longer to hatch, so more *Varroa* offsprings can developed into their sexually mature state. These two factors combined are probably the reason for higher drone brood infestation rate.

The size of the Varroa mite population is decisive during the winterization (preparing for the winter) period of the bee colonies.

With a decrease of the bee population towards the end of summer, the bee colonies need to be strong and healthy to breed the population of winter bees, which in turn will sustain the bee colony during the winter *(dormant)* period.

We have been experiencing warmer autumns and winters in the last years. The longer cultivation periods of oilseeds for green fuels and feed provide bees with enough pollen until the late autumn. The warmer autumn periods allow bees to take care of their brood 9 to 10 months a year. Due to these ongoing climate changes and the resulting longer breeding periods, the *Varroa* mites can also reproduce much longer and intensively than before these changes.

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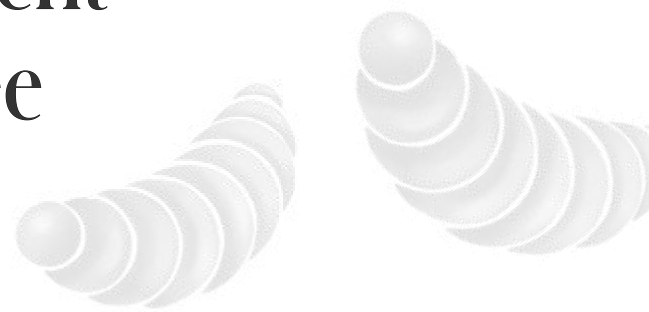
The importance of the fat body for the development and life of the honeybee



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VITAL FUNCTIONS OF THE FAT BODY

The Varroa mites feed on the tissue of the fat body of the larva and adult honeybees. This section explains the basic functions of the fat body and the consequences of its damage for the larva and the adult bees.

The fat body is not a fat cell or a tissue, it is an important organ of the honeybee, with similar functions as the liver in the human body. It is composed of groups of cells predominantly found in the belly of the bee, particularly under the cuticle on the back of the body and the abdomen, and it covers the digestive system. In addition to fat, glycogen *(animal sugar)* and proteins are stored in the fat body. In the prepupal stage there is a complete transformation of all organs. In this stage of a large body transformation the fat body plays an important function as excretion system. Some fat body cells specialize in absorbing wastes from the hemolymph.

① It is a reservoir of energy and mobilizes nutrients. ② It detoxifies pesticides. ③ It assists with osmo-regulation. ④ It helps in the functioning of the immune system. ⑤ It regulates a temperature. ⑥ It contributes to metabolic activities. ⑦ It is important in the synthesis of proteins and fat. ⑧ It is a reservoir of the protein vitellogenin, that regulates hormonal dynamics of the worker bees, the immune function, and longevity in worker honeybees and queens. ⑨ It is a reservoir of juvenile hormone that directly influences development and metamorphosis of a honeybee.

Honeybees store vitellogenin and juvenile hormone molecules in the fat body at their abdomen and heads, which then move into the honey bee blood *(hemolymph)*. Vitellogenin protein and juvenile hormone likely work antagonistically in the honey bee to regulate the honeybee´s development and behaviour. Suppression of one leads to high titers of the other. The health of a honeybee colony is dependent on the vitellogenin reserves of the nursing bees *(the foragers have low levels of vitellogenin)*. Vitellogenin levels are important during the nest stages and thus influence honeybee worker division of labor.

As expendable laborers, foraging bees are fed just enough protein to keep collecting nectar and pollen. A nurse bee's vitellogenin titer, which develops during the first four days after hatching, has an effect on its age to begin foraging, and whether it preferentially forages for nectar or for pollen. If young worker bees are short on food in their first days of life, they tend to begin foraging early and preferentially for nectar. If they are moderately fed, they will forage at normal age preferentially for nectar. If they are abundantly fed immediately after emergence, their vitellogenin titer is high and they begin foraging later in life, preferentially collecting pollen. Pollen is the only available protein source for honey bees. The queens have 300 times higher vitellogenin titres compared to foraging bees. On the other hand, the juvenile hormone is involved in many physiological processes in the development of honeybees, for example, for the gradual onset of the larval metamorphosis from pupa to adult bee. In addition, it is involved in the regulation of ovarian development, sexual maturation, pheromone production, diapause, immunity, and also for caste differentiation of social insects.

Coming back to the fat body, this organ also serves as a reservoir of energy. A large part of the body larva is composed of the fat body. The pre-pupa and the pupa no longer receive food but survive on the reserves in their fat bodies.

The formation of wax originates also in the fat cells of the fat body. The liquid wax *(a mixture of hydrocarbons, acids, esters, alcohols and aromatic substances)* travels to the surfaces of the wax-forming plates *(also called as "wax mirrors")*.

Beside supplying energy In the cold period, fat body cells provide heat insulation and protection to other internal bee organs. Bees with a large fat body thus survive easier the winter period. Bees must be "fat" to guarantee a healthy and good spring development and colony population growth. Without enough energy supply bees develop later in spring, and start rearing brood only after the spring nectar and pollen sources are again available in nature.

The honeybee longevity is directly related to the size and proper functioning of the fat body. When bees had good conditions to develop their fat body in autumn, they have a far greater chance of living longer than the physically exhausted "poor" foraging bees. It is therefore clear that the fat body is of considerable importance in the bee development phase as well as in the further functioning of the bee colonies. Consequently, the longevity and survival are not only a matter of inheritance but, above all, a matter of timely and good treatment against *Varroa* mite and proper preparation for the winter.

THE FAT BODY OF WINTER BEES WILL BE WELL DEVELOPED UNDER THE CONDITION THAT THIS GENERATION OF BEES:

①

Was well prepared for winter by receiving high quality food, a lot of divers pollen available from the vicinity of the apiary.

②

Was developed without Varroa mites.

③

Was not too exhausted by work, e.g., by longer brood rearing activity and/or collecting and storing winter supplies.

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The food composition during the development of a bee and its importance for parazitising by the Varroa

This section explains the composition of the diet in relation to the development of the honey bees castes, and the reasons why the *Varroa* mite parasitizes more nursing bees and drone brood over foraging bees.

During its first three days the larva is fed with the secretions of the hypopharyngeal glands of the young, nursing bees. The first food is rather clear and is stored in the cell, all around the larva. The second day the food has a milky colour, and on the third day the larva is fed with a mixture of pollen and honey. On the fourth day the protein supply reaches its maximum. The larva of worker bees and drones are fed with honey and pollen directly into the mouth. The amount of fat reserves in their bodies significantly increases. Once brood is capped there is no more feeding and the larva *(and later the pupa)* survive only from their body reserves.

The larva of the future queens receive only royal jelly as food while in in their cells, in such a large quantity that they cannot completely consume it. After hatching a queen bee, a visible amount of royal jelly remains at the bottom of the queen cell.

The different quality *(compositions)* and quantity of food that nursing bees have to give to larvae of different bee castes consequently affect the amount of time that nursing bees spent with different brood castes. The nutritional value of the food can be observed from the growth rate of the larva. The larva grows so fast that it changes its tight cocoon six times and leaves it in the cell.

Pollen fermented with honey, as it is stored in the combs of the colony, is a powerful source of energy for bees, which then transforms into fat. From the previous section we know that the female *Varroa* mites consume the tissue of the fat body of the honeybees. The body of the bee larva is formed almost 100 % by the fat body organ. This is why whenever there is bee brood in the bee colony female mites prefer to parasitize it instead of parasitizing the adult foragers. Foraging bees, especially the summer ones are exhausted by the arduous collection of nectar, pollen and water. Their fat body is small compared to the fat body of the larva and bees. Nursing bees must have enough energy to produce royal jelly and wax.

Drone larvae are nurished with protein rich food 3 – 4 times more compared to worker brood larvae. Consequently their fat body is larger than the fat body of the worker bee larva. We already mentioned that the developmental stage of a drone is three days longer. This makes it possible to sexually mature more Varroa females. These are probably the main reasons for female mites to parasitize drone brood more than worker brood.

The larva of the future queens are not attractive to foundress mites, as the queen develops in only 16 days, five days faster than a worker bee. This would mean that no female mites would develop as sexually mature in the queen cells. In addition, the *Varroa* mites avoid queen cells probably due to the excessive amounts of royal jelly, and a specific pheromone of the developing young queen.

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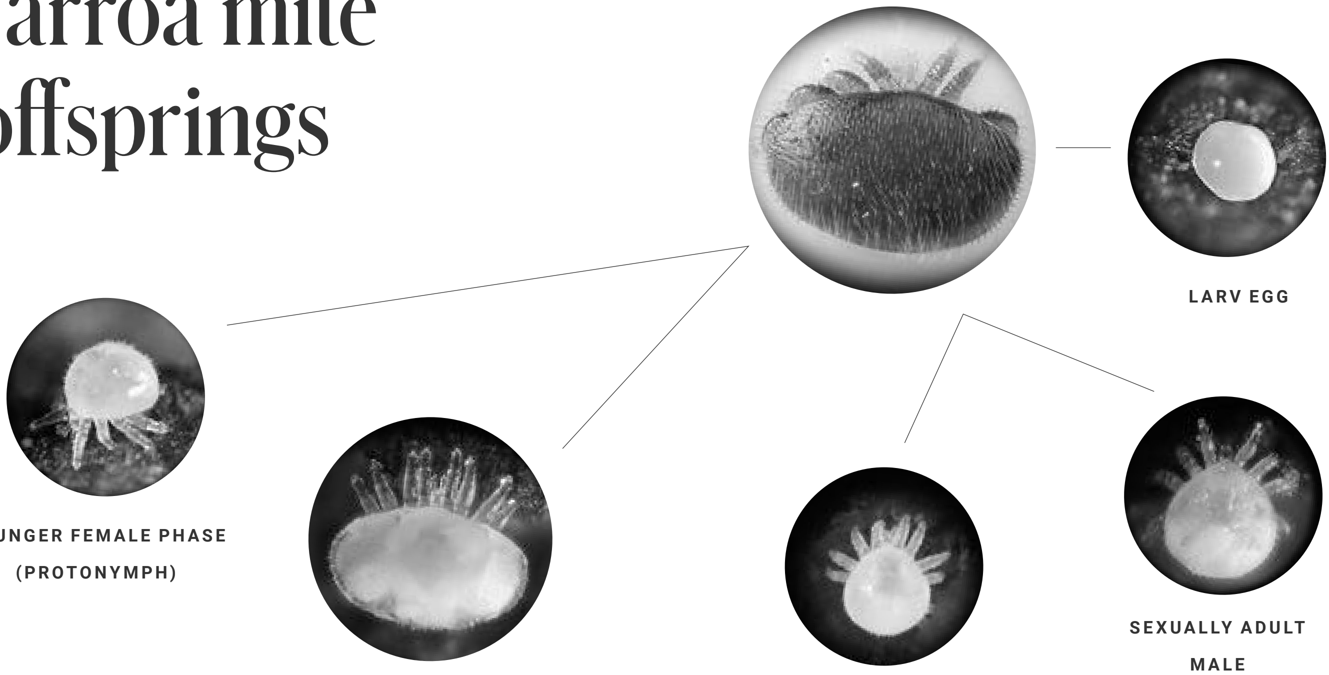
Varroa mite offsprings



There are clear physiological differences among mature, immature females and mature males of *Varroa*.

First line, left to right, shows mature daughter mite and mother mite; second line left to right shows an immature *(deutonymph)* daughter and two mature males. A younger stage *(protonymph)* of female is not in this photo.

The most commonly encountered life stage of the *Varroa* is the mature female mite. These *Varroa* mites are darkish red, oval in shape, approximately 1 x 1.5 mm wide, with the body surface covered in fine hairs and eight legs located at the front of the body. Males are smaller than females throughout the whole ontogenetic development and have longer legs in relation to the body size.

The development stages of the mite offspring pass through proto- and deutonymph stages; the developmental time is about 5.8 and 6.6 days for female and male mites, respectively. For the beekeeping practice, it is recommended to observe the sticky monitoring board and see if there are white, almost transparent mites beside the brown female mites. This indicates that the colony is brood rearing and the mites can continu reproducing. It is especially important during early spring *(population increase phase)* and late autumn *(October and November)*.

TIME OF GROWTH PHASE

Female ~5 days 19 hours

Male ~6 days 14 hours

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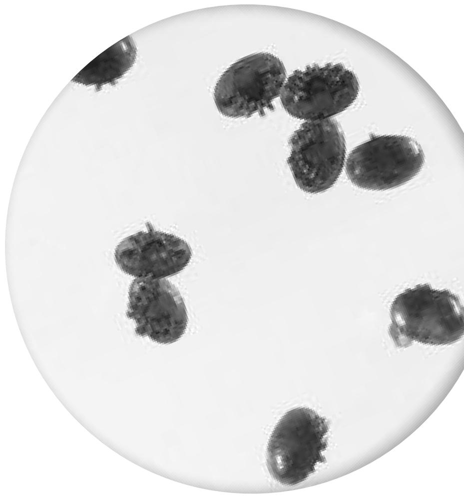
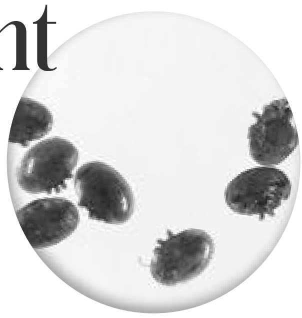
Varroa mite population growth during the seasonal colony development

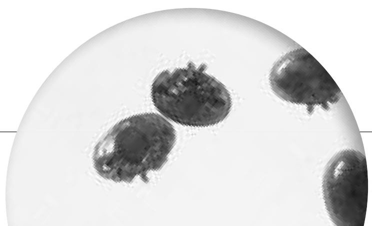




This part explains the main tactics that the Varroa mites use to grow into thousands of individuals if nothing happens to control them.

The mite population multiplies progressively — **internally**, in a brood nest, during the reproduction cycles under capped brood cells and **externally**, by drifting, robbing or swarming.

The mite's success is based upon four main tactics: ① A mite hides and reproduces under capped brood cells *(therefore they are invisible for the beekeepers during the intensive brood rearing period)*. ② It spreads by weakening or killing the colony so that it can hitchhike on robber bees to infest neighbouring colonies. ③ Sibling mating of mites may result into more resistant mites with highly virulent viruses *(therefore even a low mite population of 300 mites can kill the entire bee colony already in early autumn)*. ④ It reproduces exponentially, especially in the drone brood, once a colony is without drone cells, the worker brood cells might be infested with several female mites wanting to reproduce there. ⑤ Mite females prefer drone brood, because they have enough nutrients from the fat body tissue, and the development of a drone lasts three days longer than the development of a worker bee, allowing sexually mature more female offsprings. Parasitized drones loses their fertility, vitality, immunity and lifespan.



If a colony continues breeding in September and October and at the same time a reinfestation occurs, despite of the complete anti-Varroa summer treatment in July, an entire colony often collapses in late autumn, or early spring.

This suggests regularly monitoring the natural daily mite fall during the autumn period (September and October and in lowlands with warmer weather and agricultural landfields also in November).

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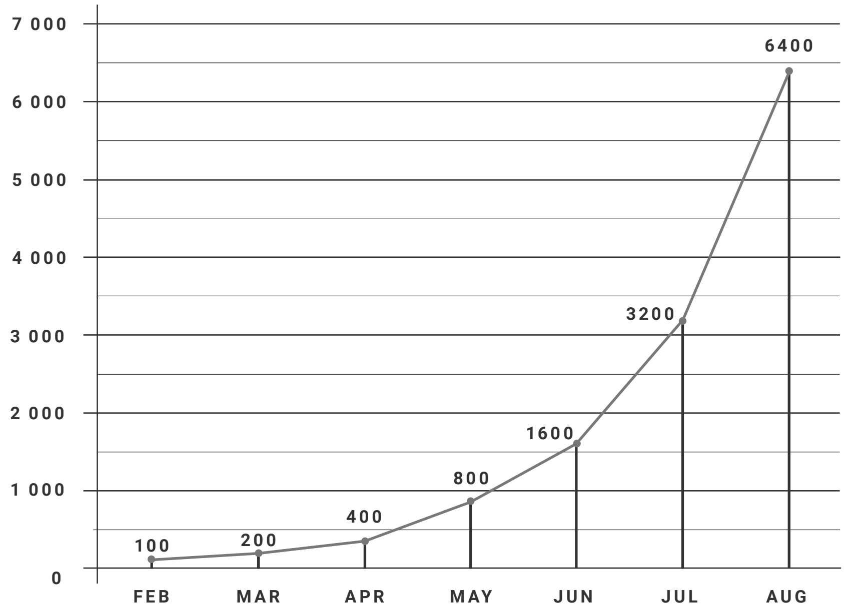
Reproduction rate of the Varroa mites

CHART

Reproductive rate – doubling per month



This part explains the reproduction rate of Varroa mites in a bee colony.

In general, the duration of brood rearing is a limiting factor for the development of the mites. The longer the brood is present in the colony, the longer the reproduction cycles of *Varroa* mites may last.

The reproduction rate is though highly variable and depends on all of the traits of the host and the parasite, and factors such as the reproductive capacity during the mite's lifetime, the lifespan, brood availability, presence of drone brood, swarming, level of defense behaviour, overall imunity of a bee colony. This all may influence the reproductive rate and the overall population dynamics of mites in a colony.

Although significant correlations between the amount of brood and/or the fertility of the mites and population growth exist, prediction to what extent a starting mite population in the spring will increase until autumn stays more theoretical.

Nevertheless, based on the scientific observations and practical tests, the population of mites in a colony is doubling per month when nothing is done against the mites. Nonetheless during the colony population decrease, when the number of adult bees decreases and the brood nest becomes smaller, the number of mites dramatically increases. This is because of well developed fat body of larvae of winter generation bees, which provides energy-rich food for the female mites, and robbing behaviour when healthy bees try to steal food reserves of the *Varroa*-infested *(collapsing)* colonies. By robbying a few hundreds of female *Varroa* mites per week can infest a hive.

As it is seen in the graphic above, the *Varroa* mite is able to double each month its total population in the colony. From each *Varroa* mite in the month 1 will be four mites in the month 4, 32 *Varroa* mites in the month 5 and 64 *Varroa* mites in the month 6. In theory, if in February the colony counts 100 mites, in September there will be 6.400 mites, under the condition we do nothing to control the mites. In practice, in between July and August the colony will reach the level of mite infestation leading to the colony collapse. There will be the level of bee viruses so high that the colony will crash already in August or beginning of September.

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Damages caused by the Varroa mites to the individual bees and the entire bee colony

There are two types of damage: one is the immediate damage to the brood itself and to the adult bee, and one is a long-term damage to the entire bee colony.

DAMAGE OF BROOD

① Experimental studies have shown that parasitized drones have lower flying abilities and a detrimental effect on spermatogenesis with fewer spermatozoa produced.

② While feeding on the bee larva and pupa, mites decrease the total level of the proteins in the bee heamolymph, especially low molecular weight proteins, so a parasitized bee has a lower body weight by approx. 1/3 compared to a healthy bee. Therefore, worker bees have a lowered capacity to bring food reserves.

③ Mites cause a decrease in the amount of arylphorin, a protein present in the bee nymphs necessary for the development of the cuticle at the time of the fledging. A *Varroa* mite can consume all the reserves of this molecule. The consequence will be a more fragile cuticle and therefore less protection against external aggressions *(physical, chemical and infectious)*.

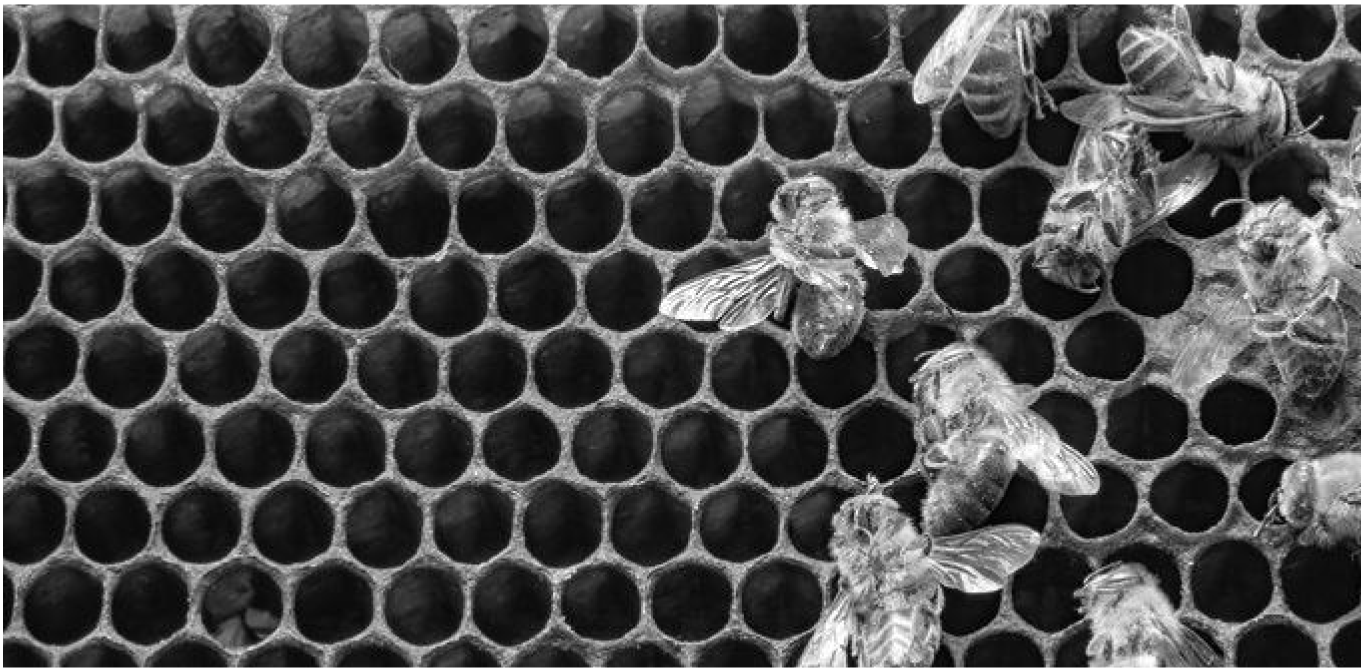
④ *Varroa* mites feed on the tissue of the fat body of the larva and pupa which is a reservoir of protein vitellogenin; the lack of vitellogenin reduces the lifespan of bees, especially winter bees and significantly reduces the immune system of the bee. In addition, at the cellular level, there is a reduction in the number of protein granules per cell.

⑤ The size of the acini of the hypopharyngeal glands of nursing bees is reduced by an average of 10 %. These glands are needed in the production of royal jelly, therefore the social function of nursing bees is compromised.

⑥ Born bees have deformed wings, antennas and legs, shorter abdomen, damaged sight, smell and other senses.

⑦ The *Varroa* mite is a vector of pathogens, especially the most commonly found Deformed Wing Virus *("DWV")* and other viruses.

⑧ Parasitized bees show a decreased level of the enzymes lysozymes and phenol oxidases, which are involved in the encapsulation process, as well as of the enzymes abaecine and defensin. Defensin has an antibacterial effect and is important for the well-functioning of the immune system. All these enzymes are produced in the fat body.



Not only individual bees are damaged, but the entire honeybee colony as the *Varroa* mite causes pathogenicity on both levels. Parasitized brood and adult bees are part of a colony that suffers as a whole organism. The whole colony is at high risks, especially after summer solstice, when the bee population is in decline, but the mite population continues to increase dramatically and parasitize the entire colony. Multiple *Varroa* mites parasitizing on a reduced brood nest often lead to a colony collapse, which is why the *Varroa* treatment of brood by means of chemical free methods in mid-summer and late autumn is highly recommended. We will come back to it later.

Even a relatively low level of mites in a colony has the following negative consequences:

① The slow growth of the bee population associated with lower honey production and pollen collection. ② A discontinuous brood accompanying with a lower replacement rate of new honeybees. ③ Various behaviour disorders, for example swarming in late autumn. ④ Deformed wings. ⑤ Shorter lifespan. ⑥ Decreased drone fertility. ⑦ Weakened thermoregulatory ability. ⑧ Reduced collective immunity of the colony. ⑨ Reduced ability to detoxify pesticides to which honeybees are exposed to.

In order to keep these damages at the lowest level possible, it is necessary to keep the population of mites under a certain threshold. This threshold highly depends on factors such as bees' hygienic behaviour, quality of queen, length and intensity of brood rearing, the ratio of summer and winter bee larvae, the presence of viruses and others. Therefore, the beekeeper is responsible to control the mite population in the bee colony, especially after the summer solstice.

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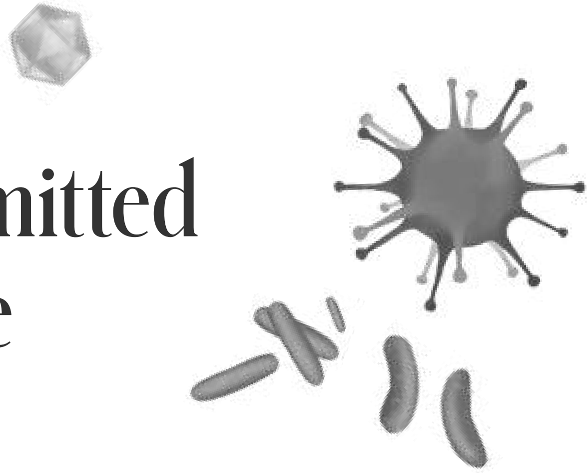


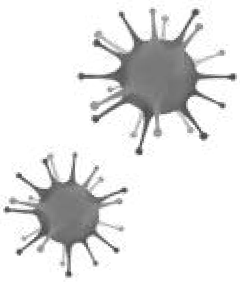
Bee viruses transmitted by the Varroa mite

The mites also activate, propagate and transmit bee viruses. In this part we explain virus transmission mechanisms and we name the most harmful bee viruses.

Viruses can be transmitted by queen eggs that have been affected by viruses. This may happen when virus infected drones mated with the queen. Another way of virus transmission is the oral-fecal way, when bees consume infected food inside of the hives. The third way how viruses can be spread among bees is when parasitized nursing bees feed young brood.

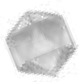
Researchers found that the high levels of Deformed Wing Virus are often inside of the brood cells and young larvae and pupea. This gives us a good reason to replace old dark brood frames by clean, chemical free wax combs in the brood nest.

We already learned that the population *Varroa* mites doubles per month, but bee viruses can multiply in millions in a few hours and can also mutate, making them extremely dangerous for the colony.



6

VARROA MITES
TRANSMIT VARIOUS VIRUSES



DWV – Deformed Wing Virus

Is present in almost 100 % of *Varroa* mites. DWV has been shown to go directly to the bee brain, and to cause premature exit of infected bees from the hive, as well as affecting their orientation and communication.

SBV – Sacbrood Virus

Found in about 50 % of *Varroa* mites.

ABPV – Acute Bee Paralysis Virus

Found in about 35 % of *Varroa* mites.

KBV – Kashmir Bee Virus

Found in 4 % of *Varroa* mites found in 4 % of *Varroa* mites.

CPV – Chronical Paralysis Virus

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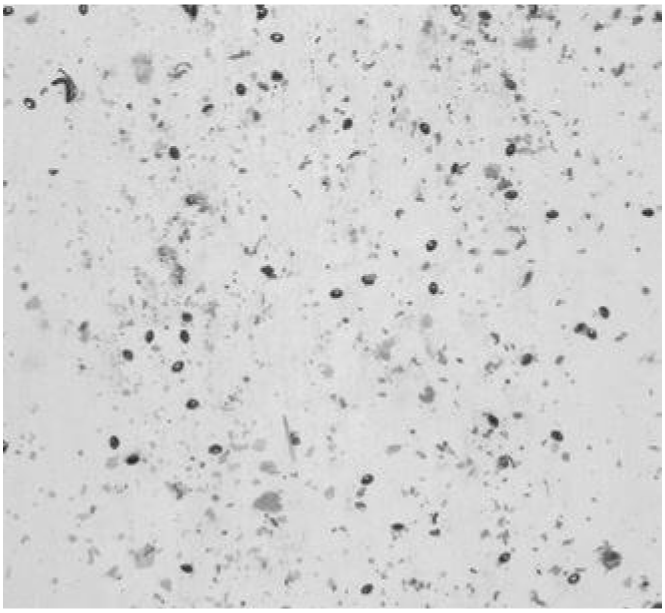
Monitoring the natural daily mite fall and calculating the total mite population in a colony



SOURCE: WIMMER, HANDBOOK ON HYPERTHERMIA.

There are different ways to monitor the mite population. This section explains how to monitor mites and how to calculate the total mite population in a colony based on the average daily natural mite fall.

In general, the colonies should be monitored at least 4 times per year. In some locations with a high density of hives, a monthly regular monitoring is highly recommended. The following method explains how one can record the natural mite fall in a beehive.

EQUIPMENT NEEDED

SCREENED BOTTOM BOARD

WHITE MONITORING TRAY WITH GLUE COATED EDGES

(ALTERNATIVELY) STICKY MESH OVER THE TRAY TO AVOID ANTS OR OTHER SCAVENGERS TO REMOVE DEAD MITES FROM THE TRAY

In order to obtain accurate numbers, it is necessary to know the average number of daily mite fall of at least one week, preferably 10 days before the treatment. Shorter observation periods could bring inaccurate results. If the average of the natural daily mite fall was identified, it is possible to calculate the approximate total mite population in the honeybee colony.

AVERAGE NATURAL FALL PER DAY

60 MITES / 10 DAYS = 6 MITES / DAY

THE CONVERSION FACTOR

x 200 Spring

x 250 Summer

x 300 Autumn

THE MITE POPULATION IN THE COLONY

6 MITES /DAY × 200 MULTIPLIER /MAY = 1200 MITES /COLONY

It is important for us to know how to calculate the total mite population in a hive and how to interpret the findings. This average number needs to be multiplied by a conversion factor to obtain a total population of *Varroa* mite in the hive. These conversion factors vary according to the season. A higher natural daily mite fall is common during the intensive breeding period, when the infested bees are hatching. During the brood free period like for e.g. in winter time, the natural mite fall is significantly lower, even zero.

ADVANTAGE

This method is useful to check the development of the mite population trends and to confirm the treatment effectiveness after a measure has been taken. It is a non invasive method. It is particularly used in spring and autumn.

DISADVANTAGE

In a beehive with two or more brood chambers *(like in the case of 2/3 Langstroth type hives)*, mites may drop on the top of the bar frames or get stacked inside of the empty cells, which leads to inaccurate results. The counting method is also time consuming when many trays need to be observed, counted and registered.

KURT TRATSH EXPLAINS HIS STRATEGY

Scan the QR code with your smartphone or enter the address in your browser.

https://youtu.be/uFCRDUGdjVg

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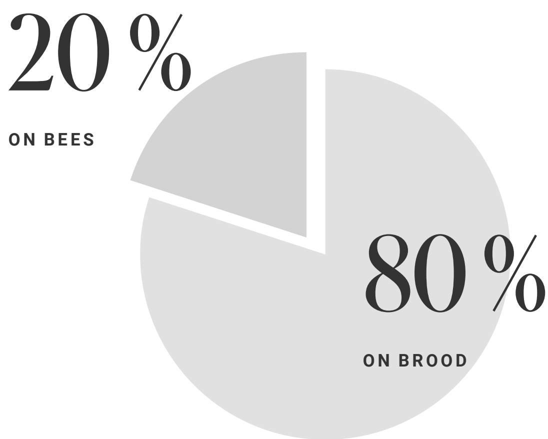
Result interpretation according to the development of the colony

Once we have calculated the total mite population in the bee colony, we need to evaluate whether the colony is at risk and a treatment is required. But, what is the threshold that determines the necessity of the treatment?

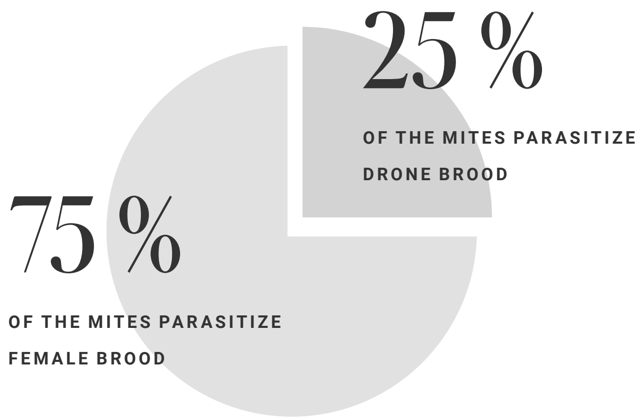
There are various thresholds indicating how many mites a colony can sustain. These limits depend on the overall health conditions of the colony and many internal *(e.g. level of viruses, number of months of brood rearing, hygienic behaviour of honeybees)* and external factors *(e.g. number of surrounding colonies with high mite infestation levels).*

The beekeeping experts from the British Food and Environmental Research Agency *(FERA)* recommend that the number of mites per colony musn't extend 1000. Reaching a higher number, the colony will be at risk of collapsing within the next weeks, if not treated properly. Below this threshold, the co- lony is safe because the number of newly hatched bees from spring to summer solstice is much greater than the number of adult female mites that are able to reproduce. In spring, 80 % of the mites parasitize brood, and only 20 % of mites are attached to adult bees. About 25 % of the mites that parasitized brood are hidden in the capped drone brood.

MITE PARASITISING



ON THE BEE BROOD



In autumn, the critical level of 1000 mites in spring and summer needs to be lower, as the number of bees decreases considerably, whereas the number of mites increases significantly. We already know that female *Varroa* mites feed on the fat body of the winter generation larvae and bees in order to reproduce. The experts from The German bee monitoring project recommended that **the critical threshold in autumn should be close to 3 % out of the total winter bee population. This means that if a colony counts about 10 000 winter bees, the number of mites in that colony should not extend 300**.

300

THE NUMBER OF MITES VARROA D.

10 000

WINTER BEES

||

THE CRITICAL THRESHOLD OF NUMBER OF THE MITES OF THE TOTAL WINTER BEE POPULATION.

LET'S HAVE A LOOK THE CRITICAL VALUES THAT DETERMINE WHETHER AN INTERVENTION AGAINST THE MITES IS NECESSARY.

MAX. NUMBER OF MITES PER DAY

1

AT ANY TIME DURING THE POPULATION GROWTH PERIOD

(ESPECIALLY) MARCH – MAY

5

AT ANY TIME DURING THE POPULATION PEAK PERIOD

JUNE – JULY

3

AT ANY TIME IN THE WINTERING PERIOD

AUGUST – SEPTEMBER

1

AT ANY TIME DURING THE WINTER REST PERIOD

OCTOBER – FEBRUARY

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Monitoring the mites in a sample of bees, calculating the infestation rate and interpreting the critical thresholds

This part explains the critical levels of Varroa mite population according to the number of mites per sample of adult nursing bees. Once the mites are removed from the bodies of nursing bees, the infestation rate can be calculated.

EQUIPMENT NEEDED

WIDE MOUTH GLASS JAR

SOLID LID REPLACED
WITH #8 SCREEN MESH

POWDERED EXTRA FINE SUGAR

WHITE PLATE
(tray, or similar device, even paper boards or sheets can be used)

WATER TO DISSOLVE
POWDERED SUGAR

COLLECTING THE SAMPLE

Open the brood chamber, select a frame from the edges of the brood nest and collect a sample of approximately 300 young bees *(avoiding collecting the queen! If she is on the frame, she has to go to another frame).*

300 BEES = 1/2 CUP OF THE GLASS

To collect bees, move the jar downward over bees so they fall backwards. Or shake bees directly from two or three brood frames into a larger container *(or honey bucket)* and scoop up 1/2 cup of bees and quickly pour them into the jar.

ONCE BEES ARE INSIDE THE JAR

① Add approximately two tablespoons of powdered sugar to the jar. ② Vigorously shake the jar for at least one minute to cover bees in sugar and dislodge the mites from bees. To improve the consistency of mite counts, shake the jar for a consistent length of time for every sample. ③ Put the jar down and wait three to five minutes *(rushing the process increases the risk of undercounting the mites).* ④ Invert the jar and shake it like a salt shaker, capturing the falling mites onto a clean plate or pan below. Shake the inverted jar until mites stop falling out. ⑤ Spray the powdered sugar deposit in the plate or pan with a water to dissolve the sugar. ⑥ Count the mites that remain on the plate. ⑦ Add an additional tablespoon of sugar to the jar, shake and roll bees again for 30+ seconds, and repeat steps 4, 5, and 6 to improve the accuracy of the count. This is not necessary when using alcohol wash. ⑧ Count the number of mites on the plate or pan. ⑨ Calculate the mite number per sample. ⑩ Sampled bees can be released back into the top of their colony or at colony entrance.

The powdered sugar shake method is non-lethal, so bees may be returned to the hive after testing. By applying the alcohol or detergent (instead of powdered sugar), bees will be sacrificed.

THE CALCULATION

12 / 300 = 0.04

MITES / SAMPLED BEES = COEFFICIENT

Multiplying this number by 100 we can say that the colony infestation rate is 4 %; in other words, 100 adult bees had 4 attached mites.

VALUES

ACCEPTABLE VALUE

Current mite population is not an immediate threat.

CAREFULNESS

The mite population reaches a level that can cause colony damage soon; continue in sampling and be ready to intervene.

DANGER

Colony loss is likely unless the beekeeper controls *Varroa* immediately!

TABLE 02

Treatment mites thresholds in relation to the colony phase (in %)

COLONY PHASE	ACCEPTABLE	CAREFULNESS	DANGER
DORMANT WITH BROOD	< 1 %	1 – 2 %	> 2 %
DORMANT WITHOUT BROOD	< 1 %	1 – 3 %	> 3 %
POPULATION INCREASE	< 1 %	1 – 3 %	> 3 %
PEAK POPULATION	< 1 %	2 – 5 %	> 5 %
POPULATION DECREASE	< 1 %	2 – 3 %	> 3 %

SOURCE

HONEY BEE HEALTH COALITION. TOOLS FOR VARROA MANAGEMENT. A GUIDE TO EFFECTIVE VARROA SAMPLING & CONTROL. 2015

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Interpretation of monitoring results with respect to the colony population development

The variable infestation rates of 1 to 3 % are based on the level of the beekeepers risk tolerance or risk perception. If the colony has many breeding frames, and/or is located at an apiary with many other uncontrolled beehives, it is then safe to treat the hives already at an infestation level of 1 % in spring. A study conducted by the National Institute for Agricultural Research *(INRA)* in France shows that if the colony infestation rate reaches 3 % in spring, the colonies will collect significantly less lavender honey, on average up to 5 kilograms less compared to colonies with fewer mites.

We already know that the critical thresholds *(the number of mites)* in a beehive vary depending on the colony development and some other factors influencing the growth of the mite population.

In a period of fast bee colony development, such as in spring and early summer, the rate of the mite infestation may be higher than in the period of the colony decline. This means that the colonies' condition in late summer and autumn determine a successful overwintering. Therefore, it is necessary to keep the number of mites as low as possible and even below the recommended 3 % of the total population of overwintering bees *(which, in our region, equals to about 300 to 500 mites in a colony of approximately 10.000 – 15. 000 winter bees).*

In October, there are only winter bees in the colony and there is little or no brood *(at least in the cold and mountainous regions).* The infestation rate in October signalizes a probability of the winter losses. The mite infestation rate of 3 % in October is considered a critical level for a successful overwintering of the monitored colony, also taking the negative efffects of the bee viruses transmitted by *Varroa* mites into account. Above this level, the treatment is needed.

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Factors influencing timing and method to combat Varroa

Based on the previous knowledge on the biology of Varroa mites and the honeybee, it is important to understand the overall situation in a colony at a particular time of the year, and also considering the climate conditions and the conditions of the location of the apiary. It is important to understand the development of the mite population during the whole beekeeping season, particularly in late summer and autumn.

A summary of the key factors and conditions contributing to the decision making about the necessity of the treatment and the chosen method:

① **Size of the brood nest in a hive in late summer and in autumn** — breeding is a limiting factor for *Varroa* mites. More brood frames mean often more *Varroa* mites. Making a brood free period in summer is becoming an important strategy when combating the mites. ② **Number of bees in a colony** — is it a new colony *(nucleus)* or a managed colony? Smaller colonies support much less mites than the productive hives. ③ **Keep colonies in a warm surrounding** — smaller colonies may struggle with temperature regulation. Keep the colonies warm in spring and if needed, also reduce the number of frames for the winter period. ④ **Pollen availability** — are your hives located in lowlands with a lot of pollen around, or in a mountainous region with less pollen available in late summer? Pollen is vital for development and life of any bee colony, particularly in August and September when the winter generation of bees develops. ⑤ **Number of uncontrolled *(managed)* hives in the flying distance of your bees.** It is good to know the beekeepers of your neighbourhood and to coordinate the *Varroa* treatments. Remember that there are always wild swarms, which can also be responsible for the reinfestation of your hives. Monitor your colonies especially in late summer and autumn. ⑥ **Weather forecast** — a warmer autumn with pollen available in nature often leads to a prolongation of the breeding period, giving the mites perfect conditions to continue reproducing. ⑦ **Virus load** — viruses mutate and become more virulent towards the end of the season. Even a relatively low level of *Varroa* mites *(300)* can generate virus epidemic situation affecting not just a single but also neighbouring hives, especially in late summer and autumn. Feed bees right after the last honey harvest and leave as much flower honey reserves as possible for each colony *(there should be at least 8–10 kgs of honey in the brood chamber at any time)*.

A successful beekeeping is directly linked to a good Varroa management strategy over the entire year.

2

Biotechnical methods to control Varroa mites

Biotechnical methods are based on the knowledge about biological characteristics of mites and honeybees and their behaviour. Their main objective is to reduce the mites without using harmful chemicals.

Varroa is present in all honeybee hives and it is impossible to eradicate it completely and definitively. However, we can control the mite so it doesn't harm the colonies. Regular monitoring and quantification of *Varroa* is necessary to optimize the timing and type of treatment as well as to verify the efficacy of the applied method.

The effectiveness of biotechnical methods can vary according to the season, the condition of the colony, and the level of infestation. None of the methods presented below allows the complete elimination of *Varroa* mites, but they can contribute to a significant reduction of mites and in a way that a beekeeper can avoid chemical treatments.

① Drone brood removal

② Brood reduction with a duplex framebox

③ Creation of new colonies by partial capped brood removal

④ Complete capped brood removal

⑤ Brood interruption by locking the queen

2 · 1

Drone brood removal

In the previous chapter on the biology of the *Varroa* mite, we learned that female *Varroa* mites prefer drone brood. The drone brood develops longer time in compare to worker brood, allowing more sexually matured female mites emerge. Also, since nurse bees spend much more time with feeding drone larvae than worker larvae, the mites have ample opportunities to come into contact with drone larvae. This is the reason why some beekeepers remove the drone cells every 24 days from April to June as a way to control the *Varroa* mite.

THE RULES

① Bees must build wax combs from scratch, so the queen can only lay a limited number of drone eggs per day. This restraint is very important for trapping to let the queen laying eggs progressively and trapping the mites continuously over an extended period of time.

② If there are also some capped worker cells, it is recommended to return the frames to the same colonies to avoid the spread of diseases by shifting the frames between different hives.

③ A full deep comb removed monthly will generally keep mite levels below threshold *(in April, May and June)*.

④ Two combs, alternately removed every other week, would likely be the best.

⑤ Drone combs must be removed in an interval of 24 days; otherwise, we will be breeding mites.

⑥ This method requires few time by preparing the trapping frames over the winter time. When the frames are marked on the top of the bars, it is easy to find them in the brood chamber. Removed drone brood combs are a good source of pure bee wax and if needed, it can be used for the production of comb honey in a later season.

Melting the drone combs, we can collect clean wax for the production of our own foundations.

It has been found that European honeybees having a good hygienic behaviour remove only infested worker pupae, not drone pupae *(1)*.

The complete removal of drone brood, if done early in the season, may reduce the population of mites up to 20 % – 25 % by each removal.

Theoretically, trapping mites with one deep drone frame once in 24 days during the period can keep the level of infestation below the critical infestation rate of 2.5 % *(2)* and *(3)*.
It should be noted that this method is applicable only for a period when a lot of brood is naturally present in the hive. It is also important to emphasize that bee colonies needs drones for the natural harmony and activity, and also for their reproductive function. By reducing the drone cells in the colony we are losing a significant part of the healthy and vital drones, potentially able to fertilize young queens.

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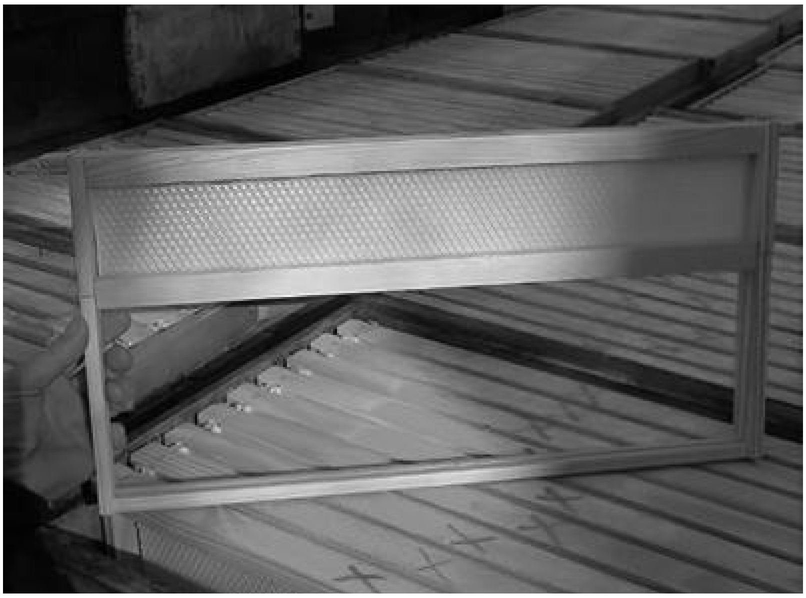
Calderone 2005.

PICTURE 01

Wired deep frame for building natural comb



PICTURE 02

Deep non-wired frame with approx. 5 cm of foundation

PICTURE 03

Shallow frame inserted into a deep brood chamber

2 · 2

Brood reduction with a duplex framebox

It is recommended to apply this method 24 days before the expected main honey harvest.

For example, in an area with many linden trees, in lowland regions, the best time to introduce the duplex framebox is the day of summer solstice, 21st of June, when the colony reaches its population peak. This method however can be used anytime from May till July depending on the expected main honey harvest.

APPLICATION

At day 0

A brood frame with fresh eggs and young larvae is taken from the hive and introduced into the duplex framebox, together with the queen. Additionally a frame with only a fresh wax foundation is given into the box. Then the lid is put in place and the duplex framebox is closed and placed in the middle of the brood chamber.

- **At day 9**

  The smell of the open breeding cells *(larvae)* in the duplex framebox is attracting the adult *Varroa* mites. Shortly before the breeding cells are capped, the adult *(female)* mite goes inside the breeding cell.

- **At day 10**

  After the capping of the cell, the mite starts reproducing.

At day + 12

after placing the duplex framebox in the hive, the brood frame inside is fully capped and the other wax foundation frame is fully built. It is now time to remove the fully capped brood frame, and to replace it by an empty, built frame inside the duplex framebox. The queen shall stay inside the framebox which is closed and put again back into the in the middle of the brood chamber.

- **During the next 12 days**

  the brood frames in the hive become empty as the new bees are born. Only the two brood frames in the duplex framebox *(where the queen did lay eggs)* will offer a chance for the adult mites to reproduce, and in this way we can "catch" them.

At day + 24

almost all adult female mites have been attracted by the open breeding cells and are now trapped inside the duplex framebox on the two remaining breeding frames.

- Now one can easily take these frames out and either do a heat treatment with the Varroa Controller or discard those two frames which contain over 90 % of all mites. The queen can be returned to the hive.

- This way one significantly reduces the *Varroa* mite population in the hive, and as there are no breeding cells anymore, one can also easily treat the mites attached to the adult bees with oxalic or lactic acid.

ADVANTAGES OF THE DUPLEX FRAMEBOX

Safe reduction of the mite population especially during the summer period.

90 % of the mites are trapped on to two frames.

Increased honey harvest.

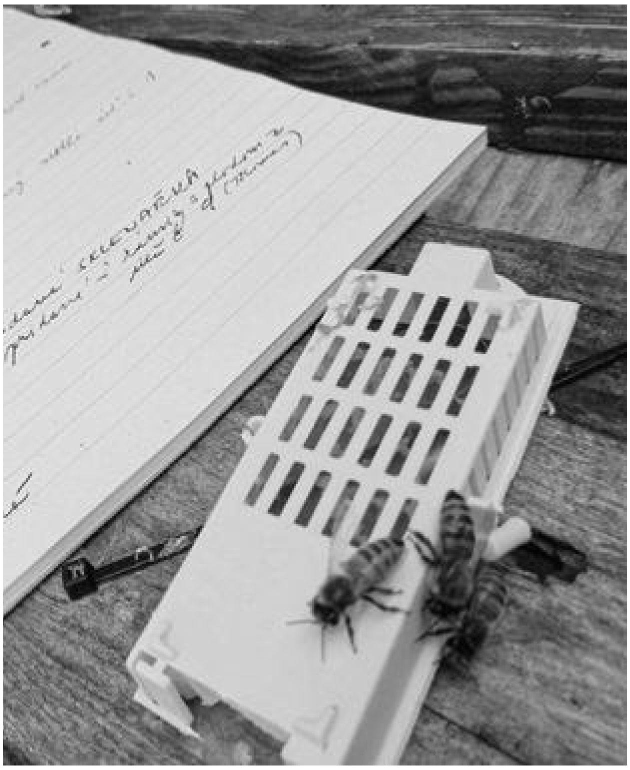
During the period of 24 days, the colony has a reduced breeding activity, leading to 20 % more honey being collected.

Easy removal of the mites attached to adult bees and, if required, easy queen exchange.

On day 24, there is no brood inside the hive anymore. The attached mites can be easily removed, and/or the queen can be exchanged without problems, because there is no open brood and bees will therefore accept the queen.

Increased hygiene of the brood nest.

The empty, old *(dark)* breeding frames outside the duplex framebox are taken out for melting and recycling of the wax, and are replaced by new, fresh frames.



VIDEO ABOUT THE DUPLEX FRAMEBOX

Scan the QR code with your smartphone or enter the address in your browser.

https://youtu.be/0DQynJa3rWg

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Wimmer, W. Praxishandbuch der thermischen Varroa-Bekämpfung. 2015.

2 · 3

Complete capped brood removal

After the removal of the brood together with the queen, the parent colony has time to breed a new queen. It is important to heat treat capped brood frames first before splitting the parent colony. By performing the heat treatment, we kill those mites that are hidden under capped brood cells – only after that, it is recommended to create a new and healthy colony. If we would leave the mites parasitizing the pupae inside the brood cells, the hatching imago will be affected by *Varroa* mites..

APPLICATION

The day 0

During the flying activity, when most of bees are foraging food, move the parent colony with all capped brood to another place *(minimum distance is 4 meters)*. The colony may have 5 to 6 capped brood frames.

At the original place of the parent colony install a new empty box with wax foundations and built frames and put the old queen here — for the sake of security lock the old queen into a queen cage with pasture. All the foraging bees will come back at the same place *(albeit it is now obviously a new hive, but still with the old queen)*, bees will soon release the queen, so she can soon form a new nest.

The day 9

On day 9, check the queen cells in the parent colony and remove all queen cells except one nicely developed cell.

On day 12, a new queen will be hatched in this hive.

The day 21

Three weeks after this artificial split, the parent colony has no capped brood anymore as all brood has now hatched. This is the certain time to extract two frames with open brood from the new colony and to put them into parent colony. Attracted by the smell, the newly introduced brood frames serve as a “trap” for the mites in the parent colony.

The day 28

After 9 days, all the brood is now capped. The beekeeper can now decide whether to treat the brood frames with the Varroa Controller or to melt down the frames in order to kill the *Varroa* under capped brood cells.

The day 31

It is possible to merge the colonies into one single colony. This is good option in particular for those beekeepers who are waiting for the late harvest as we have created a strong and Varroa-free colony with many foraging bees waiting for forest honey.

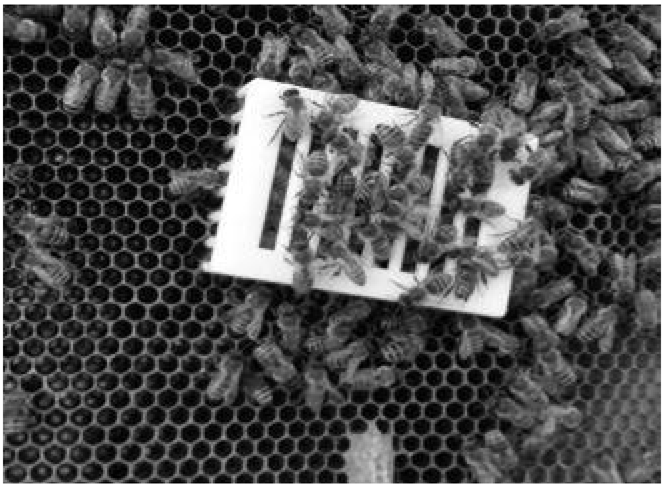
It is recommended to feed the parent colony with liquid food for a few days, since most of the foraging bees went back to the new colony with the old queen. The parent colony mainly has nursing bees taking care of capped brood.

2 · 4

Brood interruption by locking the queen

PIC 01

SCALVINI little cage



PIC 02

Other type of cage fastened to the comb

The method consists of finding the queen and locking her into a small cage.

It is inserted in the centre of a honeycomb, where a part has been cut out in order to fit the cage. The cage consists of a bottom with worker bee cells, where the queen can lay eggs during her isolation period of max. 24 days. The lid acts as a queen excluder, allowing bees to take care of the queen. It also has a cap to free the queen, without opening the cage. Although the queen lays eggs in the marked cells during her isolation, brood cannot develops as bees are not able to fully build the cells.

It is common that the queen lays over 50 eggs in one single cell as she needs to continue laying eggs. As it can be seen on the left picture above, bees started breeding a new queen cell from an egg that either they have moved from the cage or that has fallen into a cell accidentally.

During those 24 days, all brood in the brood chamber will be born and the mites attached to bees can be removed with a conventional method, for example by applying oxalic or lactic acid.

It is recommended to use this method only after the second half of July, when the swarming period is over. After being locked for 24 days, the queen is artificially forced to stop laying eggs, the colony is stressed and bees tend to replace the queen as they do not accept her in the brood nest. It often happens that the queen is killed and that bees make a new one.

This method is suitable to apply as prevention against autumn reinfestation after the main treatment in September with the device *Varroa* Controller that will be explained in the chapter about heat treatment of capped brood. It is a good solution especially for the beekeepers with beehives located in the regions where oilseeds are growing, allowing the queen to lay eggs until late autumn, often mid of November.

A good alternative to this method is locking the queen into a duplex framebox with one or two brood frames, so that the queen is not fully hindered from laying eggs. Especially new queens that are born in late June and July need to lay eggs. Using the duplex framebox, the risk of changing the queen by bees is then eliminated.

2 · 5

Creation of new colonies by partial capped brood removal

The method is easy with a duplex framebox, which accommodates two frames.

PROCESS

At day 0

a brood frame with larvae and the queen and a frame with a wax foundation *(A)* are isolated for the first period of time of 12 days.

At day + 12

the frame with the young larvae will be capped and will be heat treated with the Varroa Controller. The queen will continue laying eggs on the built wax foundation *(A)* and she will receive a second wax foundation *(B)*. The queen stays isolated in the same way for the second period of 12 days.

At day + 24

the queen will receive a third foundation *(C)*. She can now lay eggs on the second foundation *(B)* which was given 12 days before and has been built by that time, so she continues to stay isolated for more 12 days. Capped brood frame *(A)* will be removed from the hive and heat treated in the Varroa Controller.

At day + 36

the frames *(B)* and *(C)* have capped brood and can be removed from the hive. Both frames need to be heat treated in the Varroa Controller. The queen will be released back to the brood chamber and the duplex framebox will be removed from the hive.

WHAT DO YOU THINK...

Where are the Varroa mites during the period of caging the queen?

Managed, or also called parent colonies, will only have brood in the duplex framebox for a period of 36 days, meaning that all *Varroa* mites will be concentrated and trapped into a small brood nest that exists only in the duplex framebox from the second period of 12 days. By heat treating the extracted brood frames from the duplex framebox, the frames will be *Varroa*-free and can be used for formation of new colonies. The best period to apply this method is early spring, when there is an abundant quantity and greater quality of nectar and pollen *(end of April, May and June)*. Typically, this happens during the colza, acacia or linden blooming period. This method also can be considered as an anti-swarming practice since the queen cannot fly away, but she stays isolated, leaving her enough space for laying eggs. To combat the mites by means of this method, it is mandatory to respect the timing. The method is recommended for disciplined and experienced beekeepers.

What do we do with the frames after heat treatment?

The heat treatment eliminates the mites. Heat treated frames go back to their original hives or are used for the creation of new colonies. The most effective way is to synchronize this work with the formation of new, *Varroa*-free colonies. Each colony will receive 1 kilogram of bees and a young queen. These new nucleus need to be placed at least 3 km away from the original apiary. It is important to keep them warm to prevent diseases *(this can be done by creating a compact nest, keeping them tight with a wooden partition)*. They need to be continuously fed to be able to build new foundations and grow into strong and healthy mite-free colonies.

2 · 5

Seasonal applications of different biotechnical methods in spring, summer and autumn

The Varroa mite is present in all colonies and cannot be eredicated completely and definitively.

Regular monitoring and qualified estimation of the *Varroa* mite population are a pre-condition for the correct timing and selection of a suitable and effective method for mite control throughout the beekeeping season, and subsequently to evaluate the effect of the intervention in the colony.

The efficacy of the individual methods may vary, particularly by season, colony strength, intensity of breeding and the initial colony infestation by mites and other factors. None of the following methods below allow a complete removal of *Varroa* mites, but can contribute to a significant reduction in number of mites to such extent that chemical treatments can be totally avoided.

Scan the QR code with your smartphone or enter the address in your browser.

https://youtu.be/0DQynJa3rWg

LEGEND

Green color

Drone brood removal is partially effective and allows the beekeeper to keep the number of mites below the critical threshold, but only if this measure is repeated 3–5 times successively during the period when drone are present in the colony *(1)*. A study conducted in the French region of Alsace in 2010 found that after 4 repeated drone brood removal, the Varroa mite infestation in the monitored colony was reduced by approximately 25 % compated to the control group *(2)*.

Blue color

Introducing the duplex framebox to the hive in a period of 2 x 12 days, we can reduce the brood by offering only two frames to the queen where she can lay eggs on. If capped brood frames are not heat treated, the collected capped brood from the duplex framebox must be frozen/melted to obtain a clean pure beewax.

Purple color

The creation of new colonies from NON–TREATED capped brood frames can reduce the *Varroa* pressure in the original, parent colony by 25 % to 35 %, but the development of the new colony will still be damaged by mites — without the heat treatment of capped brood, there is only a "dilution of the mites" between the parent and the new colony. We cannot

TABLE

Calendar of main biotechnical methods to control Varroa mites

TYP OF A MEASURE	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
DRONE BROOD REMOVAL				●	●	●						
BROOD REDUCTION WITH A DUPLEX FRAMEBOX					●	●	●					
CREATION OF NEW COLONIES BY PARTIAL CAPPED BROOD REMOVAL					●	●	●					
COMPLETE CAPPED BROOD REMOVAL				●	●	●						
BROOD INTERRUPTION BY LOCKING THE QUEEN							●			●		

estimate the percentage of mite reduction in the parent colony by randomly removing a part of capped brood frames. Experiments have shown that this percentage is somewhere between 17 % and 78 %. Therefore, if we only remove and do not treat capped brood frames, we transfer the mites into the new colony, impeding a healthy development of the young colony as the mites will parasitize the brood *(4)*.

Yellow color

The complete removal of capped brood significantly reduces the mite pressure in the parent colony by 70 % to 80 % if the measure is taken not later than at the beginning of July. After the summer solstice, the colony will begin to produce less brood and the mite pressure will increase significantly *(multiple female mites may attack a single larva multiple times — it happens from early August to late October).* To avoid this, the brood frames must be heat treated and bees must be treated with oxalic acid or lactic acid to remove the attached mites.

Pink color

In the lowlands, brood rearing continues until November. Isolate the queen into a small cage at the beginning of October for 21 days to completely prevent the colony from breeding.

CRITICAL MONTHS

July and October — It is necessary to completely treat the colonies — eliminating mites capped brood cells as well as those attached on bees — reaching almost zero mites in the treated colonies. Therefore, these months are marked with red color.

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3

Heat Treatment of brood

This explains the basic idea behind the heat treatment respectively hyperthermia of bee brood. In the early 1990, Prof. Engels at the University of Thübigen in Germany, found out that apparently the bee pupae could sustain more temperature than the Varroa mite. This difference in the resistance to temperature has been used to develop an hyperthermia treatment against the Varroa mite.

Since both animals the bee pupae and the fertile *Varroa* mite are at one time in their development together in the cell of the breeding frame, capped with a wax lid this is the moment to expose them to a higher temperature. The idea is to kill the mite and not harm the bee pupae.

There is only a fine line between what temperature the mite cannot sustain any more and the bee pupae is still resisting. Prof. Engels found out that the best time window to do this heat treatment is from day 14 to day 18 of the development of the bee *(pupae)*. In practice one has different pupae developments in one breeding frame — especially in spring. So as long as a frame has enough cells capped with a wax lids they are good for heat treatment. If there is only a small part of the surface capped one can assume that there are not yet enough *Varroa* mites in the cells and therefor it is not worth to treat those frames.

3 • 1

Working principle of hyperthermia

This explains what happens during the heat treatment.

With hyperthermia the body of the animal *(Varroa mite)* is heated up to a temperature at which so called heat shock proteins are formed and cell damages result. The cell damages then cause the death of the mite in round about 20 hours. This is a principle is not restricted to mites only. This method of hyperthermia is also applied with human bodies. Except that there only certain body regions are heated up to result in cell damages precisely there. Clearly the cells in the human body one wants to damage are cancer cells. In this medical field Japanese oncologists are very experienced and this technique has a long tradition there.

When we heat up capped brood cells of the bee hive we also heat up the bee pupa that is located together with the female mite in capped brood cell. One could argue that the pupa is also damaged. This indeed would be the case if the temperature is too high or the exposure time at a certain temperature is too long. A successful heat treatment also means that once the brood frames are given back to the hive bees have to be strong enough to regulate the temperature of the warm brood frame back to normal temperature as soon as possible. This has to be especially considerer when giving back brood frames to weak hives with the idea of strengthening them.

Consequently we can understand hyperthermia as a process of mastering several parameters in order to achieve the intended result of damaged and finally the death of the mite, but not damaging the developing bee located in the same capped brood cell.

3 • 2

Technical requirements for a successful heat treatment

What is important, to heat treatment was it successful?

Certainly not only the temperatures the bee pupae and the mites are exposed to are relevant for a successful treatment, but also the time they experience the temperature. Additionally during the entire heat treatment it has to be ensured that the hive humidity is given all the time. It has to be avoided that the bee brood dries out during the heat treatment. This is especially relevant if there are open brood cells and clapped brood cells together on a brood frame, which is a typical situation in spring. Another critical factor for a successful heat treatment is how fast the bee pupae are warmed up. Here it has to be assured that the process of heating up runs slowly.

One important issue that has been also found out by Prof. Engels already in 1990 is that heating up the entire hive will not work. Bees will work against any heating. Such kinds of treatments are not successful.

For a successful heat treatment capped breeding frames need to be extracted and bees need to be swept off and then put into the hyperthermia device for the time of the treatment.

TECHNICAL REQUIREMENTS

A computer to run carefully the warm up procedure and controls the treatment time.

Precise temperature sensors to measure exactly.

A humidification unit to ensure enough humidity during the entire treatment.

A design that allows to heat up all frames at the same time.

3 • 3

General requirements for a successful heat treatment

It is explained here under which outside conditions a heat treatment is successful.

GENERAL CONDITIONS

The outside temperature should be more than 18 °C and less than 30 °C.

Avoiding heat treatment during the intensive honey flow, always do treatment before the main honey flow. In spring before the fruit trees are blooming.

Make sure the brood frames have enough capped cells. In spring there are open and capped cells on one frame. It is advised to heat-treat only those frames where capped brood cells cover 50 % or more of the total surface of the frame.

The weight of the treated brood frames should be more or less the same. No major difference in weight is advisable.

Freshly build up foundations with a lot of brood can cause troubles during the treatment as they may get split between brood and upper honey reserve on a single frame. It can happen in the situation when there is some honey reserve around the brood cupped cells. Especially in August.

All adult bees shall be brushed off the frames before the heat treatment — one should check that the queen is not harmed during that process.

Especially for the spring treatment it is enough to heat treat per hive two/three frames with capped brood.

3 • 4

Application of a heat treatment with the Varroa Controller — step by step

Scan the QR code with your smartphone or enter the address in your browser.

https://youtu.be/QkeXGM_wRm4

Here it is explained how the application of the Varroa Controller (VC) works.

2 hours

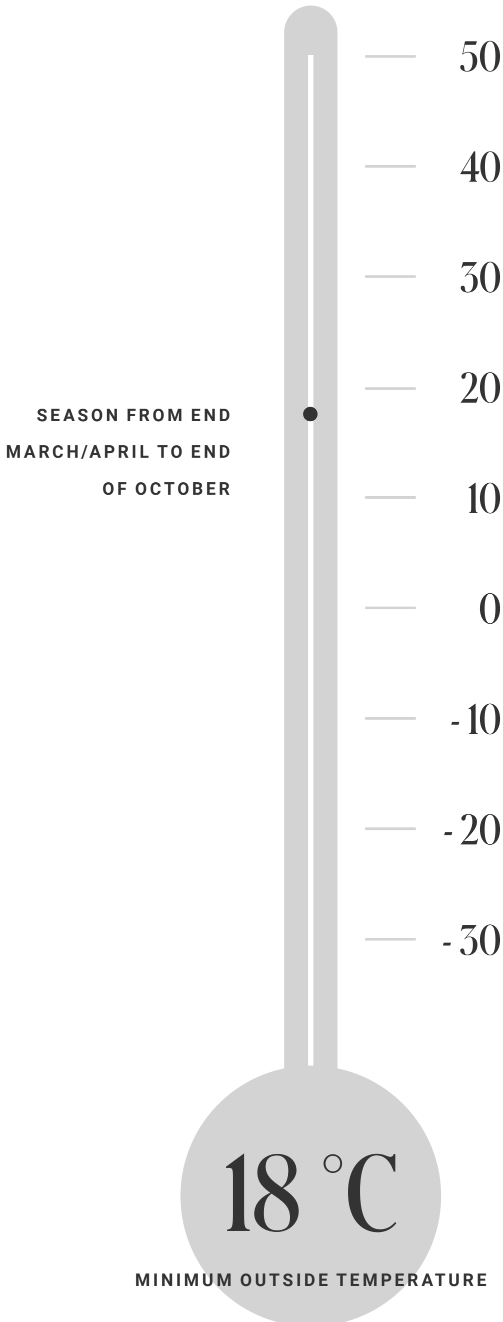
DURATION OF TREATMENT

PROCESS

① Preheat VC. ② Fill water in and switch VC on – await signal that shows the VC is ready to place brood frames. ③ Get capped brood frames and place them into VC. ④ Choose only those frames with a more or less developed capped brood surface *(50 %)*, brush off bees and place them in the VC. For each frame open and close the lid – don't park them somewhere and risk that they cool out. Mark the frames to later put them back in same hive. ⑤ Continue hive by hive until VC is full. ⑥ Avoid any cooling out of the brood frames before putting them into the VC. ⑦ Place sensor correctly and run the treatment. ⑧ Check correct setting of frames and sensor before closing the VC and starting the treatment. The treatment runs automatically for the treatment period, which is 2 hours for regular frame sizes and 2:20 for large frames. ⑨ Put frames back preferably the frames are given back into the hive they are coming from.

3 • 5

Seasonal applications of hyperthermia



Time windows are explained suitable for a heat treatment.

In general the heat treatment can be applied any time as soon and as long as there are capped breeding cells in the hive and the outside temperature is 18 °C or more. This is means from March/April to End of October. It is advisable to do a preventive treatment in spring since the reproduction of the mite is severely reduced.

Another time slot in the beekeeping year is when new colonies are made, by taking brood from several hives and they are brought together in a new colony. Here it is most advisable to do the heat treatment of capped brood frames to give the new colony a good start.

During the bee year one may discover in hive — usually those standing at the left or right end of a row of hives that the mite level increases significantly. This can be easily detected by consequently follow the diagnostic results *(e.g. screening board)*. Way before such hive can intestate other hives on the stand they can be heat-treated, without endangering the honey harvest from these hives.

The next time window is the summer treatment after reducing the brood frames with the Duplex-Framebox. This treatment is highly effective since the mites are concentrated in those frames coming from the Duplex-Frameboxes.

The autumn treatment finally is most relevant for overwintering of the hives. In autumn the most significant danger is a potential re-infestation from other hives. Here the heat treatment is a valuable help to get rid of newly introduced mites.

3 • 5 • 1

Preparing for spring treatment

It is explained how to achieve a compact brood nest for effective spring treatment.

After winter once bees start breeding again it is very important to provide them conditions that they can keep the temperature they need for the brood—even when the weather is still changing from cold to warm and back to cold. The application of insulating separators is very useful. Bees can keep the temperature much better within the brood nest and they have a much more compact brood nest then without these separators.

There are 5–6 frames within the separators and more frames full of food is located outside. The queen is not changing to out to the colder area. Bees can freely move back and forward.

With the insulating separators a compact brood nest on less frames is achieved. That helps a lot when later in the spring the heat treatment needs to be done since there are less brood frames. It is important to understand that there are less frames but not less brood cells. Simply the brood cells are distributed among less frames. This has many advantages.

INSULATING PARTITION

3 • 5 • 2

Spring treatment

It is explained what are the effects of a spring treatment. Since the mite is reproducing in a way that the mite population is doubling every month the spring treatment is most effective. One mite killed in April means 32 mites not available in September!

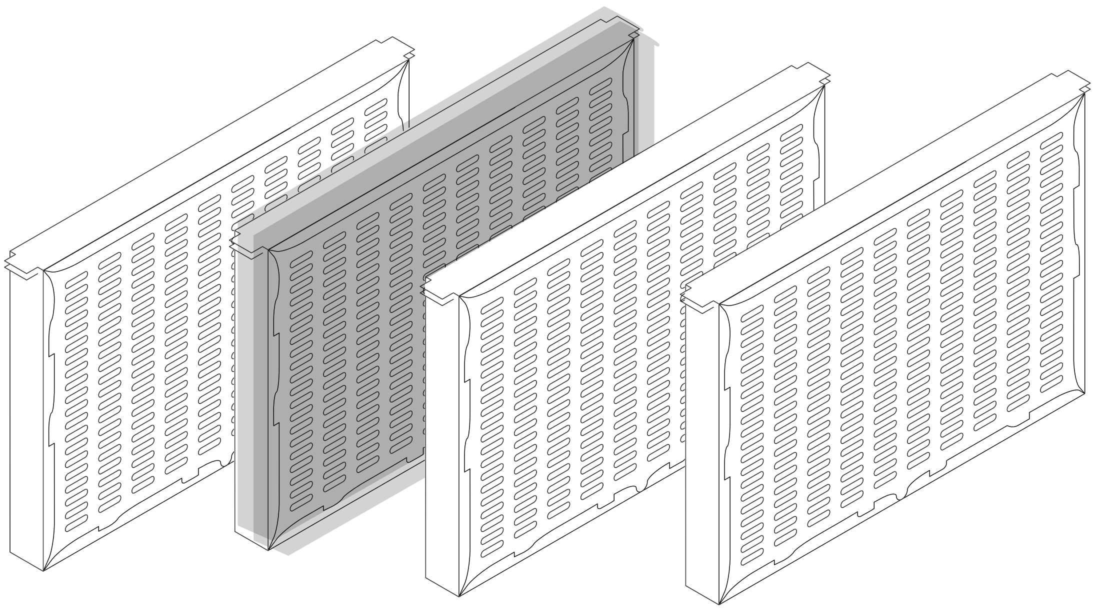
Mid of March to mid of April the spring treatment should be done. At that time, depending on the location and weather the hive has two to three frames of capped breeding cells. These frames contain the mother mites ready for reproduction. Within the spring treatment theses mother mites are killed and cannot reproduce that fast. Since the spring weather might be still with cold temperatures or wind one has to be very careful not to cool down the brood frames too much when taking then out of the hives. Actually the brood frames should be taken from the warm hive directly to the warm *Varroa* Controller so that they are not losing the temperature.

Usually the heat treatment can be nicely combined with other work at the hives such as cleaning the bottoms, checking for food, etc. That way the treatment time can be used for these works.

After the treatment is finished and the frames can be given back to the hives one can decide to equalize the brood among the different hives. The weaker hives, with less brood frames are given back maybe one frame more. The stronger ones are given back one frame less than it has been extracted. This is a nice side effect that helps to have more or less equally strong hives who need more or less the same attention during the rest of the bee season.

3 • 5 • 3

Preparing for summer treatment



It is explained how to reduce brood for the summer treatment with the Duplex-Framebox.

The summer treatment has to be prepared in a way that there are only two frames of brood per hive to treat. This can be done with the Duplex-Framebox.

The Duplex-Framebox is introduced into the hive in the third week of June and locks the nquuen on two frames only. One frame with fresh eggs and small larvae and one foundation are given into the Duplex-Framebox. After 12 days the one frame has capped breeding cells and is taken out for heat treatment. This frame is replaced with an already built, but empty frame. This way the queen has two frames to lay eggs, since the foundation has been also built and can be used for putting eggs. After another 12 days all the brood outside the Duplex-Framebox is empty — all bees are born and the *Varroa* mite can only go to the remaining two frames in the Duplex-Framebox, which serve as a trap. Now on day 24 *(2 x 12)* these two frames are heat treated and are later used as bee material for creating new colonies.

During the 24 days the queen is inside the Duplex-Framebox she can lay only a reduced amount to eggs and bees need to take care of less brood. This means they can go out and look for nectar. Usually this results in a higher honey yield of about 20 %.

At the same time the brood frames that have been in use for a long time *(dark brown)* are empty on day 24 after introducing the Duplex-Framebox. This is now the time to extract those frames and replace them with new foundations.

+ 20 %

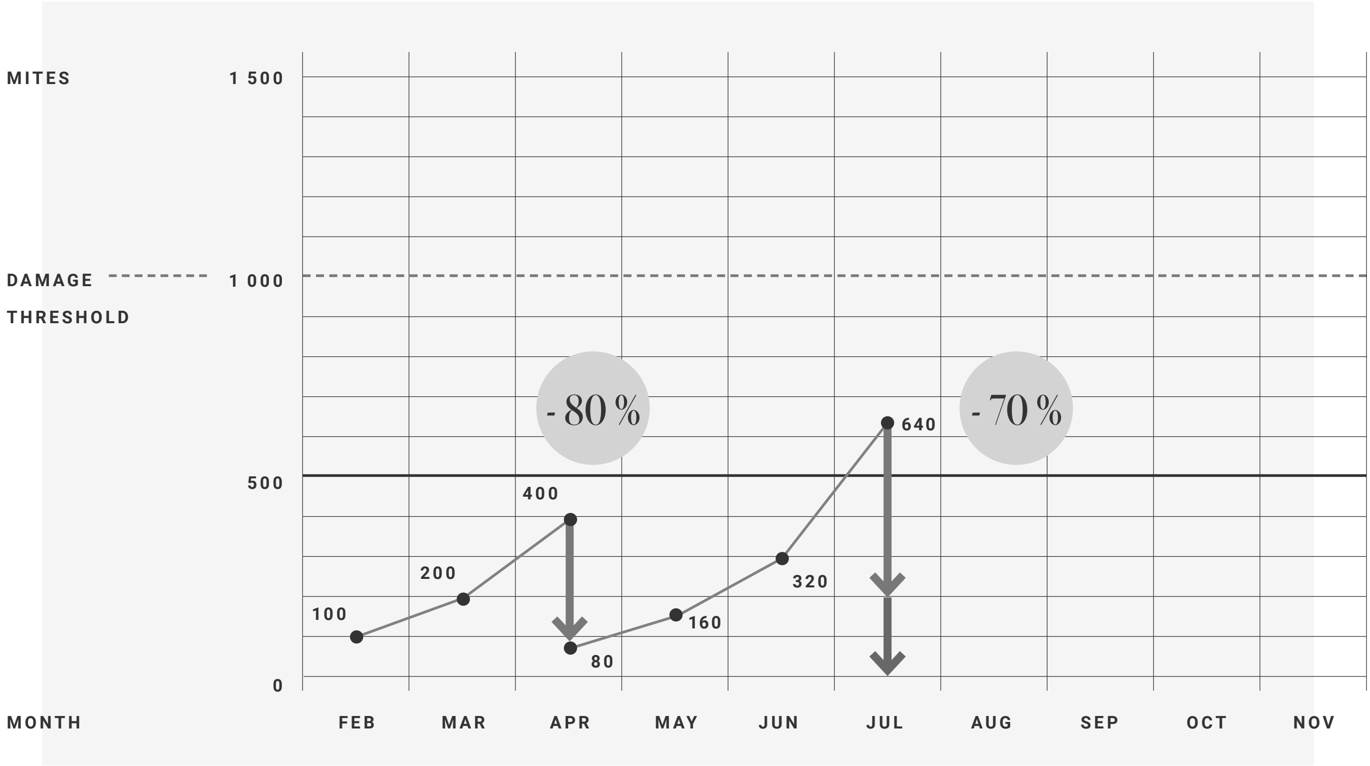
A HIGHER HONEY YIELD

3·5·4

Summer treatment

CHART

July treatment



It is explained how to reduce the mites to almost zero.

Mid of July when the Duplex-Framebox is taken out of the hives on day 24 there are two frames full of brood available to heat treat. Depending on the frame dimensions we are talking about 10 – 15.000 unborn bees per hive. Those frames are heat treated and then serve as material for new colonies. 6 of those frames + 1 kg of bees + 1 queen build a very strong new hive for the next season.

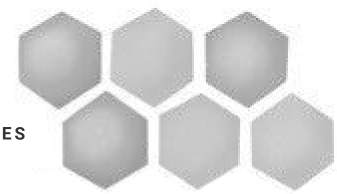
At the same time the now brood free colony is treated against phoretic mites. With this approach the mite population is reduced to almost zero at that point in time. This serves as a basis for a new start into the autumn with many more months ahead with breeding and consequently mite reproduction.

Ideally the application of the Duplex-Framebox and the summer treatment are planned in a way that the same day also the honey frames are extracted.

VERY STRONG NEW HIVE

6

HEAT TREATED FRAMES



+

1 kg

ADULT BEES

+

1

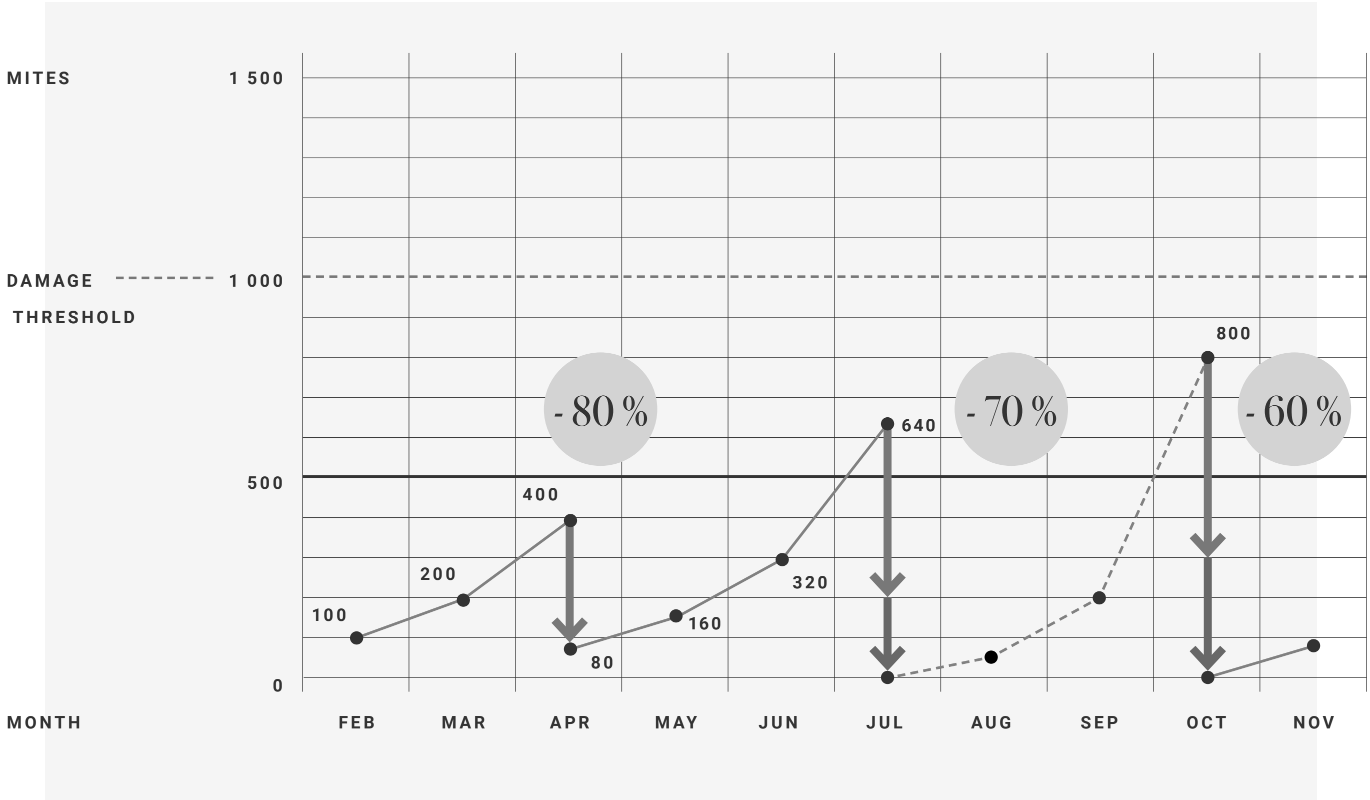
QUEEN

3 • 5 • 5

Autumn treatment

CHART

Year-long plan of hyperthermia treatment



It is explained how to combat infestation.

Although the mite population has been reduced to almost zero in mid of July it may increase again dramatically within a very short period of time. Why is that so if the reproduction rate would indicate a doubling per month and doubling a very small number still is a small number?

Well there is the effect of re-infestation. Bees are escaping from hives that have very high mite populations and come in to our healthy hives. Each of those bees can bring easily one or two mites into the hive causing a dramatic problem within several weeks only.

So the plan here is to do end of September/mid of October another, final heat treatment to kill the mites in the brood but at the same time the phorectic mites have to be taken away as well. This combined approach is a necessary measure for all of hive locations where re-infestation takes place.

If you are lucky and there are no other hives in your neighborhood serving as a source of re-infestation you are lucky and no autumn treatment is needed.

Be aware that swarms which escaped in May have to be considered as a potential source of re-infestation as well. So it is definitely needed to precisely monitor the mite population in the hives — minimum once a week and in case there is sign for re-infestation one needs to perform heat treatment and phoretic mite treatment immediately.

3 • 5 • 6

Successful annual treatment plan with hyperthermia

An overview is given how to apply heat treatment in a beehive throughout the year.

The objective of the annual treatment plan is to avoid that the *Varroa* mite can cause a damage to the hive. This is a complex task since there are many external influencing factors to that aim. Strong and healthy bees can certainly resist more mites than weak bees suffering already from e.g. bee viruses, which are transmitted again from the mites.

A pragmatic approach could be to try to manage the *Varroa* population in a way that it stays below 1000 mites all the time. With this relatively low threshold the mite is not able to endanger a healthy strong colony. One tools to keeps the threshold low is definitely the heat treatment since it can be applied any time in the year, if needed even during the honey period since there are no chemical involved.

Nevertheless it is advised that the first treatment is done in spring, the second at the time of last the honey harvest in mid of June and a final treatment shall be done end of September/ mid of October. With the mentioned three treatments the mite has no chance to harm the hive and colonies stay healthy.

4 • A • TREATMENT OF VARROA MITES ATTACHED ON ADULT BEES

Extra powdered sugar dusting

1ST STEP - POWDERING THROUGH THE MESH

2ND STEP - IT IS IMPORTANT TO GET THE SUGAR IN BETWEEN THE FRAMES

Sugar dusting is known more as a Varroa diagnostic method rather than as a treatment. It is considered as a complementary to other treatment methods.

Although sugar dusting can indeed cause a substantial proportion of the phoretic mites to drop off bees, in order to effectively manage *Varroa*, dusting requires more effort and weekly repetition. Based on the methodical experiments of Randy Oliver sugar dusting clearly causes a rapid drop of mites — most mites fall in the first hour, then the rate tapers off until it returns to baseline in about 24 hours. The method is much more efficient if applied in a single brood chamber than in doubles. During the brood rearing season, weekly sugar dusting would not be expected to substantially drop the mite population — at best it would hold it steady. However, at times when there is no brood present or in combination with heat treatment of capped brood frames, weekly dusting would be expected to accelerate the decline of the mite population.

It is a good way to quickly determine the level of mite infestation of a colony as it monitors virtually all bees in a colony, as opposed to just a sample of 300 bees. Removal of phoretic mites from the adult bees by using extra dry powdered sugar *(humidity can be avoided by adding about 20 % of rice flour and mix it with powdered sugar)* applied on the entire colony works well, but it is not "powerful" enough to remove enough mites. It has to be combined with the heat treatment of capped cells, in order to keep mites from eventually causing damage to the colonies. It is hard to image to apply this method for large scale apiaries due to frequency of its application, but for a small size apiaries, it is a method which can be well integrated into the whole concept of the annual plan.

HOW TO APPLY POWDERED SUGAR DUSTING

Scan the QR code with your smartphone or enter the address in your browser.

https://youtu.be/2lePAF54Zhk

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4 • B • TREATMENT OF VARROA MITES ATTACHED ON ADULT BEES

Oxalic acid $C_2H_2O_4$

Although organic acids are permitted for use in the treatment of bees against *Varroa* mites, their negative effects on the honeybee colony may be similar to that of synthetic drugs.

Oxalic acid is absorbed through the skin, therefore the necessary precautions should be taken when handling it. The use of protective equipment is strongly recommended.

Oxalic acid remains in the colony for a relatively long time after its application and it has subliminal effects on honeybee colonies.

A variety of chemicals are used in treatments against *Varroa* mites, including organic acids and some fragrances such as thymol. The ultimate objective is to remove the mites attached on adult honeybees. In this section, we point out only a few negative effects that application of oxalic acid may have on honeybee colony.

The use of oxalic acid is used for more than 20 years. It is most commonly applied in control of *Varroa* mites between November and January, when there is no brood in the colony. Since oxalic acid is slowly transformed, it remains in the colony for a relatively long time, sometimes until spring. Therefore, several researches focus on both the lethal and sublethal effects of oxalic acid on honeybee colony, particularly on honeybees' longevity, their ability to learn, or the pH of their digestive system and hemolymph. Even with a low concentration *(30–50 ml of 3.5% solution, corresponding to 175 micrograms/bee)* of oxalic acid, and despite of an increase in hygienic behaviour of bees, it has been observed that there is a reduction in worker activity, a decrease in brood rearing activity and a decline in longevity.

Schneider, Eisenhardt et al. *(2012)*, Papezikova *(2016)*, Rademacher, et al. *(2017)*, Gregorc, A. et al. *(2018)*, Tihelka *(2018)*, Diaz, del-Val et al. *(2019)* found that after treatment with oxalic acid there is a significant shortening of bee lifespan. Oxalic acid can reach the internal organs, alter the structure of microorganisms in the bee digestive tract, and affect the nervous system and consequently affect honeybees´ learning ability.

When summarizing the known facts about the effects of oxalic acid on honeybees, then the effect of the substances used in combating the *Varroa* mites can lead to a significant colony damage if there is even a low mite infestation. The application of oxalic acid may have long-term adverse effects on bee health as well as on beekeepers *(namely, a formation of oxalate kidney stones and other diseases)*.

Other aspect to consider is the methods of application of oxalic acid. Most often it is the sublimation or dripping of bees with a solution of oxalic acid in sugar water, glycerine or other substances. Safety instructions should be followed in all applications because oxalic acid is a substance that dissolves in water and can pass through the skin of the body. According to the information that can be found in all chemical safety data sheets, the maximum permissible concentration in the working environment is 5 mg/m^3. As a result, **always wear protective rubber gloves, a half mask with a filter, safety goggles and disposable work clothes**.

An increasing number of beekeepers are looking for chemical-free methods to control *Varroa* mites. A very efficient and safe method, such as hyperthermic *(thermal)* treatment of capped brood and the breeding of *Varroa* tolerant queen bees and colonies, can be already applied these days. It has been observed that more and more beekeepers around the world are becoming familiar with these new chemical-free methods, which is a pre-requisite for sustainability in beekeeping.

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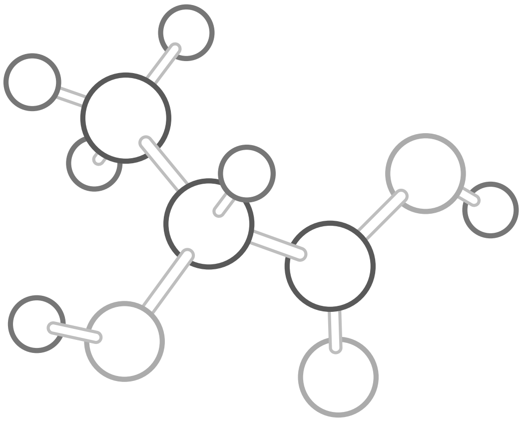
4 • C • TREATMENT OF VARROA MITES ATTACHED ON ADULT BEES

Lactic acid $C_3H_6O_3$



In this section we explain the method of applying 15 % lactic acid against Varroa mites attached on the adult bees.

Mixing of 1 part of 80 % lactic acid and 4,33 parts distilled water will give us 5,33 parts of 15 % lactic acid. This concentration will be applied directly on the adult bees against the mites parasitizing on bees.

Example
So maybe you use 100 ml 80 % lactic acid and add 433 ml of distilled water to start with. Please be careful with the 80 % lactic acid — in any case always wear protection glasses and gloves. Pour the acid slowly into the water while stirring.
(NO ACID INTO WATER!)

You only mix as much as you need — not more since a 15 % lactic acid is not very stable. Lactic acid diluted is not a stable substance. Then once you have the 15 % lactic acid put it in a sprayer that can be adjusted. Make sure you set it to a very fine spray.

Then open the hive, get the first frame and spray bees left and right gently — only that they get a light mist do not water them heavily.

Then spray the next frame and so on. Once done you close the hive and clean the Varroa-tray. Within 2 days you will see a result. Do all the hives also the ones with the hand full of breeding cells. After 12 days you repeat the exercise since then bees in the closed breeding cells are for sure born and you catch the remaining mites.

Don't wait and do it. It takes a few minutes per hive. The advantage of the lactic acid in 15 % is that it is very mild and no problem to bees. On the contrary the oxalic acid is a contact poison that is also poisoning bees and you can only apply in one max. two times. With the lactic acid 15 % you can repeat the treatment without any problems to bees.

APPLICATION OF LACTIC ACID

Scan the QR code with your smartphone or enter the address in your browser.

https://youtu.be/4aJ1z4hWFDg

Questions

1. On which day does the *Varroa* female mite go into the drone cell and on which day into the worker bee cell?
2. How does the female *Varroa* mite know when it is the right time to be capped together with the larva?
3. How can the mites survive the winter?
4. Why do Varroa mites "prefer" the reproduction phase?
5. Where does a mite reproduce?
6. How many sexually mature female mites develop in the worker's brood cell?
7. In which period of the beekeeper's year does the mite population reach its highest level?
8. What are the critical periods to treat the bee colony against the *Varroa* mites?
9. What happens when the mite feeds from the fat body tissue of the larva?
10. What are the main reasons why the female *Varroa* mite prefers the drone brood?
11. What does it mean for the practical beekeeping that the female mite attacks the drone brood more often than the worker bee brood?
12. How do you recognize the female *Varroa*?
13. What are the best tactics for mites to succeed?
14. What are two main ways of how the *Varroa* mite population develops in a colony?
15. What is the major limiting factor for the development of mites in a colony?
16. How long a Varroa female stays attached to a nursing bee when a colony still has some brood?
17. Is it necessary to treat the colonies already in spring, when there is a relatively low population of *Varroa* mites?
18. What are the consequences of *Varroa* mites that parasitize brood?
19. How can beekeepers control the bee viruses in the colonies?
20. Which viruses are the most damaging to bees?
21. What are the consequences of the high virus titre in a bee colony?
22. Suppose the average natural daily mite fall is 9 mites per day in July. Is it necessary to intervene against the mite immediately, or we can postpone the treatment for 5–6 weeks later?
23. How large is the total mite population in the colony if the average natural daily mite fall is 6 in July?
24. How large is the total mite population in the colony, if in August, the average natural daily mite fall is 15 and the colony still has a few capped brood frames?
25. Imagine a mite infestation level reaches already 2 % in March. Is this level considered to be dangerous at this period of time?
26. Is the mite infestation rate of 5 % dangerous for the colony during the population growth phase?
27. Imagine that the mite infestation rate is 2 % in March. How many mites are found in a sample of 300 bees?
28. Which factors influence the most the population of mites in autumn?
29. What are the negative aspects of the regular drone brood removal?
30. How can you create a hive free from brood during the bee population growth?
31. When it is the most effective to reduce the number of the brood frames?
32. What will happen to the Varroa mites at day +24?
33. What is the effectiveness of a complete capped brood removal?
34. Why is it risky to block the queen completely from laying eggs during the swarming period?
35. What needs to be done with capped brood frames before forming a new colony?
36. What is the heat treatment method based on?
37. What is the goal of heat treatment?
38. Which frames are suitable for heat treatment?
39. What is the main condition of successful heat treatment?
40. What are the technical requirements of the heat treatment device to effectively kill the Varroa mites?
41. What are the main conditions for a successful heat treatment?
42. What is the aim of spring heat treatment of capped brood frames?
43. List the main periods when it is advisable to heat treat capped brood frames and why?

44. What is the function of the isolated partitions in the brood chamber in spring period?
45. Why it is important to minimize the opening of the brood nest and rearranging the brood frames in spring or in autumn period?
46. What is important to consider when doing spring heat treatment?
47. What the duplex framebox is good for?
48. What are the key advantages of using a duplex framebox?
49. What happen if the brood frames in the duplex framebox is left in the brood nest longer than 24 days?
50. For what reason it is recommended to do autumn heat treatment of capped brood frames?
51. When it is not necessary to heat treat capped brood frames in autumn?
52. What is the best time to do treatment?
53. What are the advantages and disadvantages of the powdered sugar diagnostic method?

Co-funded by the Erasmus+ Programme of the European Union

3

FACTORS THAT CONTRIBUTE TO BEE HEALTH

BEEKEEPING IS A MULTIDISCIPLINARY FIELD THAT COMBINES MANY FACTORS AND KNOWLEDGE, WHICH INFLUENCE EACH OTHER. A PRESUMABLE WEAK FACTOR MIGHT BECOME DOMINANT AND DETERMINE THE SUCCESS OF BEEKEEPING. NOT ONLY GENETICS OF QUEEN BEE, BUT ALSO THE WAY OF THINKING OF THE BEEKEEPER AS WELL AS HIS OR HER ABILITY TO RESPOND TO CHANGES AND NEW KNOWLEDGE.

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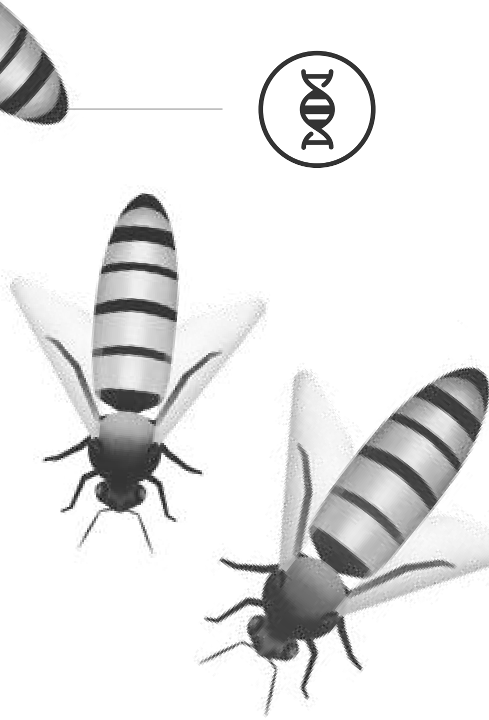


1 • QUEEN BREEDING AND GENETICS

1 • 1

Importance of queen breeding adapted to the local environment

In this section we explain the importance of locally adapted queens for sustainability in beekeeping.

In recent years, significant progress in breeding, especially to increase honey yields, has also brought important consequences:

① A SUBSTANTIAL REDUCTION IN GENETIC DIVERSITY,

② DECLINING VITALITY OF BEES,

③ AN INCREASE OF COLONIES LOSSES DURING WINTER .

Europe is home of at least 10 subspecies of honey bees, each of which shows a wide range of locally adapted populations and ecotypes.
It is recommended to breed the ecotypes best adapted to the given altitude and landscape type. It is not recommended to import queens from more distant regions of Europe belonging to other subspecies. Imported queens from remote regions and non-native subspecies have a problem with adaptation, leading to increased mortality and unsustainability of this hybridization, either in the short or long term.
In the central part of Europe *(including Austria, the Czech Republic and Slovakia)*, the most widespread subspecies is the Carnolian honey bee.

The beekeepers can use bee populations which are best adapted to the local environmental conditions, and can make selections to improve beekeeping characteristics within the locally adapted lines. Such appropriate selection supports the conservation of the genetic diversity. To achieve this goal, the method of appropriate selection of colonies can be used to prevent the extinction of the endangered subspecies and ecotypes of bee colonies in Europe, as identified in the framework of the project "Smartbees" *(www.smartbees.fp7.eu)*.

1 • 2

Selection of queens for their resistance to diseases

In this section, we highlight the importance of giving attention to the rearing of local queens with increased hygienic behavior.

At present, almost all bee colonies in Europe are kept by bee-keepers, and their breeding methods include, among other things, regular chemical treatments against mites (or other diseases). This contributes to a larger number of colonies which are not able to survive without beekeepers´ intervention. One of the major solutions is the preference of colonies with increased hygiene behaviour.

Most queen breeding programs look to promote characteristics such as honey productions and characteristics such as honey production and colony strength have priority, alongside with other desirable characteristics for commercially managed colony breeds *(e.g., docile and with low swarming behavior)*. On the other hand, disease resistance, viability and adaptation to the local conditions were considered to be less important, as the deficiencies in these features could be compensated by treatments, artificial feeding or other methods.

SELECTION

A pre-selection from a larger population of bee colonies.

Testing the viability of selected breeding colonies.

A selection of drones.

A practical solution for ordinary beekeepers is to keep bee colonies with increased hygienic behaviour. Hygienic behaviour means that the managed bees have the ability to recognize capped brood cells with a dead or infected brood, and subsequently remove such damaged brood. The most common procedure for testing hygienic behaviour of a bee colony consists of killing *(by piercing or freezing)* a number of pupae, usually 50 to 100 per colony. Subsequently, the removal rate of the dead pupae by the bees is monitored over a period of time *(12 hours and 24 hours)*, and the higher the removal rate of the dead brood, the stronger the hygienic behaviour of the colony is the hygiene behaviour of the bee colony.
The breeding of the bee colonies that survive without treatment is still functional only in areas where the flying from other colonies *(usually on isolated islands)* is prevented, and as such diseases are avoided .

1 · 3

Importance of the selection of queens and the therapeutic colony treatment

In this section, we explain the importance and the evaluation crietria for the selection of queens.

It is necessary to monitor the population of Varra mites in the bee colonies art regular, monthly intervals during the period from spring to late autumn, to identify and breed colonies where the mite poluplation remains low and below critical levels.

In particular, observing the natural daily mite fall, the level of the total mite population and hygienic behaviour can help to reduce dependence on treatments and biological methods to control the mites in colonies.

A comprehensive bee-testing performance protocol — a guide for European bee breeders — was created by members of the Smartbees project consortium and is available in several languages at www.smartbees-fp7.eu/extension/performance/. The protocol focuses on identifying, breeding and locally spreading of adapted colonies with high performance and resistance to the mites. The protocol describes the establishment of a test apiary *(its location, size, origin of queens, hives layout)*, the work with colonies *(establishment of test colonies, interventions in the bee colonies, prevention of swarming, disease prevention and treatment, evaluation of natural mite fall)*, as well as details of testing for specific performance characteristics. .

SMARTBEES PROJECT

Scan the QR code with your smartphone or enter the address in your browser.

http://www.smartbees-fp7.eu/

PERFORMANCE TESTS

① COLONY DEVELOPMENT

② TENDENCY TO STINGING

③ DIVERGENCE *("SITTING" ON HONEY COMB)*

④ TENDENCY TO SWARMING

⑤ HONEY PRODUCTION

⑥ NATURAL MITE FALL

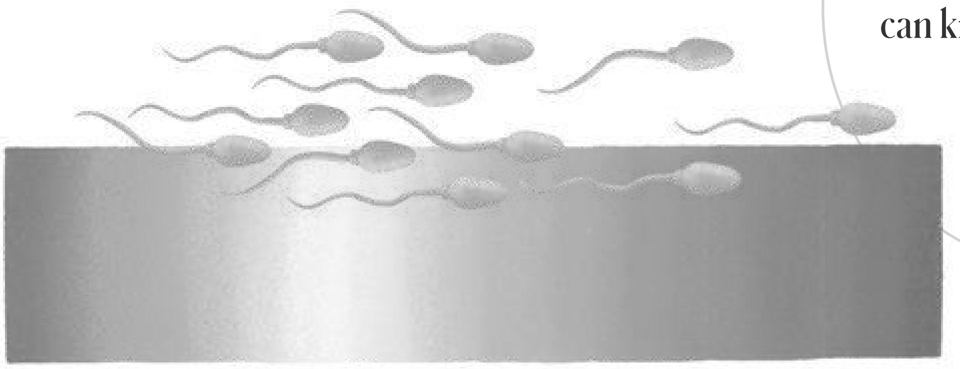
⑦ LEVEL OF MITE INFESTATION

⑧ HYGIENIC BEHAVIOUR

In particular, the last three characteristics can help to reduce dependence to reduce treatments of the colonies. In summer, it is necessary to monitor the occurrence of mite in the tested colonies at regular monthly intervals, to identify the colonies with a mite infestation **below a specified threshold** *(below 1000 mites)*. Subsequently, the selected colonies will be winterized without treatment against the mite. Those colonies which successfully survived the winter are preferred for further breeding work. The level of mite infestation at the testing apiary is continuously monitored and the most infested colonies are unwanted and treated against the mites. This approach will reduce the risk of transmission of mite among colonies *("domino effect")* and will support an objective identification of resistant colonies. The powder sugar method is an easily applicable method to monitor the level of mite infestation in a bee colony.

1 · 4

Dealing with queen failing and poor sperm quality of drones - Practical measures



Even short exposure of the queen to high temperatures can kill over 50% of sperm in the spermatheca.

In this section, we will explain the reasons for queen failure, and measures to deal with it.

On average the number of drones needed for fertilization of one queen is increasing. The queen mates with about 12 to 17 drones during 1 to 5 mating flights. In commercial beekeeping ope- rations in USA, up to 50 % of queens are replaced within half a year. The causes may vary, such as e. g. insufficient fertilization, low sperm viability, queens' infections, including semen-born viral infections, possibly nosemosis, temperature stress or impact of pesticides. The origin of low sperm viability may be due to the impact of drones or the effect of high temperatures to which queens have been exposed during commercial transportation. When sending queens, the queens are often exposed to temperature extremes *(<8 and >40 °C)*, and even the short-term exposure to such temperatures can kill more than 50 % of sperm in the spermatheca of the queen.

Drone colony breeders produce and distribute a large number of offspring only from selected queens — line founders that reduce genetic variability in the bee population. Sufficient genetic variability in the population and high levels of maternal polyandry are important for disease resistance, homeostasis, thermoregulation, and of the good physical condition of colonies. High polyandry also increases the frequency of communication signals among worker bees, resulting in better use of food resources. In inbreeding *(close family breeding)*, the proportion of diploid drones laid by the queen increases, which triggers cannibalism in the worker bees, thus eliminating and eating such genetically anomalous male, which in turn results in so called "shot brood" *(other names used are also: "pepperpot brood", or "pepperbox brood")*.

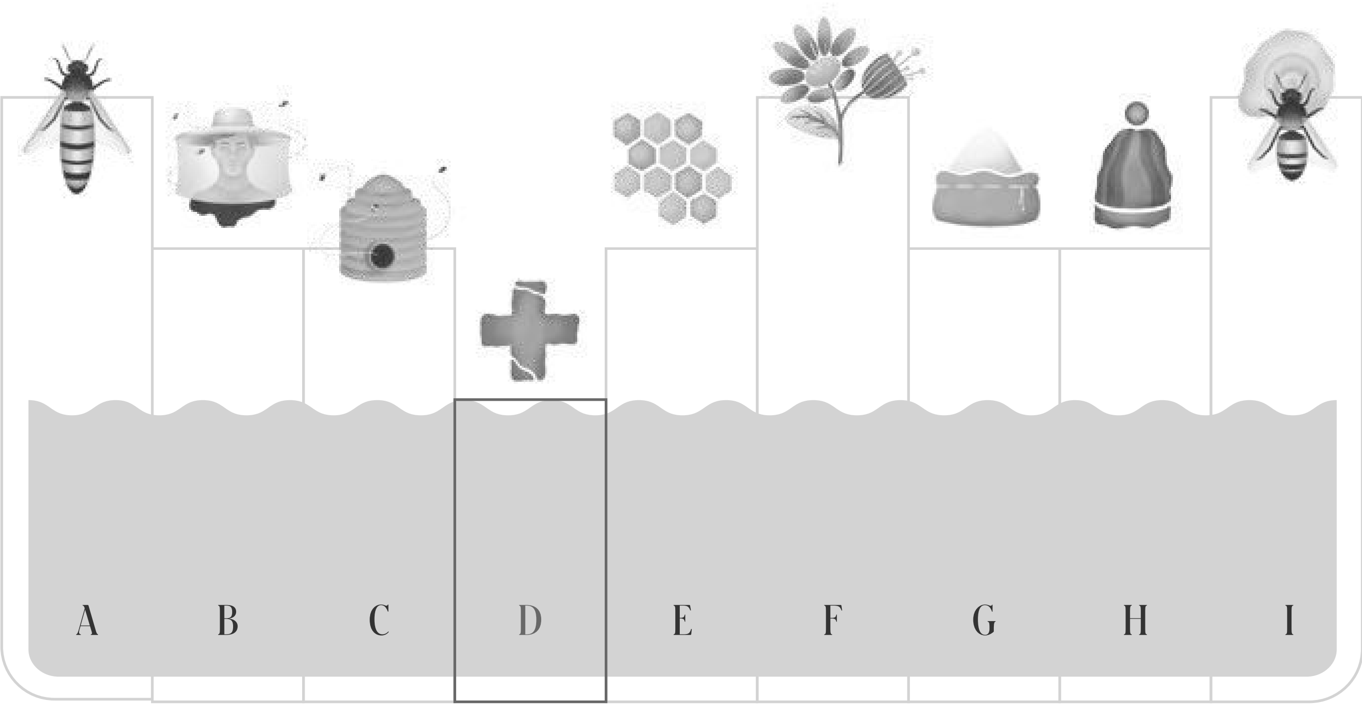
Exchange of queens by beekeepers is considered to be one of the main factors influencing successful overwintering. The beekeepers who do not regularly change queens have a much higher risk of winter deaths compared to beekeepers who change their queens every year, at least in half of their hives. In summary, young queens increase the likelihood of successful overwintering.

2

Hive management

CHART

Law of the Minimum



A • Quality of the queen B • Skills and knowledge of a beekeeper C • Location D • Bee colony health

E • Quality of wax F • Pollen and nectar flow G • Winter preparation H • Overwintering I • Availability of water

Management of hives includes many different strategies and tactics. In this section we focus on factors that significantly impact success in beekeeping.

If success in beekeeping may be represented with an analogy of a barrel made of wood, with wooden boards of different heights, the final volume and amount of honey produced by a colony will be determined by the lowest board. This lowest board is the weakest point in someone´s beekeeping practices, and it cannot be compensated by any other boards or factors. The German scientist *Justus von Liebig* refers to this concept as the "law of the minimum".

The significatly weaker health of colonies in the recent years might be attributed to the parasitic mite *Varroa destructor*. The mite causes, not only direct damage to the individual bees in the colony, but also is a vector of diseases.

Since every year might be different, and this is observed since over 20 years, the height of the wooden boards describe before is also changing every year. The impact of the beekeeper reflects his knowledge, but also his ability to respond to changes in the development of the beekeeping year. At the end of the beekeeping season the beekeeper who looks with awareness to the reasons why he has not fulfilled his beekeeping expectations, should take lessons for the next season when analyzing its annual "wooden barrels of honey".
The individual factors for successful beekeeping, as shown in the picure above, are explained one by one in the following chapters.

2·1

Role of various hive systems in the context of the colony development

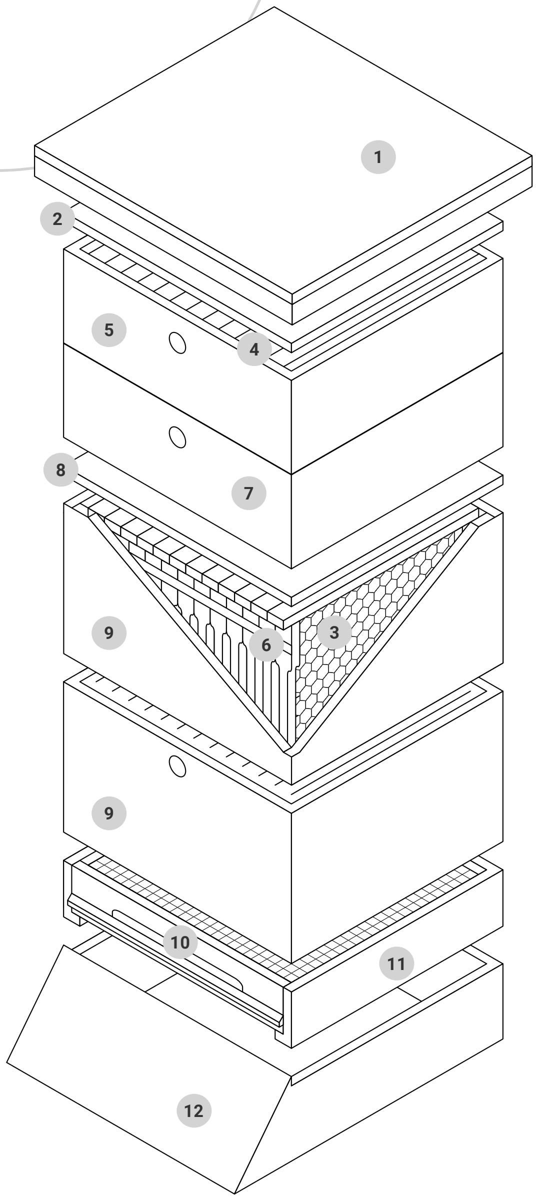
The question "What kind of the beehive system is the best to use?" has been challenging beekeepers for centuries.

This section adresses key aspects and features of the vertical hive systems.

The basic beehive parts *(a beehive system)* are: screened bottom board, a broad supper, a honey super, the outer cover and ancilliary parts such as a queen excluder, frames, entrance reducer, or inner cover with a hole (in the middle). It is possible to choose different dimensions of supers and frames in a way that they are compatible and functional. In ecological beekeeping, wood is the only allowed construction material for the frames and supers. The outer cover should have a thick insulation to avoid heat losses.

BASIC HIVE STRUCTURE

1. OUTER LID
2. INNER LID
3. FRAMES
4. FOUNDATIONS
5. SHALLOW SUPER
6. FRAME SPACERS
7. MEDIUM SUPER
8. QUEEN EXCLUDER
9. BROOD CHAMBER
10. ENTRANCE REDUCER
11. ENTRANCE REDUCER
12. STAND PLATE

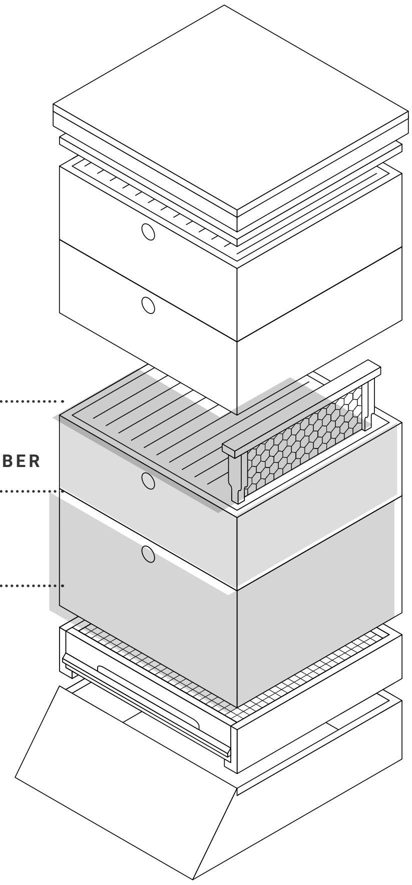


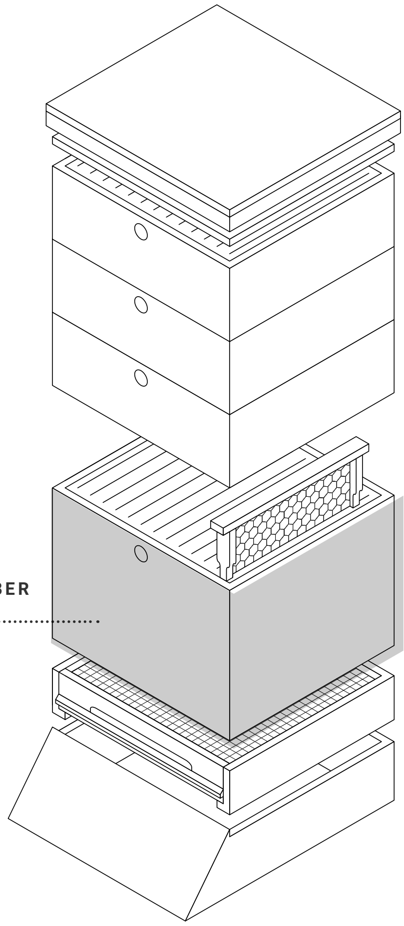
FIG

Sample hive system - Langstroth type

2·1·1

Type of hive systems — Part I.





Depending on the apiary location and the colony vitality, it is important to estimate the size of brood nest that the colony will need in the period of fast growth. Various options for the nest brood development are explained and explained in this section.

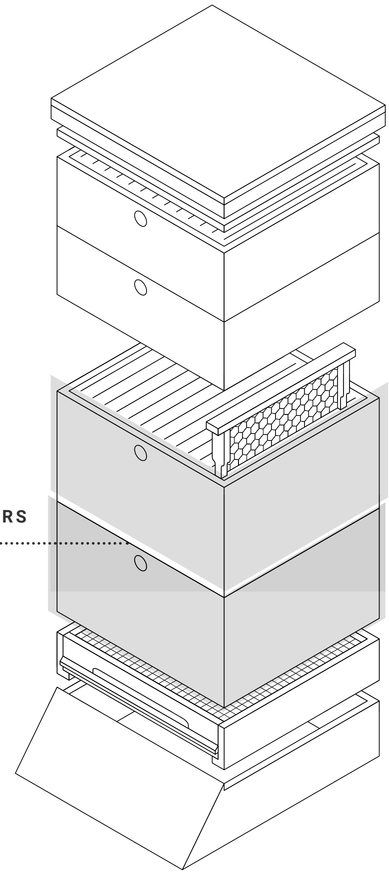
Model 01 shows a large brood chamber with 10 frames and with a size of **a brood frame up to 10 dm², in combination with a shallow honey super serving as food reserve for bees from brood chamber**. The disadvantage of a relatively small space for the development of a brood nest with one deep single chamber can be compensated by keeping honey only in the added shallow honey supers. This is especially the case when we do not have a fast pollen flow from (e.g., from clover) that could significantly reduce the empty area for the queen to lay the eggs.

Model 02 shows the use of a frame size of more than 11 dm², we can leave out the shallow honey super, and get a standard **Dadant hive with a large brood chamber**, and shallow honey supers.

2·1·2

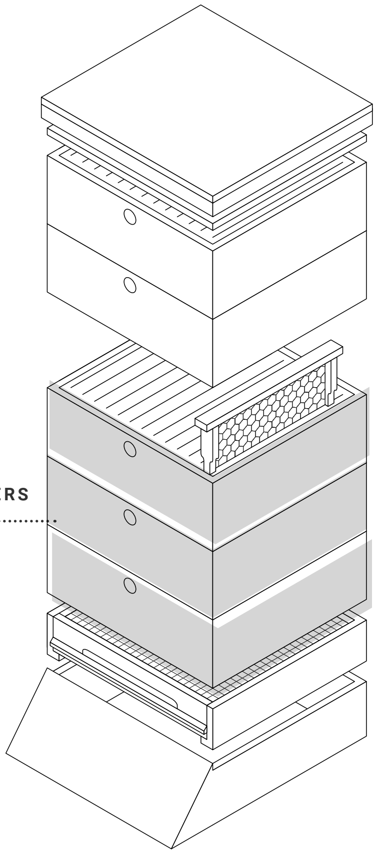
Type of hive systems — Part II.

MODEL 03



DEEP BROOD CHAMBERS

MODEL 04



SHALLOW BROOD CHAMBERS

This section discusses two vertical hive systems with brood chambers using different supers.

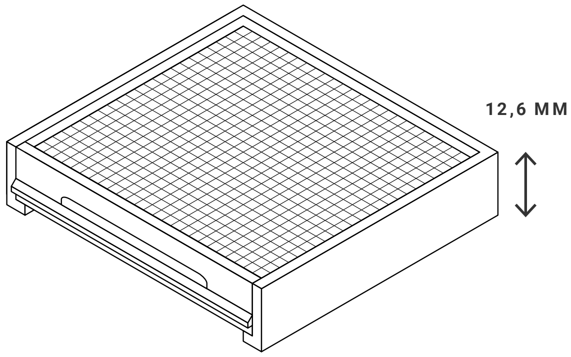
Two deep brood chambers are used in areas with an intensive pollen flow, which requires storage space and often restricts the queen from laying the eggs so the colony does not have the ability to develop into strength. Colonies in such hives store honey reserves in the upper deep honey super, which can lead to swarming, as the switching of two deep supers is unclear. It is also more difficult to find a queen in two-deep brood chambers of 20 frames. Beekeepers who use the same frame size for the brood super and the honey super often use the second brood super as a honey super, once the most intense egg laying period is over, as shown in Model 03.

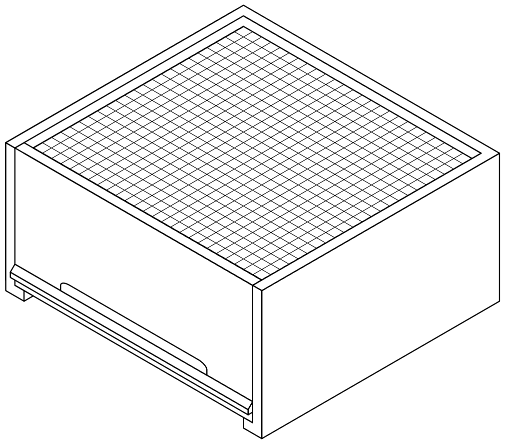
The 3 to 4 shallow supers used as brood chamber as shown in Model 04 are suitable for easier handling, making it lighter for beekeepers to lift these supers. **The advantage** of this system is also the use of the same frame size in the whole hive system. Also, the mechanization during honey harvest in commercial beekeeping is easier with this hive system. Queen breeding and creating new colonies do not require additional types of hives because they are simply done from one narrow brood chamber. When collecting the late honey harvest, there is a possibility to merge fully productive colony with the new colony, having already winter honey reserves with a young queen of that year. The disadvantages of this system are higher costs *(almost double)* and, with the larger number of hives, the possibly higher investment into a cooling room to keep frames and supers.

2·1·3

Bottom board types

FEAURES AND COMPARISON





A LOW BOTTOM BOARD

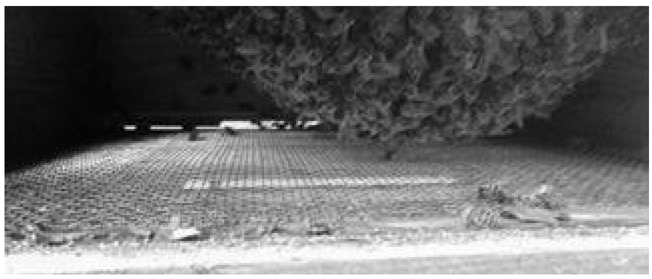
Is easier and cheaper compared to the high bottom board. It allows faster access of foraging bees to brood chamber, and bees can better and easier manage the heat insight the brood nest. Howeever, when a colony develops very fast, there might be atendency to swarm. It was also proved that in late summer, when colonies are standing one next to the other in one line, it is easier for bees to enter into neighboring hives for stealing (Robery). Moving the hives from one location to another with a low bottom board would require to include additional ventilation. The minimun height should consider the distance for two bees to move in parallel, so at least 12,6 mm.

The selection of the bottom board depends on the location and strength of a colony, and on its overall development. Experience has shown that healthy and strong colonies that occupy more than 8 standard Langstroth frame sizes (or Slovak "B-size" frames) develop well in spring, independently of the type of bottom board.

A HIGH BOTTOM BOARD

This type of bottom board is more natural to bees because it allows foraging bees to hang in a bee cluster at the free space of the bottom board. It also allows better *Varroa* monitoring, and in the hot days, when moving hives, it is advantageous to have high meshed bottom board for the ventilation of the bee colonies. In case of a large bee cluster, a space beneath of the winter cluster allows for better himidity and air flow and exchange during the winter period. The risk of a high and meshed bottom board is that under extremely wet conditions it can increase the water content in honey stored in the hney supers..

A good compromise in the selection of the bottom board is the one of Karl Pfefferle, a German beekeeper. It is a high bottom board, but from the back side of hive, it is possible to insert individual partitions, so the bottom board becomes a low bottom, which allows foraging bees to easily entry into the hive. In spring, when the colony needs to keep warm, a low bottom suits better and bees can regulate the micro-climate inside of the nest much easier. Later, the partition is replaced with the mesh partition, so it can be used for *Varroa* monitoring.



2·1·4

Inner cover and its functions

In this section we explain different functionalities of the inner cover throughout the year. The inner cover is placed on the top of honey supers throughout the year. It consists of a wooden frame in which a plywood board with a minimum thickness of 6 mm is embedded. The hole in the middle of the plywood is used for different purposes during the year.

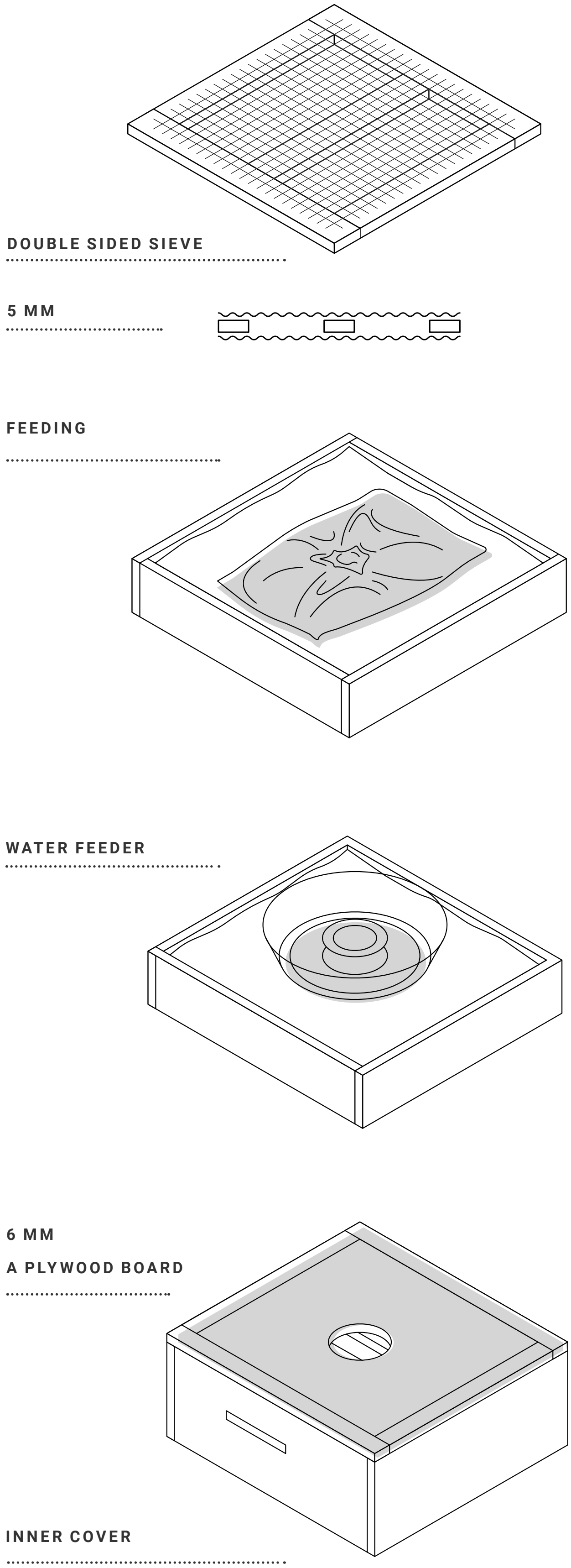
① Releasing water vapor of the wintering colony, as the hole has a chimney effect for permanent air exchange. This works only under the condition that the outer cover is permeable, respectively, it has water vapor openings.

② Feeding the colony with water in cold spring and hot summer days with inner feeder.

③ Providing food for bees during nectar shortage, by feeding them with solid food pastry.

④ The possibility of controlling *Varroa* mites during the mid-summer period *(after creating an artificial brood free colony)* with using a device for sublimation of oxalic acid.

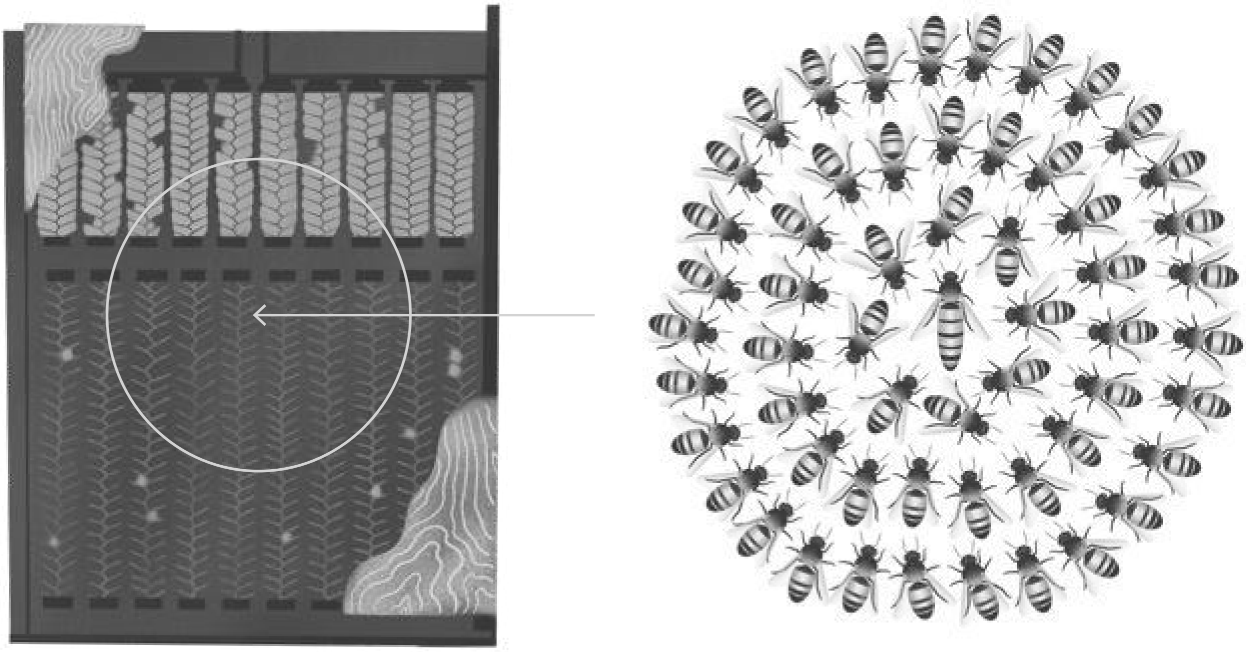
By replacing a plywood board with a double sided meshed board, one can place a new colony over a strong one, or overwinter a weaker colony on top of the stronger one. The prerequisite of such system is that the thickness of the wooden frame between the lower and the upper meshes is at least 5 mm, so that the lower and the upper bees can not touch each other and exchange information about their respective queens (to avoid conflicts). Otherwise, one of the queens is at risk of being killed.



2 • 2 INFLUENCE OF WEATHER AND NECTAR SOURCES ON THE COLONY DEVELOPMENT

2 • 2 • 1

Conditions for successful overwintering



As a social organism, a bee colony survives winter in a winter cluster, in which the creation of and the organization of individual honeybees inside of the winter cluster is very important. In this section we explain the functioning of the winter cluster and the conditions for successful overwintering.

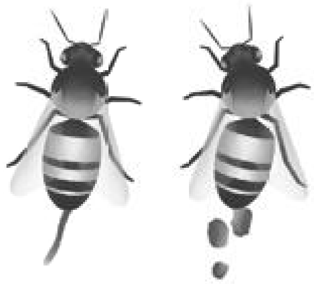
Brood rearing ends in autumn, latest 21 days after the first frosts. When temperatures fall below 10 °C, a winter cluster is formed, in which individual bees have different functions depending on their position and outside temperature. In the picture above, the ideal situation of a wintering bee colony is shown. When the outside temperature falls below zero degree, bees form a winter cluster similar in shape to a ball (sphere). This allows for a given volume of wintering bees to have the smallest surface. Only a small part of the cluster can reach the winter reserves in the hive. The greater part of bees *(the cluster)* is sitting on dry combs.

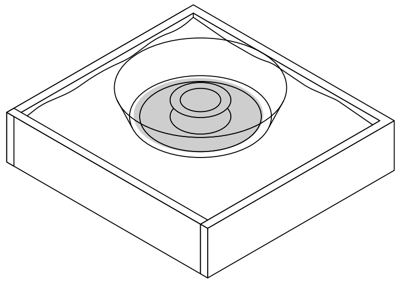
REQUIREMENTS FOR SUCCESSFUL WINTERING

① Quality *(literally fat and not overworked winter bees)*, and sufficient number of wintering bees at the range of 8.000 to 12 000 worker bees and the queen. ② Enough food reserves stored around the winter cluster, of partly stored honey, with available pollen for early spring brood rearing. ③ Honey reserves should be located mainly above the winter cluster and not seppared by the upper or lower parts of the frames; if they are more on the sides of the frames *(in case of the shallow and long type of the frames)* and the winter is long and freezing *(less than – 5°C)*, there is a risk that the winter cluster tears away from honey reserves and dies of hunger, even if it has sufficient reserves 5 cm away from the outer layer of the winter cluster. ④ A colony is protected from the pests, animals and also tree branches. ⑤ In the last years, a new problem occurs in connection with warm autumns and longer period of blooming plants; the queens continue laying eggs until late autumn. This problem can be partially solved by placing colonies at the locations with colder climate or placing the colonies in the underground rooms with a stable temperature. Into some extend it is possible to lock the queen into thin cage between frames, and place it exactly in the middle of the winter cluster, so that the queen can move together with the winter cluster. This method is risky and requires good beekeeping skills.

2·2·2

After the first spring fly out





In this section we explain the tasks of a beekeeper after the first spring fly so that we minimize disturbance to the bee colony.

SPRING HIVE INSPECTION

Is the winter cluster alive?
Hear the soft buzzing.

Is the queen breeding? Place a hand above the winter cluster super and feel the warmth of the cluster.

How much winter reserves do the bees still have? Identify the position of the cluster with respect to the flight entrance of the hive.

Is the colony looking healthy? Observe the sahpe of bee excrements after first flights to look for signs of nosemosis.

Is the colony strong? Observe the number of frames and supers occupied by the bees.

These five important features can be inspected without removing any frames. The first two aspects can be assessed can be discovered by placing a hand on the inner cover or inner sheet over the frames and by moving the hand over the frames over the frames, to feel the heat generated at the brood nest, in the middle of the cluster. The winter cluster moving away from the hive entrance to the back of the hive, in the case of "cold" allignment of frames, indicates that the cluster still has winter food reserves. The extent of nosema can be determined by the shape of the bee excrements, see the image right above. Thin and long excrements means that a colony is in a healthy condition, but round excrements points out the increased disease pressure.

The picture in chapter 2.1.4 demonstrates the functionalities of the inner cover with a feeder. The first sugar syrup can be served in small amounts. This will fulfill two needs: The bees will learn to visit the feeder and store the watery syrup over the brood nest, securing bees the access to water during the next cold spring days. The subsequent feeding is then continous, but only with just a small amount of added salt.

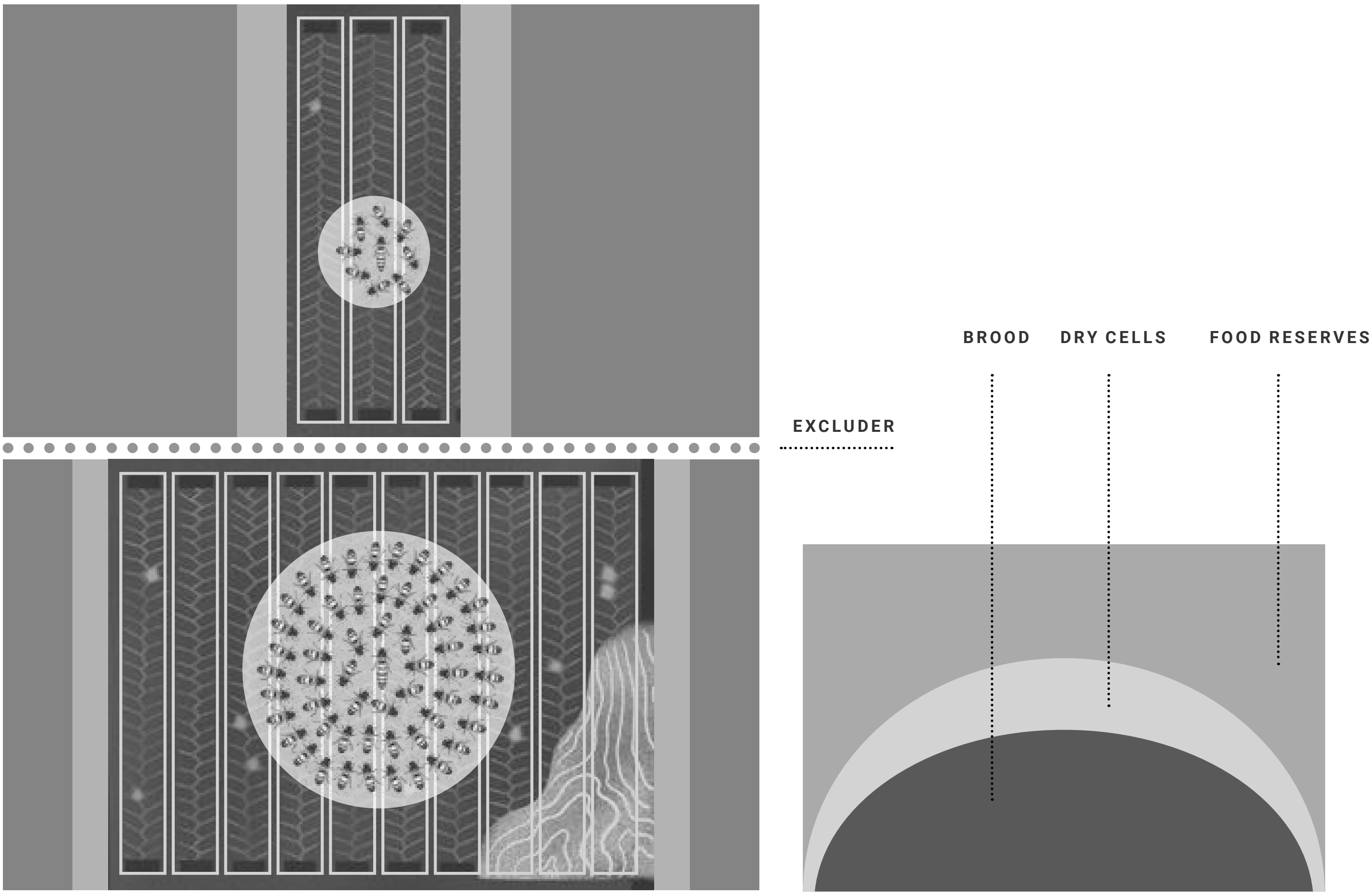
The overwintered colonies that occupy 8 frames of the standard Langstroth frame size develop independently without tightening the frames inside of the brood chamber. Medium size colonies, between 5 to 7 frames, should be isolated with the partitions from each side of the brood nest, and placed so that the center of the cluster is under the feeder, which is placed on top of the inner cover with the hole.

Concentration 1:1

WATER : SUGAR

2·2·3

Helping weaker colonies in spring



In this section we explain how to help weak colonies to get into strength and discuss the measures to prevent swarming in spring.

A weaker colony in spring **can be placed above the brood chamber** of a strong colony, so that there is only a queen excluder between them. Both queens should be about the same quality. The nursing bees can freely move from the stronger colony to the upper weaker colony, which then develops very quickly. This works best if the upper colony has shallow frames and the queen is not infected with nosema. Other necessary adjustments are tightening and placing the upper *(shallow)* frames in the center above the frames from the lower super, so that nursing bees do not have a barrier to move between the upper and the lower breeding nests.

Around the end of March, the number of newly hatched bees is higher than the number of dead winter bees. The result is a steadily growing colony. The fact that the enlarged nest is not only built by converting winter reserves above brood, but also by bringing new nectar.

If the collected nectar is stored in empty cells, these block the development of the brood nest and it is the first signal triggering the bee colony to swarming. Therefore, it is recommended, in case of strong bee colonies, to put on the top of the brood chamber another super with empty frames for newly brought nectar and thus to allow full utilization of the brood area for new brood.

2 · 2 · 4

Development of colonies after flowering of fruit trees and managing swarming

TABLE

The colony´s ability to collect nectar - capped brood to foraging bees:

ADULT, FORAGING BEES	10 000	40 000	60 000
CAPPED BROOD CELLS	8 500	22 400	18 000
PORTION OF BROOD CELLS (%)	85 %	56 %	30 %

In this section, we explain the gradual build-up of the colony in spring to profit from the main honey flow.

The young bees perform different tasks inside the brood nest, almost as if it was an own unit. Respecting this process is is key to manage swarming later on. This includes: expanding the brood nest with the wax foundations at the edges of brood nest, and once these are built, they are inserted between the frames with capped brood cells. If a colony has sufficient strength, then a disruption of inner colony harmony, like i.e. the food reserves not being in contact with the brood nest, or putting an empty built frame between the brood frames, etc. will likely stop a colony from swarming.

All swarming countermeasures are based on the disruption of inner colony harmony so that the colony does not prepare to swarm, and it is "postponed" for the next season. Timely addition of honey supers, a creation of space for for storing above the brood nest will not allow the creation of food reserves in the brood frames. Creating a distance between brood combs and honey combs halt the preparation for swarming. This leads to a collection of nectar surplus to survive winter, which is actually the profits of the beekeeper.

2·2·5

Management of colonies after summer solstice

In this section we look at preparing the colonies, after summer solstice, for the winter.

The preparation for the winter is critical because there are two opposing mechanism in parallel, which means they have to be carefully managed to avoid collapsing colonies in autumn. The first process is the gradual building of winter bees that need a large amount of pollen. The second process is the gradual increase in the mite population, especially in the untreated colonies where the mite levels exceed the recommended threshold and the colonies collapse due to viral diseases. These processes are affected by a lack of proper nutrition for the colonies, especially in areas with intensive farming, where the season ends with the end of the linden or the sunflower blooming periods. While waiting for sunflower or honeydew flows, the beekeeper can apply **three strategies** to support the colonies.

SUPPORT FROM BEEKEEPER

Control Varroa mites in a manner that does not compromise the quality of honeybee products.

Keep enough food reserves, e.g., at least 8 to 10 kg of honey per hive.

Supporting feeding during the nectar pauses in a way that does not endanger the quality of honey.

The winter reserves should be supplied immediately after the harvest, so that short-time living summer bees can be used for storage of the winter reserves. September to mid October, when the seond peak of honey flow occurs *(forest harvest)* proper management measures to control Varroa overpopulation and strengthening the colonies by merging with younger ones at the end of the season, are especially needed.

2 · 3

Selecting a suitable location for the bee hives

In this section we explain the basic criteria for selecting a suitable location for the bee colonies in terms of different seasonal needs of honeybees.

IDEAL APIARY LOCATION

Water quality and availability.

A source of clean water available all the beekeeping season.

Pollen diversity and availability.

The different pollen sources need to be reachable from the surrounding trees and bushes. The existence of gardens with fruit trees is very useful, as well as flowering meadows that are not cut before their blooming. Ideally, the agricultural plants should bloom gradually and compensate for the nectar breaks during the summer, and the farmers' technological practices should be "bee-friendly". In the later season availability of forest honeydew is an ideal condition for keeping bees.

Bee protection.

The bee colonies should not be located near water flows, because cold air from the water flows can affect the local micro-climate. It is also recommended to avoid the hill tops as the blowing wind can also negatively affect the colonies. It is recommended to choose a rather dry and partially sunny location to avoid brood calcification. It is also important that the country sides have dominant landscapes for better orientation of bees.

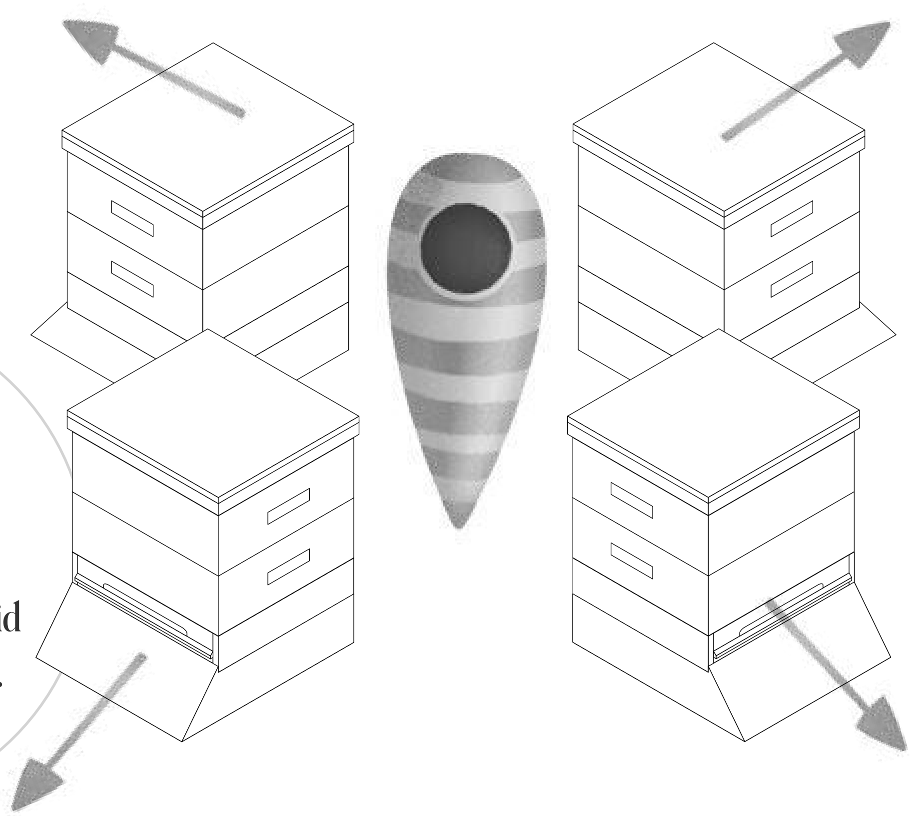
Access for the beekeeper.

Access with a vehicle can be also important criterium.

Such location is ideal, but nowadays it is difficult to find. More and more, more and more, availability of pollen and nectar pollen and nectar can be secured for bees only by moving the hives from one place to another.

2·3·1

The beehive orientation

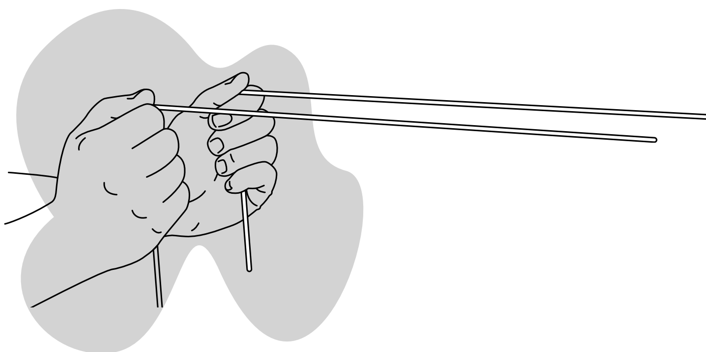


In this section, we discuss the topic of appropriate hive orientation to avoid drifting and robbing.

Experience, as well as the recent observations from studies on the orientation of the hives indicate that there is a connection between the increase of the mite population and the hives traditionally placed in line, next to each other. The mutual exchange of bees in such hives is up to 80 % in contrast to hives placed approximately 10 meters apart. In this case, the exchange of bees will be reduced to just a few percent. These studies examined the development of the mite population by experimentally feeding the bee colonies with different kinds of food, for different groups of colonies at the same location. In spring, all food reserves were examined and a mix of food was found in all hives at the location. For a practical beekeeper, this fact means that all treatments of the bee colonies against mites are ineffective, if the bee colonies are not in the same condition of capped brood. Thus, it is strongly recommended to keep the queens into duplex frame boxes for a period of about 3 weeks, and subsequently treat all colonies against the mites at the same time and at the same location. The instinct of the swarm, which refuses to settle near the “parent colony” affected by the high infestation of mites, shows that it is important to consider the hive orientation and hive distances. A beekeeper often does not have large space, and he/she can solve this problem by grouping hives by four, each hive having the entrance into different cardinal points. Different colony development can also be an advantage for a beekeeper, as a smart beekeeper will manage and treat the hives by groups / by cardinal points.

BEES AND GEOMAGNETIC RADIATION

The geomagnetic radiation is associated to ores and metals, but also to underground water flows and tectonic breaks. Bees and ants seek such zones and draw energy from them. Here, bees are much milder and hardworking. The bee hives could be placed along the aquifer (source) and with the hive entrance to the south.



3 • TYPE AND QUALITY OF FOOD AND THE IMPORTANCE OF FLORAL DIVERSITY

3 • 1

Influence of nutrition on wintering bees

Intensive agricultural practices have negative impacts in the pollen supply for the bees, which in turn affects the colonies ability to overwinter. Pollen from crops might be available only at limited periods of time, and its quality might be affected by pesticides (e.g., corn and rapeseed pollen). In the pictures below, you can see how intensively cultivated landscapes over most of the season turn into a desert for the bees.

When bees are under stress, as e.g. infected with Nosema ceranae, their lifespan is directly proportional to the possibility of consuming various types of pollen. It is not possible to compensate diverse pollen sources withpollen from monocultures. Mointain meadows, offering highly nutritious pollen rich in proteins, carbohydrates and antioxidants, are ideal for prearing bees for the winter.

Bees prepare themselves for the winter periodright after the summer solstice. Gradually, winter bees born, and are not immediatelly involved in any work inside the hive. These long-living honeybees have the character of nursing bees at an early stage, but from the consumed pollen the body of the winter bees can create a protein-fat organ, called fat body.

Winter bees look like the summer bees, but they have a much lower level of the juvenile hormone in the hemolymph. Nevertheless, they have high levels of vitellogenin — a protein that affects their lifespan. The shorter days at the end of the summer with lower night temepratures as well as decreasing reproductive activity of the queen, contribute to changes in the biology of the winter bees. If the colony does not have enough food at this time, or the queen is not healthy enough, the colony remains active and produces less winter bees of the corresponding quality. It is a similar situation when the mites infest the brood, and the colony cannot produce a sufficient number of healthy winter bees able to survive over the winter.

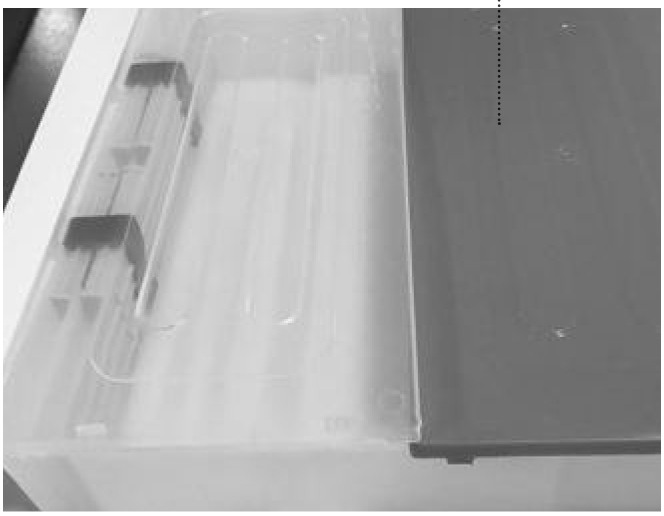
If long-living bees are reared under lack of pollen, they are malnourished and not able to resist diseases. Winter bees live from July to the end of April of the following year.

3 • 2

Safe feeding in summer without affecting the quality of honey

FOOD DOUGH

FEEDER FOR LIQUID FOOD



Nectar and pollen show interruptions in the summer. The beekeeper has to supplement the diet of the colonies without impauring the qulaity of the honey. This section explains how to proceed safely with this feeding. In this section you will learn how to proceed safely.

It is necessary to maintain a minimum of 8 to 10 kg of food reserves, so that the bee colony does not switch into "the saving mode". The pollen consumption in July and August is only slightly lower than in April and May, when the colony goes through the fast growing development phase.

8 – 10 kg

MINIMUM QUANTITY OF HONEY RESERVES

An intensive feeding prior to the expected nectar flow is not appropriate, as the fed syrup or any other non-honey food can deteriorate the quality of the real honey. Feeding shall be controlled and kept below the daily consumption, so that the food it is not attractive or accesible to a large number of bees at the same time. It is best to use a high quality dough without any not digestible substances *(polysaccharides, such as starch and lactose)*, and the hydroxymethylfurfural level (HMF) value must be comparable to honey. It is more effective and safe to place the dought near the brood nest. This can be ensured by using a well-placed frame-type feeder. Experience shows that a more comfortable solution is to place 2.5 kg of food dough with a 5 x 5 cm hole on the wooden inner cover. This dough is taken in smaller doses than the daily consumption of the colony, and in the case of discovering new nectar sources, it is less frequently visited by bees *(photo, left above)*. Recently, a feeder for liquid food has been developed, where it is possible to open the entrance for a one single bee. It is possible to place it on the inner cover, and to fill it without contact with bees and the supers *(photo, right above)*.

3 • 3

Risks from feeding with sugar substitutes

There are risks associated with feeding bees with different types of food. Bee food selection requires maximum care.
This section explains the types of bee food and the most approriate way of feeding the bees.

The best type of food is still crystal clean sugar, nevertheless, many suppliers also offer liquid food for the bees. This type of food needs to be evaluated for feeding bees in Central European conditions, as follows:

FOOD FOR THE BEES - WHAT TO LOOK FOR

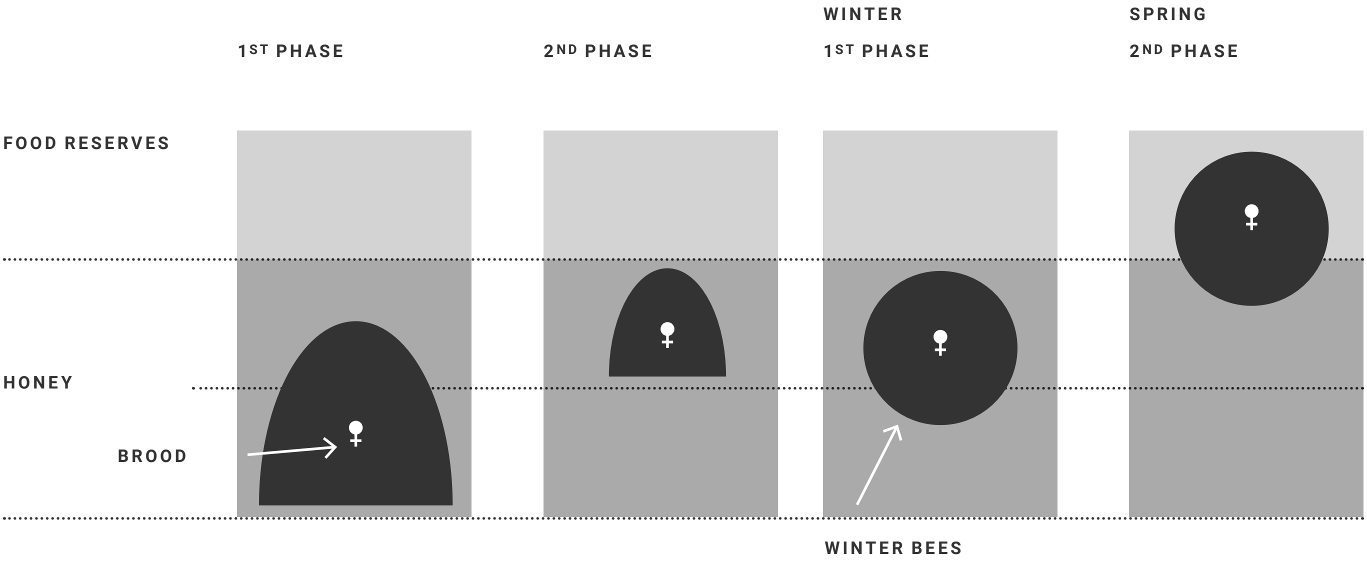
① Origins of food – food from sugar beet contains poorly digestible ingredients such as fructose, glucose and sucrose, in a concentration of about 75 %. Ideally, there should be a higher fructose than glucose concentration in the food for the bees, to avoid crystallization of food supplies.

② Cereal food contains maltose and polysaccharides which are substances not digestible for the bees, and which may lead to bad wintering of the bee colonies over a longer winter period.

③ After processing 1 kg of crystal sugar, bees keep only 0,75 kg for winter reserves. For other types of food, this share can be even lower.

3 • 4

Winter feeding strategies



The best quality of food for bees is pure honey. In case we feed bees for the winter with some other type of food (other than with honey from our own bees), it is important to ensure that the large part of the winter period bees over winter on their own honey reserves.

In principle, bees keep honey as detached as possible from the entrance to the hive — most probably for strategic reasons. In the case of a warm positioning of frames, the first frames are without food reserves, followed by the brood nest, and only behind it there are the honey reserves. In the vertical hives, at the bottom of the hive there is a brood nest, and honey is on the top of it. This is the case in a natural honeybee habitat. The reserves should be above the brood nest, because it keeps them warm and it's easier to reach. The winter cluster could ventually not reach the honey reserves during really cold winter days and die. Therefore, it is better already in autumn, to place the brood frames under the reserves frames.

Colonies that overwinter with reserves of floral honey have a well developed inmune system, and can handle better the diseases associated with the Varroa mite, and the changes in nutrition. For this reason, we have to adapt the **winter feeding strategy**. The last honey harvest shall not include honey combs from the brood chamber, so that honey reamins for the colony to consume. A part of the former honey super will be filled with sugar food, as future food reserves. In this way, winter bees will emerge **in two phases, as shown above**.

Phase 1

Is a quick feeding with sugar syrup at doses over 5 liters, so that the bees can store them in the free super aobve the brood nest. A complete capping of these reserves is ideal, so that bees do not store the food (sugar) reserves in the brood chamber, after the gradual reduction of the brood.

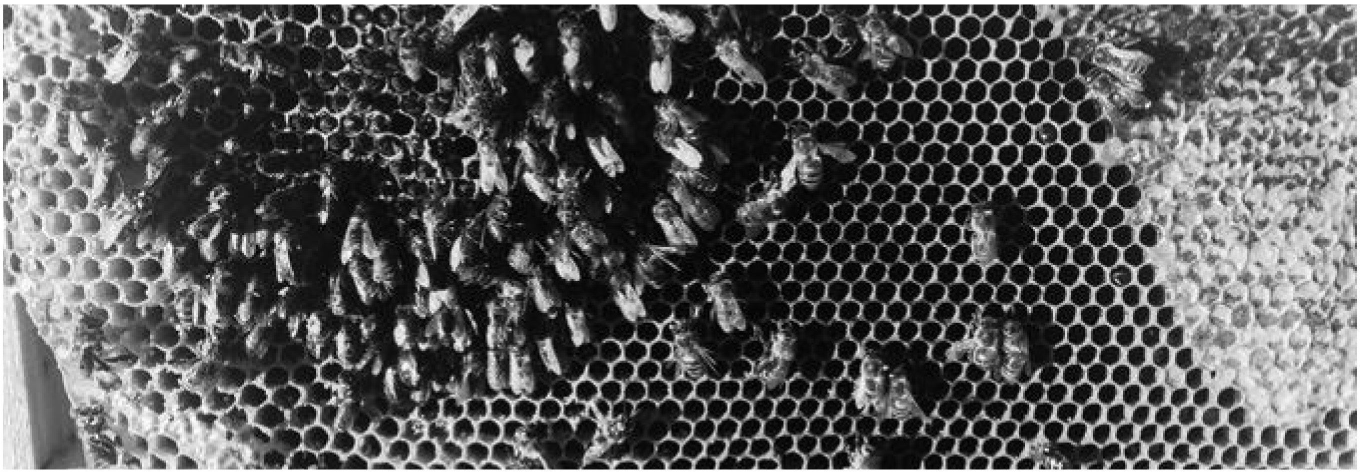
Phase 2

At the end of the summer, beginning of autumn the brood is reducing gradually. This might take place at the same time of a reduced nectar flow. Honey produced in this period is better placed directly above, in the same frames of the decreasing brood nest.

During winter, the bees consume honey reserves created in the phase 2 in the brood chamber, and in early *(first)* spring they consume sugar reserves of lower quality from the phase 1. At the same time, the collection of the first pollen starts.

3 • 5

Successful wintering and the role various factors play



This section explains the necessary conditions for successful wintering of strong colonies. It considers factors such as position of the hive entrance and air exchange from the winter cluster.

WHAT TO WATCH OUT FOR?

① Strength of the wintering colony: At least 8 standard Langsthroth frames *(or Slovak B10)*, exquivalent of 7 Jumbo Dadant frames populated with bees are needed for a successful wintering of the colony. The volume of the hive for the winter should fit to the strength of the colony and the necessary food reserves. Given this guideline values, bees and frames shall be reduced to one main chamber, and protected with thermal insulation panels (themo-sheets).

The winter cluster of very weak colonies might be far away split from the food reserve *(see the picture above)* when the temperature drops below minus degrees for a few days. Often this leads to starvation, especially when narrow and long frames are used. Less often, the colony deaths occur as a result of starvation in in wider and shorter frames, as the bees have sufficient reserves rigth next to them during the winter period. The winter cluster must be in contact with honey reserves. Possible winter losses should be detected by the beekeeper already in autumun after the inspection of the colonies.

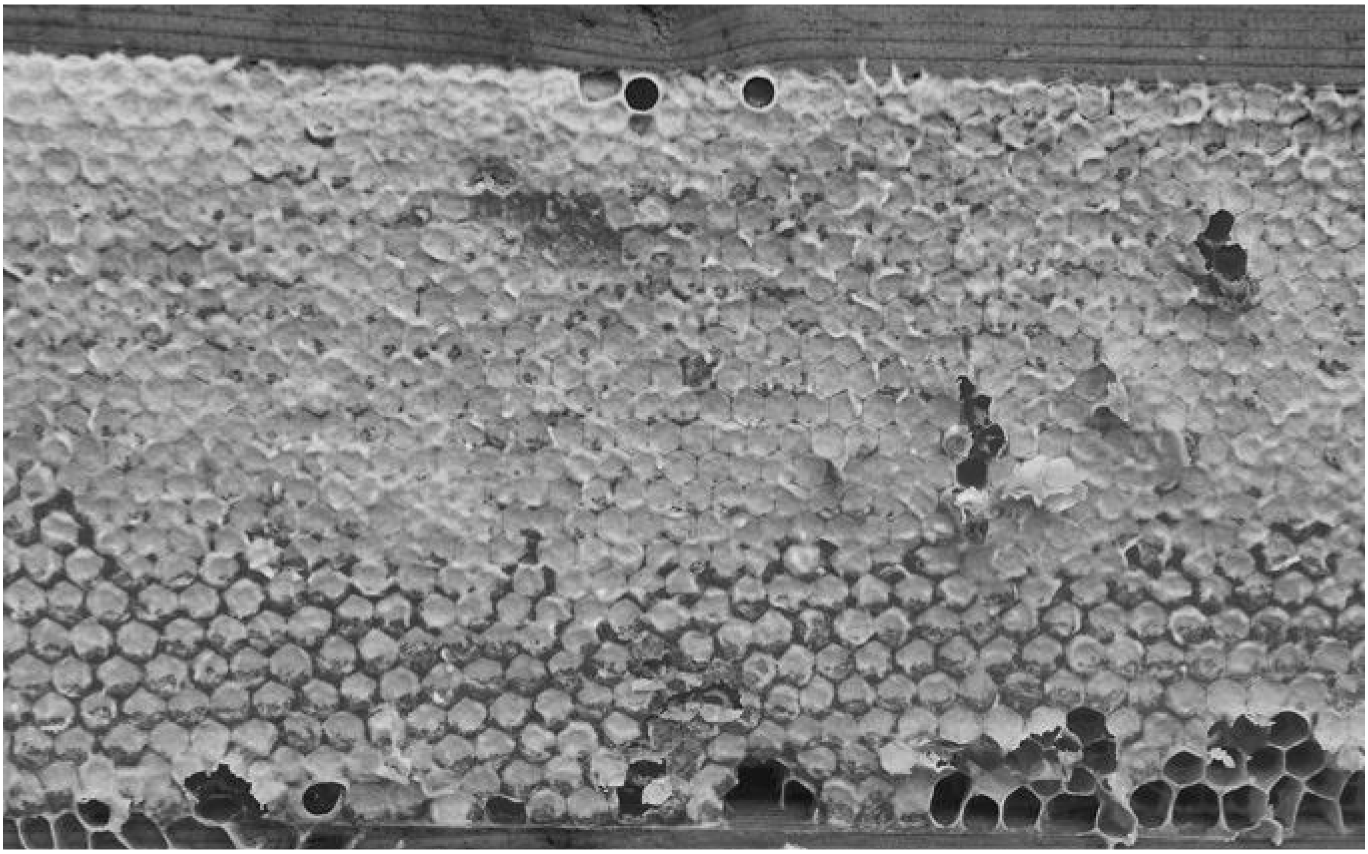
② The position of the hive entrance and the place of winter reserves. Previously we discussed the so called "cold positioning" of frames in the hive in the case of "warm positioning" of frames, of frames, to keep the position of the hive shall not be at the middle of the hive, because the winter cluster would have access to only half of the reserves. It is also necessary to prevent entry of pests *(such as mice)* into the hives and to protect the hives against flying pests *(such as hornests and wasps)*.

③ Removal of the CO_2 released by bees during wintering. Unless there is a warm winter, the cold temperatures in winter result in a break of the breeding activity. It is important though to keep in mind that the lid of the hives helps insulating them against the cold, and that thermal bridges must be avoided. Moreover, ventilation of the CO2 from the bee´s own breathing, and the flow of warm air towards the upper part of the hive are both not possible when using a closed bottom board.

Water condensation on the frames and walls not covered with propolis will favour the growth of mold inside the hive. As a matter of fact, when wintering the colony with a closed bottom board, it is necessary that the lid is permeable to air. When the bottom board is meshed and the colony is healthy and strong, or it is overwintered in several supers, the lid does not need to be permeable to air.

3 • 6

Winter melecitose honey and its impact of colony development in spring



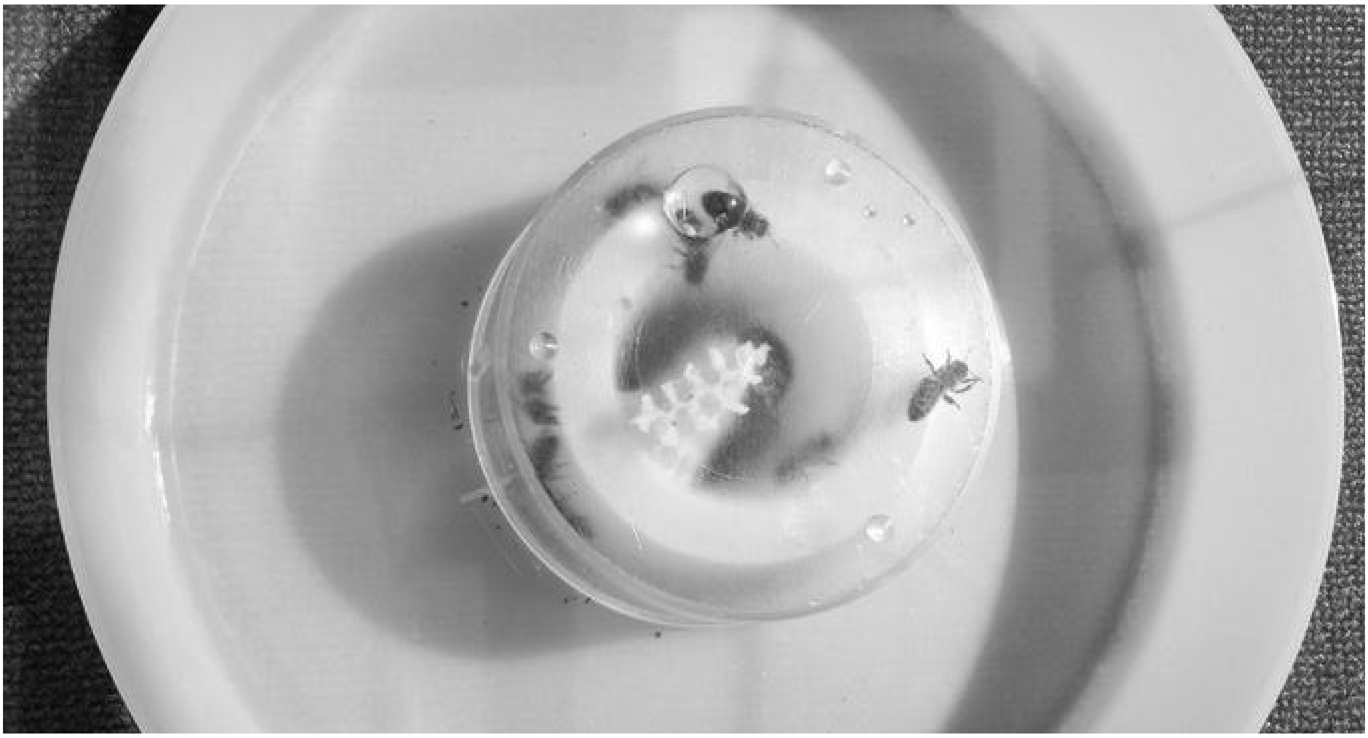
Due to climate change more melecitose honey is produced in nature. This section explains what melecitose honey is and how to deal with it.

Melecitose is a triple sugar consisting of two molecules of glucose and one molecule of fructose. It is produced during the honeydew flow and cristalizes quickly, so bees often remove it from the storage cells. It is a problem in late summer because it is collected even from the tree leaves, and it is stored for winter reserves, but the bees are not able to process it properly at this time. In the spring the bees can produce an enzyme to decompose it, which also has a significant positive effect on the spring development *(A. Roman – Poland)*. Consumption of melecitose can cause Black sickness *(Alopetia nigricans apis)*, which is often confused with viral diseases, such a deformed wing virus. The symptoms are similar, bees show loss of hair and damaged wings.

Bees infected with Nosema ceranae prefer flower honey to honeydew - during experiments they preferred sunflower honey, and had the smallest amount of *Apis ceranea* spores. *(The Self-curative Effect of Flower Honey - Silvio Erler, Deutsches Bienenjournal)*.

3 • 7

Importance of water for colony development and optimal relative humidity



Water is very impiortant for the development of the colonies and for keeping an optimal relative humidity in the hives, especially during transport of bees and under changing climatic conditions. This section discusses the proper supply of water for the bees.

Water in vital for the bee colonies, especially in early spring when nectar is still missing. Water can be provided directly in the hives, by means of sugar syrup with a proportion of 1:1 sugar to water. This will secure the water spply even during cold days. Between February to end of April a colony could cunsume up to 20 liters of water. Water availability also has an influence on the number of foraging bees in early spring.

When the wintering takes pace in air permeable hives, it is recommended to replace the lid by a non-permeable one, to aovid losses of water with the warm air flow out of the hive. This is important to keep the optimum level of relative humidity, especially in the presence of bee larvae in the brood.

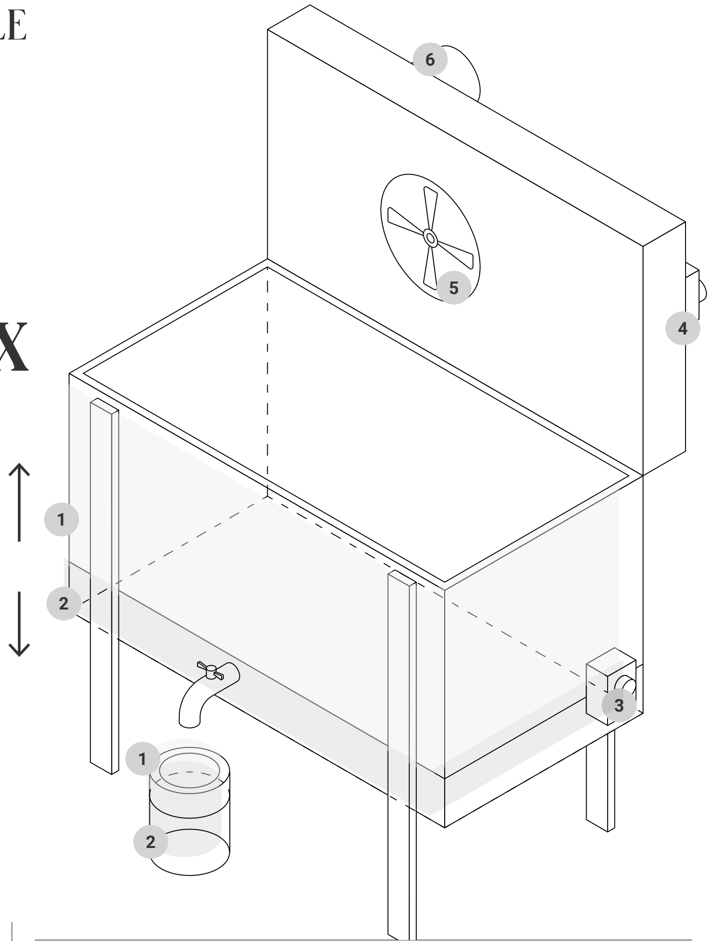
WATER NEEDS DURING THE SEASON

If nectar flow occurs, the need for water for the brood is secured from the collected nectar. Bee hives placed in the sun during the summer shall be cooled be cooled to prevent brood damage due to lower relative humidity. Water is also important for foraging bees. Foraging bees can bring water from remote sources, but this could cause compains from owners of swimming pools and garden ponds where bees go collect the water.

4 • THE IMPORTANCE OF THE WAY CYCLE

4 • 1

Collecting and producing own wax



WAX EXTRACTION DEVICE

1 LIQUID, MELTING WAX
2 HONEY
3 40 °C
4 65 °C
5 FAN
6 MOTOR 0,045 KW/1350 RPM

Wax combs are an integral part of the bee colony. Therefore, it is necessary to ensure that colonies have only pure bee *(own)* wax without any foreign impurities. In this section, we explain how to obtain wax from the beewax cappings and how to further process it.

ADVANTAGES OF AN OWN BEEWAX CYCLE

① Beeswax management with partial replacement using paraffin (or other substances) is done to reduce costs, but the used substances are often toxic for the colonies.
Even for an experienced beekeeper, such adulterated can be very difficult, often impossible to distinguish from pure beeswax, especially when it has an appealing color/smell and texture.

② Dark wax is often infected with pathogens and viruses, but also with residues from the conventional chemical treatments against bee diseases, mainly used against the *Varroa* mites..

③ Synthetic pesticides such as *fluvalinate, flumethrin, amitraz, coumaphos* and *cymiazole* leave residues in beewax. The increased dose of acaricides in approved veterinary products can result in even higher ammounts of such chemicals accumulating in the beewax.

The least polluted wax (*from the pollution in the vicinity of the beehives)* is the beewax from the honeycomb cappings, which can be used as raw material of high quality for the production of the wax foundations.

The extraction of wax from honeycomb cappings by means of a wax extraction device is shown in the picture above.The devices separates honey from wax in a gentle way. Heavier honey is at the bottom, where temperature is about 40 °C, and it is protected by lighter wax floating on the rim at a temperature above 65 °C. The wax obtained in this way is light yellow with no added substances, and it is not overheated.

4 • 2

Production of own foundations

In this section we focus on ways of self-production of wax foundations for hobby and commercial beekeepers.

This production method is suitable for beekeepers with a stable number of bee colonies so that they can take advantage of the closed cycle of own beeswax.

WAX PROCESSING OPTIONS

① Processing the wax at a trusted wax processor where there is a guarantee that the produced wax foundations are only of the own wax.

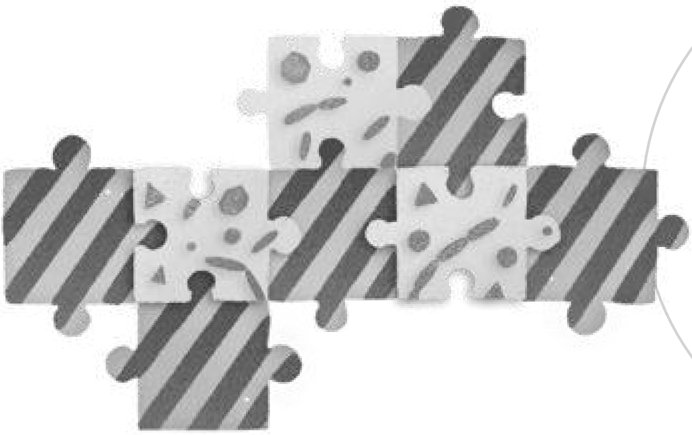
② Own production of wax foundations by casting the wax into the silicone mold *(photo left above)* or by rolling, first on smooth rollers, and then on the marked rollers *(photo right above)*. Such foundations are thicker but more flexible than standard foundations produced by casting the wax in a mold.

Both methods require **careful handling** of the wax in terms of melting temperature of max. 70 °C, and use of stainless steel or plastic containers. In the second method, if we also process the wax of somebody else, it must be sterilized against spores of foulbrood. Heating should be at about 107 °C for 60 minutes, or 150 °C for 5 to 10 minutes.
Smaller cells of 5.1 or 4.9 mm have been suggested, for reducing the reproduction of the Varroa mites in the brood. This though has not been fully proven.

5 • BENEFICIAL SYMBIOTIC ORGANISMS

5 • 1

Beneficial intestinal microorganisms and their impact of the bee health



For bee nutrition and for improvement of bee immunity, it is necessary to better understand the role of intestinal microorganisms and nutritional supplements.

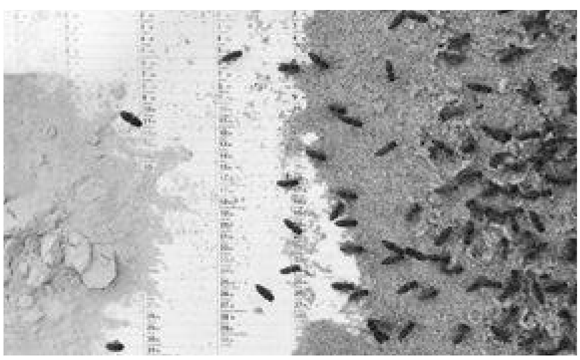
In this section we focus on the impact of microorganisms on the bee's health.

The presence and role of microorganisms in mammals has already been extensively studied, and some strains are used in food and supplements for human and animal nutrition. In invertebrate nutrition, including bees, such research is still in its infancy, and the main focus is on the strains that are naturally occurring in the bee's digestive tract *(e.g. Lactobacilus)*.

Today, the relationship between these microorganisms and bee immunity has been proven. When large numbers of probiotic bacteria attach to the intestinal tissue of the bees, they occupy the receptor sites that otherwise would be occupied by pathogenic bacteria, which in turn are not capable of binding to the intestine.

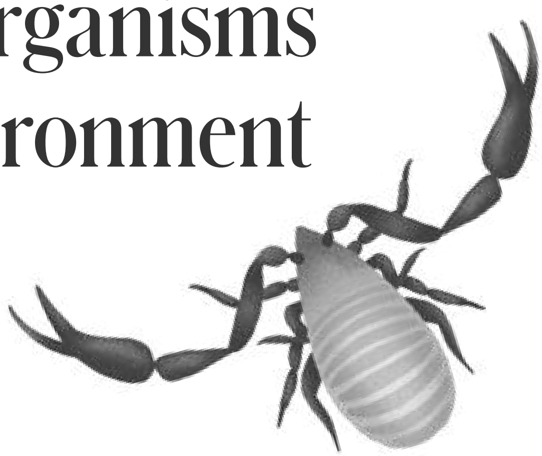
In the experiments carried out at a group of beehives, three different species of strains were given to bees in sugar syrup and the result was a significant increase in the number of microorganisms in the digestive tract of bees There is no systematic use of references so please delete or harmonize in all the places where references would be needed. So far, the impact on the amount of brood produced by these colonies, bee longevity and honey yield has not been investigated. On the other hand, the complexity of the use of these microorganisms in bee nutrition should be pointed out, these microorganims subsequently could become part of bee products consumed by humans.

For decades, efforts have been made to improve the bee nutrition with nutritional supplement, but no full replacement of pollen for bee´s nutrition has been found so far. pollen in bee nutrition has been found so far. Latest development of include algae-based supplements produced in the USA and the Czech Republic. First results show that, if they are applied in the feed dough or in the syrup, they contribute to the development of immunity of individual bees, but do not increase the amount of brood in the colonies. Conversely, if offered in a dry form in spring, the increase in brood quantity is evident. Whenever bees are offered an alternative to pollen, they still prefer pollen *(see photo below)* and ignore this nutritional supplement. When pollen was mixed with an algae-based nutritional supplement, this mixture was taken all.



5 • 2

Symbiotic macro-organisms in the bee hive environment



Restoring a suitable structure of natural fauna in the hive environment in our colonies could be another strategy to preserve the bee colonies. In this section, we will present an example of symbiotic organisms that are calling scientists' attention again.

Predatory mites, parasitoids and entomopathogens *(nematodes, protozoa, viruses, Bacillus thuringiensis, rickettsia and fungi)* were tested for their potential to fight againts the Varroa mite *(Varroa destructor)*. Most attention was paid to the natural enemies of the Varroa mites, the taxonomically related species from the mite group. Entomopathogenic fungi, which attack a wide range of mite species, also have great potential. Further research is also needed on the Bacillus thuringiensis, especially the strains producing toxins againts specific non-insect hosts.

In the hive environment there are, not only parasitic mites, but also beneficial mite species that can protect bees from diseases, other parasites, and cleptoparasites. These beneficial mites provide cleaning services to developing bee larvae, e.g., removing harmful fungi and other microorganisms. Knowing more about these beneficial species in the hives, can also help improve the conservation of pollinators in the future. The list of mites species found in colonies is at: http://idtools.org/id/mites/beemites/

After spreading the frames from the planed wooden boards about 130 years ago, along with the spread of chemical treatments in the hive environment, the European hives have not only inadvertently lost beneficial species as the acaro-fauna *("cleaning mites")* but also false scorpion *(Pseudoscorpiones)*, which the colonies probably protected from many small enemies. The effectiveness of the false scorpion in the fight against the Varroa mite has not *(yet)* been sufficiently proven. However, as recent research suggests, one false scorpion is able to search, and kill from one to ten *Varroa* mites per day in the hive.

The initial co-existence with bees was described at the end of the 19th century, when the false scorpion was referred to as "a kind guest in a bee colony". Symbiosis was interrupted by the use of modern hives as well as as by the use of acaricides. In 1951, Dr. Max Beier wrote about it as a small assistant who helped to dispose of wax moths in a hive. Attempts have shown that *Pseudoscorpions* truly feed on *Varroa* mites and a decrease in mite population has been reported. The origin of these false scorpions is the leaf mould *(phasmatodea)*. Across Europe, up to 760 different species were identified.

MITES INSIDE THE HIVES

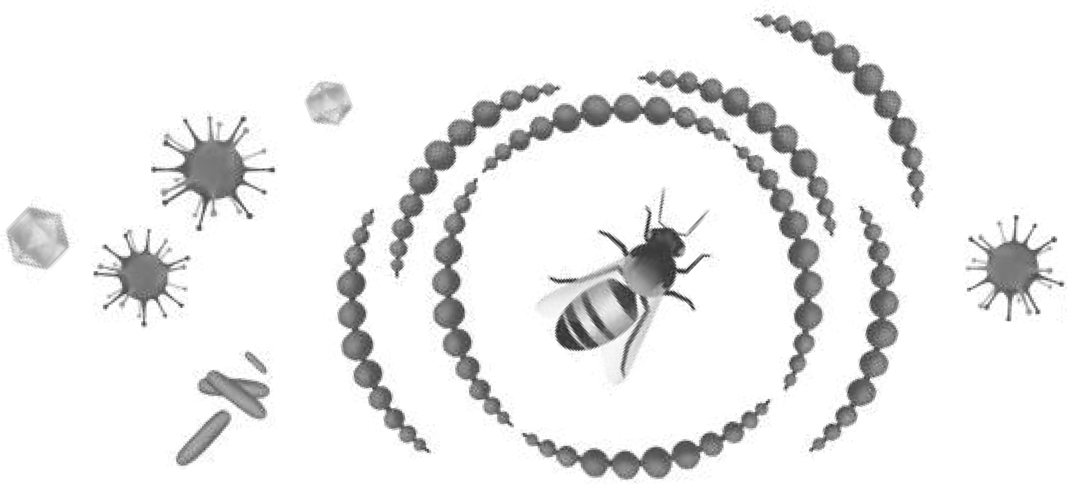
Scan the QR code with your smartphone or enter the address in your browser.

http://idtools.org/id/mites/beemites/

6 • PROPOLIS

6 • 1

The role of propolis as a natural barrier against pathogens



In this section we explain the role of propolis, especially as an effective pathogen barrier.

One example of social immunity is the collection of plant resins by honey bees, and its placement on the inner walls of the nest, also referred to as the propolis layer. Collecting and storing resins in the nest architecture has an effect on the individual immunity, the overall colony health, and supports the bee's antimicrobial mechanisms. Plants are not only a source of food for bees, but also a source of "medicinal" substances.

The process of human domestication of honey bees using smooth hive walls has also affected a very important natural colony defence mechanism, namely the formation of a propolis protective layer.

The propolis protective layer serves as the outer antimicrobial barrier around the colony, providing an improvement in the immunity of adult bees, better physical condition of colonies in spring after the winter, and a natural defence mechanisms of the bee against foulbrood and calcification of brood. In nursing bees - feeding brood, it helps to induce an effective immune response after brood infection, leading to a reduction in infection about two months after such a bacterial challenge. After the outbreak of bacterial or fungal infections, bees increase the contribution of resins to the colony.

Propolis is not only a construction material, but has a significant protective function in creating a protective atmosphere in the hive. The volatile substances from propolis have antibacterial effects on many bacteria, salmonella, staphylococci and other pathogenic and non-pathogenic organisms. Also interesting is the combination of these effects and the enhancing effect of propolis on junctions, where bees create resonant gaps for communication and transfer of information.

Leaving **the propolis mesh** on the hive ceiling is not recommended for a longer period of time, especially during winter, as it weakens the colony's health. It is also not recommended to use the propolis tincture to bees, as this may lead to the destruction of beneficial microbioma in bees' intestine, which is essential for bee health and their survival.

6 • 2

Impact of construction and selection of materials on the amount of propolis in the hive

Propolis forms part of the construction of the wax combs to reinforce the combs, as well as for better communication between bees. In this section, we explain how it is possible to support the amount of propolis in the hive environment.

Domestication of honey bees has resulted in a reduction in the collection of resins, probably due to the fact that beekeepers have selected colonies that have less propolis around the frames, to facilitate hive inspections. The collection of propolis is a relatively rare phenomenon, especially in the European honey bee populations.

Bees do not form a propolis layer in standard hives because the inner walls of the supers are solid and smooth, so no sealing is needed. Instead, bees store propolis in scattered cracks and crevices in the hive, and not as a continuous inner layer, as would be seen in a tree cavity occupied by bees.

It is possible to artificially create a propolis layer by coating the inner walls of the hive with a propolis extract *(approx. 13 % propolis in 70 % ethanol)*. Also, the bee colonies can be encouraged to create a natural propolis envelope in a standard hive by cutting the propolis grids so that the hive's inner walls can be overlaid with them. The smooth side of the propolis grid is attached to the wall, and the rough side faces the colony. After filling the holes with propolis, the grid can be removed from the walls. In this method, only 9 frames should be used instead of 10 frames. Even if the inner walls of the hive are left plane, scrapping them with a wire brush, by a wire brush, or if 3 mm grooves are milled into the inner walls, will stimulate bees to produce a propolis layer.

Beekeepers often prefer conditions that make their work more efficient, regardless of the needs of the bees. For example, wood is replaced as a construction for the hive, and plastic materials are used instead. Air non-permeability and higher thermal insulation of these hives can be very disadvantageus for the survival of the colonies. It has been shown that, at higher temperatures in the hive, not only pure wax is used as a construction material for wax combs, but it is a mixture of propolis and wax that is used to keep bees communicating through bee resonance. If the combs built in colder environments are inserted into styrodur hives *(not allowed in organic farming)*, where the temperature is higher, this may limit the communication of bees during honey flow. This is critical in case of using the plastic frames in brood nest where bees cannot form resonant holes at the edges of the comb.

Besides reducing brood calcification, propolis also helps combating other bacterial and fungal diseases. The photos show the amount of propolis used in the wooden hives *(picture above)* and styropor hives *(picture below)*.

Questions

1. What impact does long distances transport has when importing queen bees?
2. What do we understand by bee hygienic behaviour?
3. How can we estimate the current level of infestation by varroa mites in a colony?
4. What has the most significant impact on reducing winter colony losses?
5. What weight do the individual factors have for successful beekeeping?
6. Where is the queen excluder insterted?
7. Where is the varroa monitoring tray inserted?
8. What is the purpose of a shallow super in the hive system composed of one deep and one shallow chambers?
9. What are the advantages and disadvantages of the hive system composed of one deep chamber?
10. What characterizes the hive system composed of two deep brood chambers?
11. What characterizes the hive system composed of three or four shallow supers?
12. What are the advantages and disadvantages of a low bottom board?
13. What are the advantages and disadvantages of high meshed bottom board?
14. What are the advantages of the inner lid?
15. What is the prerequisite for successful overwintering?
16. What are the task of a beekeper after the first spring flight?
17. What needs to be done in case of a weak developing colony in spring?
18. How do we develop a standard bee colony at the end of March?
19. What are the measures to prevent swarming in spring?
20. What is important to do with a bee colony during the main spring honey flow?
21. What should be done after summer solstice?
22. What needs to be done during the late forest honey flow?
23. What is the ideal location for an apiary?
24. How does the location of the hives affect the varroa mites population growth?
25. How does the increasing pollen diversity influence the bee's health?
26. Why artificial feeding is not allowed before and during the main honey flow?
27. What kind of bee food is better to secure good quality honey?
28. What are the risks associated with different types of artificial bee food?
29. How do we feed bees before winter?
30. How does inadequate food reserves affect wintering bees?
31. How is it possible to prevent the creation of melecitose reserves?
32. Why is it important to provide clean water and optimal relative humidity for colony development?
33. What type of bee wax is best suited for wax foundations?
34. What is the advantage of producing your own wax foundations?
35. How can microorganisms be used in supporting the bee's health?
36. What is the difference between microorganisms and nutritional supplements?
37. What effect do symbiotic mites have in the hive environment?
38. What are the benefits of propolis?
39. Is it recommended to keep propolis grid on the top of the hive during winter?
40. How is it possible to enlarge the propolis layer inside of a hive?

Co-funded by the Erasmus+ Programme of the European Union

④

PRAKTICAL BEEKEEPING CALENDAR

THE CALENDAR DISTINGUISHES 10 SEASONS. THE PHENOLOGICAL CALENDAR IS ORIENTED TOWARDS RECURRING STAGES OF DEVELOPMENT IN THE PLANT WORLD. THE TRANSITION FROM ONE PHENOLOGICAL SEASON TO THE NEXT IS INDICATED BY CERTAIN INDICATOR PLANTS.

THE LENGTH OF THE 10 SEASONS VARIES FROM YEAR TO YEAR AND FROM REGION TO REGION. THUS, DIFFERENT SITUATIONS RESULT IN DIFFERENT LENGTHS AND BEGINNINGS OF THE PHENOLOGICAL SEASONS EACH YEAR. THE HONEYBEE DEVELOPMENT IS CLOSELY LINKED TO ITS LIVING CONDITIONS.

WINTER 21. 12. WINTER SOLSTICE	SPRING 20. 3. SPRING, DAY AND NIGHT EQUINOX	SUMMER 21. 6. SUMMER SOLSTICE	AUTUMN 23. 9. AUTUMN, DAY AND NIGHT EQUINOX

WINTER	EARLY SPRING	SPRING	FULL SPRING	EARLY SUMMER	SUMMER SOLSTICE	LATE SUMMER	EARLY AUTUMN	FULL AUTUMN	LATE AUTUMN	WINTER
AVERAGE DURATION	37 days	32 days	30 days	22 days	44 days	23 days	27 days	25 days	19 days	106 days
POINTER PLANT	HAZEL	FORSYTHIA (BLOOMING)	EARLY APPLES (BLOOMING)	ELDERBERRY (BLOOMING)	LARGELEAF LINDEN (BLOOMING)	EARLY APPLE (RIPE ENOUGH TO PICK)	ELDERBERRY (RIPE ENOUGH TO PICK)	COMMON OAK (FRUITS)	COMMON OAK (LEAF COLORING)	COMMON OAK (LEAVES FALL)

January
- **Hive entrance control**
- Wax stocktake material
- Planning bee colonies and marketing
- **Further education and reading**
- Maintenance and Reparations

February
- Hive entrance control
- Proper sealing of dead hives
- Colony losses/determination of the cause
- **Food resources inspection**
- Hives and frames preparation

March
- ***Varroa* mite monitoring**
- **Food reserves inspection**
- **Compact brood nest**
- Strengthening of weak colonies
- Cleaning of Varroa tray
- Planting phacelia
- Preparation of hive record cards
- **Reporting winter losses to veterinary authorities**

April
- ***Varroa* mite monitoring**
- **Food inspection**
- Compact brood nest
- First honey super

May
- **VIS-report**
- Adapt honey and brood spaces
- Avoid swarming or catch and treat
- Division of colonies
- Creation of nucs
- Honey harvest

June
- **Honey flow / Food supply**
- Avoid swarming or catch and treat
- Preparation of Varroa-treatment
- Honey harvest
- Getting queens of pure breed

July
- Honey harvest
- **Complete *Varroa*-treatment**
- **Feeding**
- Nucleus

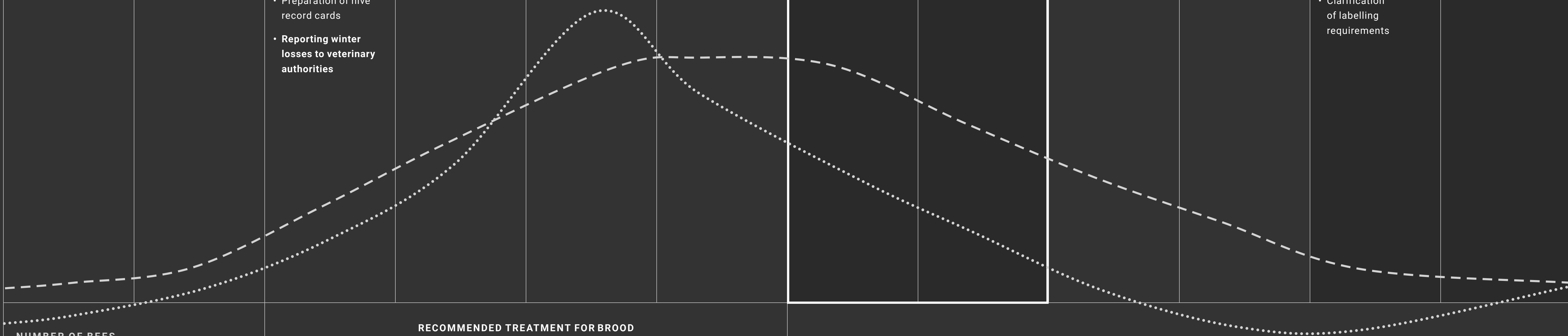
August
- **Feeding**
- Hive inspections
- Only strong colonies should be kept for winter

September
- Hive inspections
- **Only strong colonies should be prepared for winter**
- Finish feeding
- **Varroa monitoring**
- ***Varroa*-after treatment**

October
- Observe hive entrance
- Knocking test
- *Varroa* mite control
- Food control
- **Last revision open hive entrance and hive bottom**
- Fill and sell honey

November
- **VIS-report**
- Hive entrance inspection
- "Brood free hives" ?
- **Last Varroa treatment**
- Prepare bee products for sale
- Sale Advent/ Christmas
- Clarification of labelling requirements

December
- **Last Varroa treatment** *(if not done end of November)*



NUMBER OF BEES *(8 000 – 50 000)* - - - -

NUMBER OF BREEDING CELLS *(0 – 60 000)* ••••••

RECOMMENDED TREATMENT FOR BROOD

Method: The mite infestation is based on a natural daily mite fall calculated from the number of mites found on the monitoring tray after a period of 7 days. There are other diagnostic methods. The following values show the critical limits *(= average daily mite fall in 7-10 days)*. **Recommendation**: 7 days are sufficient, in case of uncertainty observe for another 3 days.

CLEAN VARROA MONITORING TRAY, REMOVE BUILDING RESTRICTION. INSPECTION AFTER 3 DAYS, COUNT MITES PER DAY - ONLY RED FEMALE MITES COUNT.

	January–February	March–April	May–June	July–August	September	October	November–December
NO PROBLEM		0 – 1	1 – 2	MAIN SUMMER TREATMENT	1 – 2	0 – 1	LAST VARROA TREATMENT (IN CASE OF BROOD FREE HIVES)
TREATMENT NEEDED SOON		~ 2	~ 5		~ 3	~ 3	
TREATMENT IMMEDIATELY		> 4	> 6		> 4	> 4	

	March	April	May	June	July–August	September	October	November–December
RECOMMENDED TREATMENT FOR BROOD	VC	VC	VC/OA	VC/OA	VC/OA-LA	VC	VC	OA
RECOMMENDED TREATMENT FOR BEES	OA | LA	OA | LA	OA | LA	OA | LA	OA | LA	OA | LA	OA | LA	

VC = heat treatment of capped brood with the *Varroa* Controller

VC/OA or LA = isolate the queen into Duplex-framebox for 2x12 days, then heat treat the capped brood frames and treat adult bees with OA/LA

OA = OXALIC ACID – either evaporated or trickled

LA = LACTIC ACID – sprayed on the honeycombs or in the swarm box *(to be checked if it is has a VET-approval in your country)*

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