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NO BEES LIFE

EBA MAGAZINE

25 COUNTRIES

FROM WHICH EBA HAS MEMBERS

(35 beekeeping organizations)

In order of confirmation of the Statute of EBA

350.818 beekeepers

Serbia
Slovenia
North Macedonia
Bulgaria
Greece
Romania
Malta
Germany
Hungary
Ukraine
Montenegro
Lithuania
Bosnia and Hercegovina
Sweden
Croatia
Czech Republic
Poland
United Kingdom
Netherlands
Italy
Ireland
Belgium
Cyprus
Türkiye
Switzerland

BEEKEEPING CANNOT SURVIVE UNFAIR COMPETITION!

SCARY HONEY ANALYSIS DATA UP TO 80% FAKE!

Some time ago, the European Commission announced that almost 50% of the honey on the market is fake. These data should be more than an alarm for politics, which is the only one that can regulate this with appropriate legislation. At the initiative of Slovenia, the European Commission changed the directive on honey, and soon honey will have to be labeled with the country of origin. In addition, it has set itself the goal of defining a harmonized method for determining fakes and also defining reference laboratories, but unfortunately this is all too slow, as it should only be in three years. The latest data show that the proportion of fake honey has increased. The European Professional Beekeepers Association – (EPBA) did a survey in Germany and experienced a real SHOCK, as approx. 80% of the honey in hypermarkets is fake, it was the same in Serbia.

Beekeeping will not survive unfair competition from fake honey. Therefore, it is high time that we all act and cooperate, first in line are European politicians, to take strict measures against fake honey, to IMMEDIATELY establish coordinated methods of detecting fakes and reference laboratories. The method MUST be such that it will be followed by inspection in all European countries and will be able to eliminate all fake honey from the market immediately!

We all need to make consumers aware of this problem, because we consume honey to strengthen our health, and fake honey sometimes only harms it. That is why the call goes out to consumers in Europe; "We Europeans choose

European bee products, preferably from the local environment or at least from our country or at least from Europe!

All beekeeping organizations in Europe must join forces for the same goal. Unfortunately, there will be no success without cooperation and joint performance.

The European Beekeeping Association EBA is open to cooperation with everyone, our only goal is the protection of beekeepers and consumers!

Boštjan Noč

President of the Slovenian Beekeeping Association and European Beekeeping Association

Is there a rainbow in sight in European beekeeping?

80% OF FAKE HONEY IN GERMANY

EUROPEAN PROFESSIONAL BEEKEEPERS ASSOCIATION "CLEAN UP THE HONEY MARKET"

In a time of great challenges for the beekeeping sector in Europe, only with joint efforts and support in work, together we can change the reality of the beekeeping sector in Europe.

EBA fully supports the action European Professional Beekeepers Association - EPBA "Clean up the honey market" and we are concerned about the beekeeping sector in Europe, because this action gave birth to the information that 80% of the honey sampled was fake.

EBA wants to cooperate with everyone and with EPBA and Bee Life to achieve the same goal STOP ADULTERATED HONEY!

We hope that this action of our colleagues, with the support of all of us, will raise awareness of the problems we are facing.

We convey the most important parts of his presentation to you, our readers.

The President of the European Professional Beekeepers Association addressed the entire public with one specific piece of news. They tested honey from German supermarkets and got the results. They are terrifying. 80% of honey is fake. The campaign is called "Clean up the honey". In his address, the President of the European Professional Beekeepers Association described all the steps of the action taken, pointed out the fact that honey producers cannot sell their honey and that markets are flooded with honey. He stated that in some European countries up to 75% of professional beekeepers give up beekeeping, pointing out the dramatic consequences for the ecosystem and the environment.

He made a parallel with the year 2021 with the situation in America and Europe. 50,000 tons of cheap honey ended up in Europe because America closed the entrance to such honey.

Cheap honey then flooded the markets, in the exhibition he also showed pictures from German supermarkets with the prices of cheap honey, indicating that no one on the planet can produce honey with such cheap sales prices, which was also pointed out by American professional beekeepers. Also in his presentation, he showed a calculator of the production price of honey.

He pointed out that in March 2023, the European Anti Fraud Office published information that 46% of honey in markets was imported from non-EU countries of dubious origin, and that they do not comply with the standards that apply in the EU legislative. This created unfair competition. He believes that rigorous controls should be introduced at border crossings in order to stop this phenomenon and protect consumers.

He pointed to the appearance of fructose syrups in markets that have nothing to do with honey.

He noted that ordinary laboratory tests cannot detect fake honey and stated the laboratory's explanation: with regard to parameters ehamined, the honey complies with the legal reguriements (EU Honey directive 2001/110/EC).

Lab test the profile suger if the sugar profile resembles honey, it passes.

They suspected a genetically modified bacterium that is used to produce enzymes, the enzymes produce a sugar profile and that bacterium can be programmed to make any sugar profile. It is a patented method (an example is a syrup that has a sugar profile and the laboratory says it is honey).

In his presentation, he referred to companies that produce vegan honey without bees, stressing to consumers not to eat such things.

The samples they tested had a specific smell, taste and texture.

EPBA and DBIB took 30 samples from German markets from different cities and one control real honey from German beekeepers as a marker. They sent it for analysis (NMR, IRMS, Oligosaccharides), knowing that it was counterfeit, but the result of the laboratory in Germany said that it was honey.

They were looking for an alternative to the given situation and came across Estonia , new DNA mass sequencing for testing honey.

Results DNA testing:

3 samples out of 30 samples passed tha DNA test as a real honey;

2 samples barely passed but with comments about notable features;

25 samples failed-not typical DNA profile for honey;

80% OF THE SAMPLES ARE FAKE!

DNA mass sequencing: all DNA in the sample is specified: plants, humans, bacteria, viruses, bees, all organisams groups.

- 4 different models of testing authenticity
- 10-20 milion sequences for analysis
- IN THE COMPOSITION, PROPORTIONS AND CONCENTRATION OF DNA DO NOT MATCH WITH REAL HONEY PROFILES, IT IS A FALL.

DNA examples for fails:

-if the sample only contains spider and bacteria DNA;

-if the only 6 plants in the DNA spectrum

-if there is little or no honey bee DNA at all in the sample

-if the label says its from Mexico, but only has Asian DNA it

-for different date analysis models for testing and authenticity

-if the composition, proportions and concentration of the DNA do not match with the real honey profile, it is a FALL.

The President EPBA pointed out all bad aspects of this result, bad not for bees and beekeepers, but also for consumers, he pointed out that it is pure crime. In the coming days they will sharethe results with all relevant authorities in the EU, EUROPOL, INTERPOL.

Video:
https://vimeo.com/1013237687/087ac38d10?share=copy

THE LITHUANIAN BEEKEEPERS ASSOCIATION ACHIEVED FIRST RESULTS IN THE FIGHT AGAINST FAKE HONEY

The Lithuanian Beekeepers Association (LBA) takes care of the quality of honey and other bee products that are sold in Lithuania.

In the spring, LBA received a letter from the Estonian Professional Beekeepers Association that they made an analysis of the "Mazurskie miody" honey sold in the Estonian supermarket LIDL in the German laboratory QSI. The laboratory detected foreign sugar additives in it and this product was withdrawn from the market.

So, on April 18 we wrote to the Lithuanian State Food and Veterinary Service and asked them to check the quality of the same brand of honey sold in the Lithuanian supermarket LIDL.

On July 25 we received an answer to our request. The Lithuanian State Food and Veterinary Service conducted an unscheduled inspection of JSC LIDL warehouse in Lithuania.

During it they selected a sample of "Mazurskie miody" honey to determine its authenticity.

This sample was sent to the German laboratory for analysis. After receiving the research protocol, it became clear that honey does not meet the requirements for authentic honey.

Foreign sugar additives were detected in the sample. The supermarket LIDL was obliged to remove the product from the market. So, fake honey was withdrawn from the supermarket.

The Lithuanian Beekeepers Association continues active cooperation with the European Beekeeping Association, Estonian Professional Beekeepers Association and government institutions regarding the quality assurance of honey and other bee products.

Lithuanian Beekeepers Association

MEETING WITH THE HEAD OF THE CABINET OF THE PRESIDENT OF THE EUROPEAN PARLIAMENT, Mrs. ROBERTA METSOLA

On September 17, 2024, a meeting was held with the head of the cabinet of the President of the European Parliament, Mr. Kamil May, Mr. Nigel Mifsud – Communications Officer to the President.

The meeting was attended by Mr. Jorge Spiteri, Vice President and member of the Executive Board of the EBA from Malta and Mrs.Biljana Tomić from Serbia, General Secretary of the EBA.

Meeting took place in an excellent atmosphere, where EBA representatives presented the activities and work of EBA, pointed out the problems in the beekeeping sector in Europe, especially the problem of fake honey. The Head of the Cabinet expressed great gratitude for the activities of the EBA, got acquainted with the work of our EBA and wished him good luck in his further work with support in the continuation of the further activities of the EBA.

The meeting is of great importance, the head of the cabinet is the direct representative of the President of the European Parliament, Mrs. Roberta Metsola, and we thank Mrs. Roberta Metsola for the trust shown and wholle team.

Jorge Spiteri, Vice President and member of the Executive Board of the EBA

Biljana Tomić, LL.B.
General Secretary of the EBA

BRIEF REPORT ON THE WORK OF THE EUROPEAN BEEKEEPING ASSOCIATION FOR SEPTEMBER 2024.

The European Beekeeping Association (EBA) is an organization dedicated to the protection and promotion of beekeeping across Europe and is deeply committed to safeguarding the well-being of bees, beekeepers, and consumers throughout the continent.

During September 2024, the European Beekeepers Association continues its work on cooperation with the Members of the European Parliament on the issue of protecting European honey markets from counterfeit products.

All EBA members, the EBA Executive Board and the EBA Scientific Committee are discussing all possible measures necessary to consolidate the efforts of European officials, beekeepers, scientists, consumers in protecting national honey markets. Our slogan “Europeans choose European honey” works at all levels.

EBA fully supports the action of the European Professional Beekeepers Association EPBA “Clean up the honey market”. The European Beekeepers Association is ready to cooperate with Bee Life European Beekeeping Coordination, EPBA European Professional Beekeepers Association, and other European beekeeping organizations to achieve positive results in the fight against honey falsification.

In September a meeting of European Beekeeing Association was held with the Head of the cabinet of the President of the European Parliament, Mr. Kamil May, and Mr. Nigel Mifsud Communications Officer to the President of the European Parliament.

The meeting was attended by Mr. Jorge Spiteri, Vice President of the EBA and member of the Executive Board of the EBA from Malta and General Secretary of the EBA Biljana Tomić from Serbia.

At the meeting, representatives of the European Beekeepers Association informed about the

activities of the EBA, the main goals and objectives of the Association. Attention was paid to the issue of honey falsification and the problems associated with this fraud for beekeepers and consumers in all European countries.

The European Beekeeping Association continues to build its beekeeping capacity.

In September, the following beekeeping associations joined the European Beekeeping Association:

- The Foundation for the Conservation of the Maltese Honey Bee from Malta;
- Imkers Nederland;
- The Bulgarian Association of Organizations of Producers of Honey and Bee Products from Bulgaria;
- The Irish Bee Foundation from Ireland;
- The Cercle apicole de Charleroi (CAC asbl) from Belgium;
- The Pancyprian Beekeepers Association from Cyprus;
- The Turkish Association of Beekeepers.

As of October 1, 2024, the European Beekeeping Association includes 34 beekeeping associations from 24 European countries.

In September, the European Beekeeping Association's Scientific Committee met, consisting of the Beekeeping Products Safety and Quality Committee and the Bee Health Committee. The Scientific Committee brings together professionals and researchers who study issues and provide scientific advice on aspects of bee product safety and quality, bee health protection in Europe, etc.

At the end of September, all members, partners, sponsors, friends and supporters of the European Beekeepers Association will have the opportunity to read the news and events at EBA in the next fourth issue of the magazine “NO BEES, NO LIFE”.

EBA is grateful to all partners and sponsors who help in its work and thus the European Beekeepers Association is able to work and develop for the benefit of European beekeepers

Vasylkivska Tetyana
Vice President of the European Beekeeping Association

NO BEES LIFE
EBA MAGAZINE

PUBLIC CALL

FOR PROPOSALS FOR CO-ORGANISATION OF EBA MEMBERS' EVENTS IN 2025

ABOUT THE CALL

The European Beekeeping Association (EBA) is offering support to its member organisations through the EBA Events Fund 2025. The aim of the Call is to promote and raise awareness of the importance of bees and bee products through different events, as well as connect all entities that can contribute to the improvement of the beekeeping sector in Europe.

WHO CAN APPLY

The call is open to EBA member organisations (individuals are not eligible), and each organisation may submit only one application. The proposed event must align with one or more of the priority areas outlined in EBA's objectives. Events should be held in Europe and must be open to the general public. Only one application per event will be accepted. Additionally, the ap-

plicants must secure their own funding for the event since EBA, as co-organiser, provides only a contribution to its realisation.

Applicants who provide translation into at least one foreign language or lecturers who hold lectures in a foreign language with translation into the host language will have priority in selecting the event.

APPLICATION PROCEEDURE

Applicants are required to submit the following documents:

A completed application form (download on https://ebaeurope.eu/public-call-for-proposals-for-co-organisation-of-eba-members-events-in-2025);

A description of the event, including its objectives and the proposed agenda or programme;

A plan detailing how EBA's co-organiser role will be visibly acknowledged at the event.

Applications must be submitted in English via email to eba@ebaeurope.eu with the subject line "EBA EVENTS FUND 2025 – Application".

The deadline for submission of applications is November 1st, 2024.

The EBA Executive Board will select up to five events for co-organisation in 2025. Each selected event will receive a grant of 1500 EUR (gross), disbursed to the applicants after the event, following approval of a final report.

Results will be announced by the end of November 2024.

JOIN THE EBA VIBER AND WHATSAPP GROUPS FOR FREE

Anyone interested can join the EBA Viber or WhatsApp groups free of charge to inform beekeepers about EBA activities, regardless of whether you are an EBA member or not, so that you can be timely and properly informed about everything important in EBA and European beekeeping.

If you have the Viber or WhatsApp application installed, in order to join the EBA groups, you just need to click on the following link:

For Viber: https://ebaeurope.eu/join-the-eba-viber-group-for-free
For WhatsApp: https://ebaeurope.eu/join-the-eba-whatsapp-group-for-free

BEES LIFE

IT IS WRONG TO CLASSIFY HONEY AS FREE SUGARS

Free sugars include white and brown sugar, glucose, high fructose syrup (HFCS, IFIS)), inverted syrups, molasses, and other sweetening substances added during cooking or food preparation, as well as in soft drinks, alcoholic beverages, and other commercial products. Free sugars are devoid of significant nutrients, and their excessive consumption leads to cardiovascular diseases, fatty liver, type 2 diabetes, obesity, hypertension, and chronic inflammation. This is why nutrition experts recommend avoiding their consumption as much as possible.

The sugars in honey are mainly simple. In pure categories of Greek honey, glucose ranges from an average of 21% to 36% and fructose from 24% to 40%, depending on the botanical origin, approximately in a 1:1 ratio. This ratio is also true for common sugar (sucrose), which breaks down in the digestive tract into equal parts of glucose and fructose (50:50), and for high fructose corn syrup (HFCS 55%), where the ratio is fructose and glucose 55:45%. This similarity of honey to sucrose and corn syrups is often misinterpreted by some, resulting in honey being classi-

fied as a free sugar, and consumers being advised to avoid it.

Honey is a natural product that undergoes no chemical or other processing, and its biological effects on the body differ significantly from those of free sugars. The sugars in honey can resemble those found in fruits and vegetables but not sucrose or other sweeteners. In addition to simple and complex sugars, honey contains minerals, trace elements, fatty and organic acids, amino acids, aromatic substances, antibiotics, vitamins, enzymes, phenolic acids, flavonoids, and other components. The organic integration and synergistic action of more than 200 different substances in honey give it unique properties entirely different from those of common sugar and syrups, which are products of industrial processing.

The different effects of honey on the body compared to free sugars have been documented by clinical studies published in international scientific journals (Samarghandian et al., 2017; Baglio, 2018; Bobis et al., 2018; Yaghoobi et al., 2008). Honey is not just sugar. Its consumption shows a synergistic action of its components in controlling blood glucose, which includes a combination of lower glycemic impact due to the natural composition of its sugars, enhanced insulin sensitivity through antioxidants and anti-inflammatory compounds, prebiotic effects on gut health due to the oligosaccharides it contains, enzymatic activity from glucose oxidase, which has antimicrobial properties and affects metabolic processes, and the potential regulation of incretins, hormones involved in the physiological regulation of glucose homeostasis.

Honey is a healthy alternative to processed sugars, and its consumption does not cause the problems associated with free sugars..

Andreas Thrasyvoulou
Emeritus Professor
Aristotle University of Thessaloniki (AUTH)
Hellenic Republic (Greece)

References

Baglio Ettore (2018) Chemistry and Technology of Honey Production. Springer Briefs in Molecular Science ISSN 2199-689X ISSN 2199-7209 (electronic)

Bobiş O, Dezmirean DS, Moise AR. (2018) Honey and Diabetes: The Importance of Natural Simple Sugars in Diet for Preventing and Treating Different Type of Diabetes. Oxid Med Cell Longev. Feb 4;2018:4757893. doi: 10.1155/2018/4757893. PMID: 29507651; PMCID: PMC5817209.

Samarghandian S, Farkhondeh T, Samini F. Honey and Health: A Review of Recent Clinical Research. Pharmacognosy Res. 2017 Apr-Jun;9(2):121-127. doi: 10.4103/0974-8490.204647. PMID: 28539734; PMCID: PMC5424551.

Yaghoobi, N., Al-Waili, N., Ghayour-Mobarhan, M., Parizadeh, S., Abasalti, Z. & Yaghoobi, Z. (2008). Natural honey and cardiovascular risk factors: Effects on blood glucose, cholesterol, triacylglycerole, CRP, and body weight compared with sucrose. The Scientific World Journal 8: 463-469.

INTERRUPTING EGG LAYING FOR HIGHER YIELDS AND MORE EFFECTIVE CONTROL OF VARROA MITES

In my beekeeping, I have been interrupting egg laying on about 200 colonies in AŽ-beehives for quite a few years. I do this in the time before the flowering of the linden and chestnut trees (and the forest honeydew flow, if it is happening at the same time). My method of interrupting the egg laying is not the same as others that employ the confining of queens, as I take the queen and use her to make a spare colony.

During this time, I want to have as many bees in my colonies as possible that are able to collect nectar. At the same time, I also want to ensure that bees have as few hive tasks as possible, and especially as little work as possible taking care of the young brood. With my interventions, I must therefore prepare the bees to make the best use of this forage. I do this as follows. Three to five days before the planned start of flowering of linden and chestnut trees or forest honeydew flow, I perform a thorough examination of the bee colonies. This is also the last thorough inspection of bee colonies in the season. During this inspection, I take away the queens from the colonies and thus interrupt the egg laying process.

When inspecting, I do the following:

– I assess the quality of the queen (with the help of notes on the hive inspection sheet and the situation in the colony). If I assess that the queen is strong, then I use it to make a spare colony. If I judge that the queen is weak, regardless of her age, I destroy her. For oneand two-year-old queens, I pay particular attention to the quality of the brood, the gentleness, the colony's level of congestion, the breed purity and the previous honey yields. I arrange the brood chamber; I only leave combs in the brood chamber that have

a nice light brown colour with as few drone cells as possible.

– For colonies that have at least seven to nine brood combs and a large number of bees at this time, with normal development and a strong queen, I remove the queen together with the vast majority of the uncapped brood.

– I replace the removed combs with brood exclusively with comb foundations.

– During this time, I also cut out the drone comb on the natural comb, as this is a very important apitechnical measure. This is the last cutting-out, as there will be no queen in the hive in the following period, and afterwards the young queen will usually no longer lay eggs in the drone cells.

– In the colony, I mark the combs that have some eggs on the edge of the combs. From these eggs, the bee colonies will pull out the queen cells so that they can breed a new queen.

The next inspection of the same colony follows no later than the eighth day after the removal of the queen. At that time, under favourable conditions, honeydew forage already takes place in nature and the bees are diligently bringing in nectar. This inspection should be carried out in order to inspect all the combs with brood on them, to see where the bees have pulled out the queen cells in order to breed the new queen. I destroy all the queen cells that have been pulled out of the older brood, but leave the two queen cells on the comb that I had previously marked as having eggs on the edge of it. This tells me that the bees pulled quality queen cells from these eggs, which were supplied with royal jelly from the beginning.

I always leave two queen cells: one in the brood chamber, and the other I cut off and place in the honey chamber, so that at least one queen will definitely hatch and, of course, at least one will also mate. Of course, I open the honey chamber so that the young queen, when she hatches in the honey chamber, will be able to fly away to mate.

The next inspection is necessary only after 15 to 18 days from the first inspection or on the 23rd to the 26th day from the removal of the queen. There is no point in doing it earlier, as we have already inspected the colonies on the eighth day after the queen has been removed, and in the meantime the colonies should have a quiet period until the next inspection, so that they can

make the most of the honey flow in peace. Any unnecessary intervention during this time reduces the yield of honey by a few kilogrammes. The queen will hatch on the 15th to 16th day after the queen has been removed, and it is not until about 10 days after the queen has hatched that it is realistic to expect the first brood, i.e. it is possible to expect the brood of a new young queen hatched from the queen cell sometime around the 26th day after the queen has been removed.

At the third inspection, I look to see if the queen has mated in this colony, I look at the honey chamber and the brood chamber, as I left the queen cell in both parts of the hive.

As soon as I realise that the queen has started to lay eggs after days 23 to 26, I have to get ready to extract the honey as soon as possible. This should be done at least two to three days before the brood is capped in the hive, so that I still have time to deal with the varroa mites. Usually, 30 days after the queen is removed, the brood is not yet capped. Since there is no brood in the hive at this time, or the young brood is only present on a comb or two, there will be honey in all the combs in the hive if the honeybee forage is good. This way, I can extract the entire honey chamber and for a maximum of four combs also from the brood chamber. I leave at least six honeycombs for the bees so that they have a good quality food supply. I leave the honeycombs that have a beautiful light brown colour and extract the older ones and melt them into wax.

Since I have left one queen cell above and one queen cell below in the colony, it may happen that I get a young mated queen above and below in that colony.

If the bee colony is very congested, I partition the hive with a hardboard and leave both queens and treat them as two colonies. In this case, I take a maximum of six honeycombs from the brood chamber and the honey chamber, leaving

at least four honeycombs for the bees on each floor.

If I think there are not enough bees for two colonies (if there are not many bees in the brood chamber and if they are present in at least five combs in the honey chamber), I remove one queen and put her where I need the mated queen.

Fighting varroa mites is the beekeeper's single most important task after the last honeybee forage!

After you have extracted the honey, we immediately begin suppressing varroa mites with a registered medicine according to the instructions of the competent veterinarian. Since the brood is not capped in the hive during this time, high efficiency of varroa mite suppression is achieved. I would like to emphasise once again that we must suppress the varroa mites before the brood is capped in the hive again. This usually occurs after the 30th day after the queen has been removed!

Watch out for honeybee robbing!

It is important to determine whether or not there is a queen in the hive before extracting. If there is no queen, it is imperative to add a mated queen to the hive before extracting. If you were to extract from a queenless colony, supress varroa mites and return honeycombs during the non-foraging period, you would create a great risk of honeybee robbing.

In my beekeeping, I do not interrupt egg laying by confining the queen. In my experience, it does not make sense to keep the queen confined

during the main forage period, thus preventing her from breeding. It is significantly better to create a spare colony. In contrast to the queen-confining method, in my case we use the queen to lay eggs in a newly made spare colony during a period of abundant honeybee forage.

This will give us a quality and strong spare colony from each colony, which is very important in modern beekeeping. In a very simple way, we will get an extra spare colony with bees that the colony does not necessarily need in August and that are much more useful for making new colonies. We will be able to use this spare colony to strengthen colonies in the autumn or as a spare for the spring period.

Boštjan Noč
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The article Interrupting egg laying for higher yields and more effective control of varroa mites was published in the magazine Slovenski čebelar 6/2021, p. 175. According to readers, this was the best expert article in 2021 – https://www.czs.si/objave_podrobno_czs/12408

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VARROA DESTRUCTOR MITES REGULARLY GENERATE ULTRA-SHORT, HIGH MAGNITUDE VIBRATIONAL PULSES

Abstract

The ectoparasitic mite Varroa destructor is considered one of the greatest threats to the honeybee Apis mellifera. To successfully manage mite populations residing in the colony, beekeepers must stay informed of infestation levels in their apiaries. The remote, non-destructive detection of Varroa mites in honeybee hives would therefore be highly desirable. Here we show that an ultra-sensitive (1000 mV/g) accelerometer can detect vibrational waveforms originating from one individual mite. We further focus on a commonly observed pulsing behaviour never before described, characterising its physical features, periodicity and strength. The spectral features of the detected pulses strongly depend on the substrate on which they are produced. The characteristics of the vibrational pulse, particularly its repeatability and strength, indicate that mite vibrations could be successfully detected in a fully populated honeybee hive. These features, combined with the remarkably high varroa muscular power output (up to 810nW) indicate that this pulse may be functional for the mite. Our results uncover an exciting novel behaviour and provide a foundation for the remote detection of mites in beehives using vibration capture.

Keywords: varroa, vibration, biotremology, honeybee, behaviour

1 Introduction

The Varroa destructor mite (Anderson & Trueman 2000) is described as the most influential ectoparasite of the European honeybee, Apis mellifera, in terms of its destructive impact (Rosenkranz et al. 2010; Evans & Cook 2018). Bee health and development is negatively affected by the mite feeding on both adult bees and larvae, particularly as by doing so, it acts as a vector of viral diseases (Kevan et al. 2006; Chen & Siede 2007). Evidence has recently been found to demonstrate that mites feed primarily on the fat body of adult bees, which is an important tissue necessary for protein synthesis, pesticide detoxification and successful overwintering of colonies (Ramsey et al. 2019). Mite and bee are closely linked because the parasite lacks a free-living stage, instead adopting a two-phase life cycle: phoretic (on adult bees) and reproductive (within sealed brood cells). The adult bees carry a phoretic mite towards a brood cell, and the mite enters it a short time before the cell is capped in order to begin its reproductive phase (Rosenkranz 2010; Nazzi & Le Conte 2016). Once in the

cell, mite reproduction appears tobe synchronised with the development of bee larvae, regulated by the production of larval signals (Fr ey et al. 2013).

1.1 The importance of detecting and monitoring Varroa mites in honeybee hives

Varroa infestation can lead to the complete destruction of colonies, particularly over the winter months as high mite levels in the autumn impact upon bee lifespan. This in turn affects the bees' ability to transition from a winter bee into a summer bee, which is critical for colony success moving into the warmer months (Le Conte et al. 2010). The phenomenon known as Colony Collapse Disorder (CCD) is also thought to be linked to Varroa presence in a hive, as mites are the cause of many adverse reactions in honeybees. It is the interaction between these mite-related effects, combined with other stressors such as pesticides that is accepted to lead a colony to CCD (Le Conte et al. 2010). Beekeepers therefore need to stay on top of Varroa mite infestation in their apiaries. Many beekeepers treat preventatively, regardless of mite population size (Lee et al. 2010). However, 'blind' use of chemical control can be detrimental without knowing the full extent of the infestation level and whether treatment is truly needed at that time. This is due to the adverse effects of acaricides on honeybee processes and functions (Tihelka 2018), and mite resistance to chemical control, which has been documented around the world (Milani 1999; Martin 2004). This could be avoided through infestation checks and the use of alternative or rotated treatment types (Milani 1999; Pettis 2004). Regular monitoring is particularly useful to identify trends in the mite population (Gregorc & Sampson 2019), so that the mite control treatment can be applied at the correct time, if at all needed. However, at present, the monitoring methods available to beekeepers and honeybee/Varroa mite researchers are time-consuming, require physical visitation to hives and can be disruptive to the colony.

Counting the natural mite-fall to produce a whole colony infestation estimate, for example, is inaccurate unless it is done daily over a prolonged period (Bienkowska & Konopacka 2001; Pietropaoli et al. 2021), which leads to regular visitation requirements. Other techniques such as sugar shake, washing and brood sampling require the removal and/or destruction of adult bees and brood (Dietemann et al. 2013; Barlow & Fell 2006). Although the sugar shake method allows bees to be placed back in the hive, there is still colony disruption caused, as well as inaccuracies that can be caused by sugar quality, temperature or humidity (Fakimzadeh et al. 2011; Gregorc et al. 2017).

1.2 Remote Varroa monitoring techniques

There has been a recent move towards investigation into remote monitoring techniques for honeybee colonies, including Varroa detection. Improvements to reduce the number of hive visitations and negative impacts upon the colonies can be only be a positive step. Currently, the use of gas sensors (otherwise known as electronic noses, capable of identifying complex odours (Bąk et al. 2020)) and video for detecting mite presence in hives are being explored. At present, these sensors are being tested for their ability to discriminate between healthy and mite-infested colony odours (Bąk et al. 2020; Szczurek et al. 2020a; Szczurek et al. 2020b; Konig 2021), and in most cases these can be inserted into a hive and left to carry out continuous measurements that can be accessed remotely (Szczurek et al. 2020a; Szczurek et al. 2020b; Konig 2021). However, they are at risk of being propolised. In terms of video detection, various image processing techniques and training software have been employed to identify the presence of a mite in video recordings of the adult bees or within brood cells (Ramirez et al. 2012; Elizondo et al. 2013; Chazette et al. 2016; Bjerge et al. 2019; Bilik et al. 2021). This work is currently in a prototype phase with some limitations, including video clarity affecting the chance of a successful detection (Elizondo et al. 2013) and the requirement for powerful hardware to carry out complex com-

putations (Bilik et al. 2021). In the field, videos can only be recorded at the hive entrance (Bjerge et al. 2019), high-lighting another flaw as this type of monitoring presently cannot take place within the darkness of the hive.

Here, we employ ultra-sensitive (1000 mV/g) accelerometer sensors to capture vibrations produced by mite individuals. This technology has previously been employed by our research group for the remote detection of various individual honeybee vibratory signals, as well as providing vibration information on the status of the colony as a whole (Bencsik et al. 2015; Ramsey et al. 2018; Ramsey et al. 2020). The sensors are secured to the comb at the centre of the hive and can be left for unlimited, prolonged periods to continuously monitor and record the vibrations captured within the colony, irrespective of propolis/wax coating or darkness levels. In this present work we establish whether mite vibrations can be successfully detected with this technology, with future implications for its use within honeybee hives to remotely and continuously monitor Varroa levels. In doing so, we also present a new Varroa behaviour.

2 Methods and materials

2.1 Mite collection

Measurements were undertaken at Nottingham Trent University between August 2019 and December 2019. Live Varroa mites were identified and collected daily from the baseboard of a colony of Apis mellifera. Mites were immediately taken to the laboratory for use within one hour of collection. Live mites were weighed on a set of electronic scales (Kern ALJ 160-4NM) together, and an average taken, as a single mite was too small to register on the scales (n = 12, 0.42 mg).

2.2 Visual and vibrational measurements

Mites were placed either onto (i) a plastic Petri-dish (50mm × 10mm), (ii) a “British Standard” sized frame of fully built, but empty comb or (iii) a piece of loaded brood-comb, removed from a hive using a scalpel (two samples were used: 1 – 3.5 × 2cm diameter, containing 6 empty cells and 25 capped brood cells, and 2 – 4 × 2cm diameter, containing 17 empty cells and 20 capped brood cells). An ultra-sensitive accelerometer (4507 B 002, Brüel and Kjær, 1000 mV/g) was used to collect the vibrational data, glued to the Petri-dish or secured centrally to the frame of comb by pushing it into the comb and adding a small amount of molten wax to hold it in place. Brood-comb samples were placed on the Petri-dish with accelerometer attached. The activity of the mites on each substrate was filmed and the vibrational data synchronously recorded with a camera (Sony 4K FDR-AX100E handycam, China). Each filming session lasted from 10 to 20 minutes, for all three substrates. Mites were placed on each substrate in randomly selected groups (ranging from 1 individual to 19 individuals), dependent on the number of mites collected that day. Overall, the number of videos recorded for each are as follows: i) Petri-dish (n = 10), ii) honeycomb (n = 5), iii) brood-comb (n = 2). The full technical details for this set-up and all further experimental design can be found in supplementary material S1.

The recordings were then examined to identify behaviours of interest. Several behaviours were identified that produced a detectable vibrational trace (supplementary Fig. S2, S3, S4 and S5), but we chose to focus on those that were deemed the most promising for yielding exciting science and for further analysis. The pulse that is the main focus of this study is a vibrational trace that is produced by a novel behaviour, which we term ‘jolting’. Attempts to capture acoustic sound from this behaviour with both a standard microphone (Tascam DR-05X, USA) and ultrasonic bat detector (Magenta Bat 5, Tutbury, UK) were unsuccessful, indicating that jolting pulses produce vibratory signals only.

For all recordings (265 minutes of footage in total) where more than one mite was in the field of view, each individual mite was tracked to record the corresponding number of jolting pulses produced, in order to obtain the percentages of mites that produced the behaviour of interest.

For in-depth analysis of jolting pulse features, three videos comprising of a (different) jolting mite individual were chosen, one for each of the

three substrates. Focus was placed upon these individuals as they produced a high number of the pulses of interest in succession with visible and hearable accelerometer traces.

2.3 Comparing Varroa vibrations with honeybee vibrations

The strongest Varroa jolting accelerometer waveform on each substrate was compared with (i) mite walking vibrations, (ii) the vibrations of an individual honeybee and (iii) those originating from a full honeybee colony. The strongest jolting pulse was initially chosen to demonstrate the current best-case scenario for discriminating between the vibrational trace of jolting and those of honeybee and Varroa behaviours. The magnitude of acceleration of every jolting pulse in the three collections was then also compared to the vibrational traces of honeybees and Varroa behaviours to provide an overall comparison.

'Low' and 'high' signal recordings of the honeybee colony (depending on the comb mass density load) were collected for the full colony comparison. The entire colony is distributed on ten frames, resulting in typically 1000 to 2000 honeybees per frame, and we expect that around 10% of these individuals contributed to the measured signal.

An individual honeybee vibratory signal (a 'whooping' signal (Ramsey et al. 2017)), was also extracted from this data, for the individual honeybee comparison.

The brood-comb substrate data was chosen for visual comparison to the colony and individual honeybee vibrations as the brood-comb set-up is (i) the closest to the mites' natural environment, and (ii) is the best match of substrate types onto which accelerometers were attached. This allowed us to explore the possibility of detecting mites amidst the continuous honeybee signal.

2.4 Estimating the power required for Varroa jolting pulse production

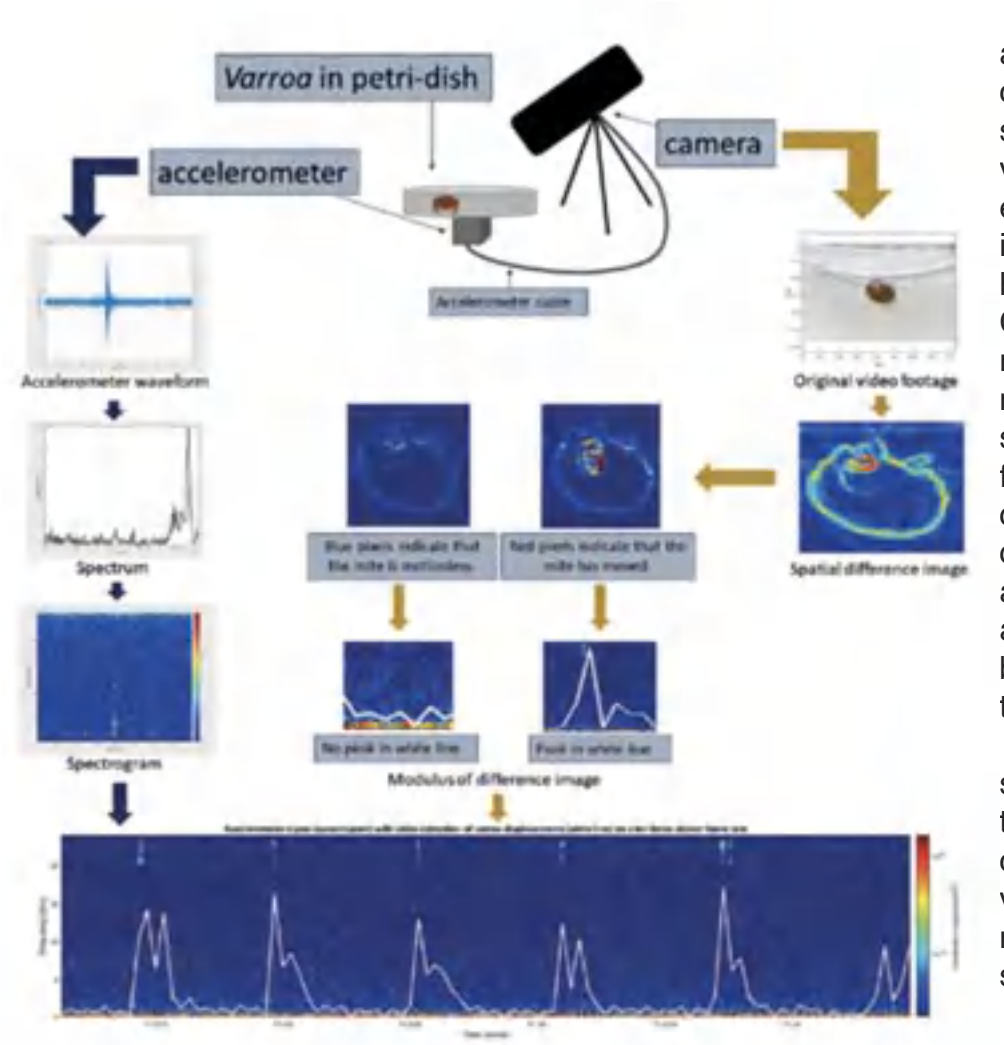
The power output of the jolting pulse (i.e. the muscular power required by the animal to produce the vibration (Michelsen et al. 1982)) was estimated by an experiment aiming at replicating the vibrational trace of the Varroa jolting signal. Glass beads (400 µm in diameter) were dropped from a height of 1cm onto the Petri-dish, both directly above and slightly offset the accelerometer, to replicate the differing positions of mites when jolting. Fifteen repeats were taken for each position.

The bead velocity was measured by estimating the distance travelled by the bead frame-by-frame as it was dropped, using a video captured at 240 frames-per-second (FPS). The average weight of a collection of glass beads was taken as a single bead was too light to be detected by the scales (8028-series professional digital jewellery scale, China) (0.07 mg).

2.5 Signal analysis

All signal analysis was conducted through Matlab (Refworks 2019a) using code written specifically for this study at Nottingham Trent University. The vibrational trace of the jolting behaviour was analysed directly from the audio track of the video footage. The synchronicity between the vibrational trace and the movement of the Varroa mite was demonstrated through the creation of a separate movie (one for each of the substrates investigated). Each jolting pulse vibrational trace was extracted from the accelerometer data and further analysed using their waveforms and spectrograms. All the pulses in each substrate collection were carefully synchronised using the cross-correlation product so that comparisons could be made within the three collections. Two methods of alignment were used, dependent on the type of analysis that was to be performed. Varroa walking pulse vibrational traces were also extracted from the three recordings for comparison against the jolting pulses on each respective substrate.

2.6 Signal to noise ratio analysis/successful detection of jolting pulses



To establish how detectable the jolting pulse is, by accelerometer evidence only, the signal-to-noise-ratio (SNR) value was first estimated for each substrate and each jolting pulse: Petri-dish, empty honeycomb and brood-comb. Critical listening of the accelerometer recording of the signals was then undertaken, separately. Playback speeds for each substrate were reduced accordingly, to best demonstrate the pulses audibly (Petri-dish reduced by a factor ten, honeycomb and brood-comb reduced by a factor 2).

The percentage of audible signals was finally calculated to pinpoint the (independently calculated) SNR threshold value, indicating where the method begins to detect jolting signals.

Fig. 1. A diagram to demonstrate the process of analysing the jolting pulse data. All three mites on each substrate underwent the same video analysis. The mite is placed on the substrate with the accelerometer attached and is filmed with the camera, pro- ducing a movie that has synchronous audio and video. The left- hand side of this diagram demonstrates how the vibrational data are processed. The raw accelerometer waveform of the movie is transformed into a spectrum, which shows the frequency (Hz) (x axis) and magnitude of acceleration (m/s2) (y axis) of the vibra- tional data. A collection of such spectra are then stacked into a spectrogram, which shows time (seconds) (x axis), frequency (Hz) (y axis) and magnitude of acceleration (logarithmic to the base 10) (pixel intensity). Dark red shows the highest magnitude and dark blue shows one 70th of this maximum. The maximum acceleration magnitude is forced to be that of the Varroa jolting pulses for better viewing, resulting in clipping of the irrelevant data at the lowest frequencies. The accelerometer recording for the entire original movie is transformed into a spectrogram, run- ning with respect to time. The bottom panel shows a two-sec- onds long section of this movie. The right-hand side of the diagram demonstrates how the visual data are processed. The original video data that is collected is further cropped to better focus on the mite for the purpose of simple edge detection (by means of the spatial gradient of the pixel intensity). The modulus (absolute value) of the difference image shows the changes in pixel intensity in two consecutive frames as seen in the spatial difference image. In the modulus of the difference image, the mite is mostly dark blue when motionless, but exhibits edges that flash red when moving abruptly.
The sum of the pixel intensities in this panel are then displayed as the white line that is superimposed on the spectrogram data of the bottom panel. This demon- strates the remarkable synchronicity between video-detected mite displacement and accelerometer trace. This processing is used to create the supplementary videos S9, S10 and S11.

2.7 Accelerometer and Petri-dish resonance analysis

To measure the inherent resonance of the accelerometer that is used in our work, a full frequency sweep (0-24000 Hz) was conducted, using the same equipment as for the rest of the study. The amplitude of the accelerometer recordings, taken at three signal magnitudes, was compared by plotting the ratio between the signal sizes. The Petri-dish with attached accelerometer was also measured with a full frequency sweep (0-24000 Hz) to establish the frequency response and to then compare it to that of the accelerometer alone. The responses of the Petri-dish and accelerometer were then visualised together.

3 Results

3.1 The behaviour of interest: the jolting pulse

The vibration that is the focus of this study, here termed 'jolting pulse', chosen because of the corresponding abrupt movement of the body, is extensively showcased and analysed. We describe this behaviour as a rapid pulsing of the body either in a left or right direction, before returning to the original central resting position (supplementary video S6). When producing a jolting pulse and viewed from a side-on position, the Varroa legs can also occasionally be observed to flex, moving the body down and then back up again off the substrate (supplementary video S7). During all video observations, mites were only observed to jolt when stationary.

Mite jolting behaviour was recorded and analysed on three substrates (empty honeycomb, brood-comb and Petridish). Overall, 55 mites were inspected for general jolting patterns. 46% of these were never observed to jolt. The remaining 54% jolted at some stage during the video footage, categorised as either jolting without an obvious trend (42%), or jolting with an obvious trend (13%) (supplementary Fig. S8). We define 'obvious trend' as a mite repeatedly jolting in quick succession, with 10 seconds or less between consecutive jolts for more than one minute following commencement of the behaviour. A substantial number of jolting vibrational pulses were measured on Petri-dish (n = 250) and on brood comb (n = 189), with a smaller sample size for honeycomb (n = 28). Other vibrational traces were also detected when mites produced recognisable motions such as walking (supplementary video S2).

All observed behaviours except one (defecation) produced measurable accelerometer traces (supplementary videos S3, S4 and S5).

3.2 Relating the Varroa vibrational pulse to the video recording of jolting

The absolute timing of each jolt accelerometer trace was assessed within the sampling rate used for that signal, i.e. 48 000 Hz. The vibrational trace is systematically seen (Fig. 1) to be produced within the time window of the videodetected mite jolting displacement, which is assessed within an interval of two successive video frames i.e. 20ms. Despite this large discrepancy in the sampling rates of the two independent assessments of the jolting, our analysis demonstrates (Fig. 1) that the observed vibrational trace and the jolting behaviour of the mite are consistently synchronous phenomena to within 20ms (Fig. 1, supplementary videos S9, S10, S11). Figure 1 also demonstrates that the visually assessed time duration of the jolting (the width of the white peaks) is substantially longer to that assessed from the vibration (the spectrogram traces).

3.3 Effect of substrate

The Varroa jolting vibrational trace that exhibited the highest magnitude was extracted from each substrate-specific collection of measurements (Fig. 2). This trace varies substantially, in shape mostly, and modestly in time duration, dependent on the substrate on which the mite resides. The substrate-specific features observed in the loudest pulses can also be seen in most of the jolting vibrational traces. On the Petri-dish, the signal is mostly found at the highest

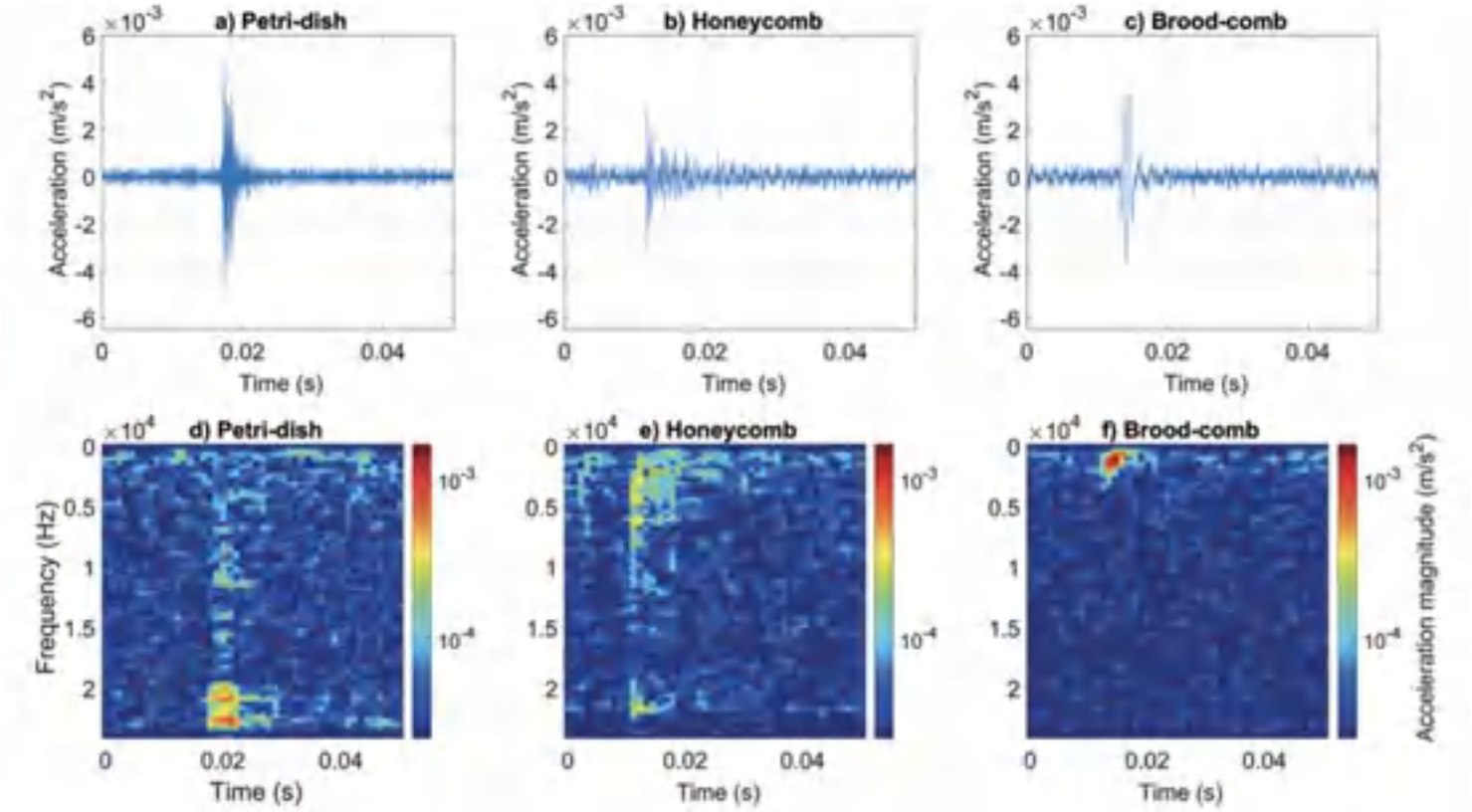


Fig. 2. A series of accelerometer waveforms (top) and spectrograms (bottom) allowing the comparison of the vibration originating from the strongest Varroa jolt, detected within three separate substrates (Petri-dish (a and d), empty honeycomb (b and e), and broodcomb (c and f). The accelerometer waveform has been high-pass filtered (500 Hz cut-off for all substrates) to remove irrelevant background vibrations that otherwise dominate the waveform. In the spectrogram, acceleration magnitude is logarithmic (to the base 10), where the highest magnitude is dark red (1.6×10-3 m/s2) and the lowest magnitude dark blue (and forced to 1/70 of the maximum). Panels found in the top, and the bottom, are scaled identically for ease of comparison

measured frequencies (19 to 23 kHz), with the strongest signal contribution found between 22 and 23 kHz (Fig. 2, supplementary video S12, supplementary Fig. S13). On the brood-comb, the opposite situation occurs, with the signal of highest magnitude found at the lowest frequencies between 1 and 1.5 kHz (Fig. 2, supplementary video S14, supplementary Fig. S15). In the honeycomb the signal is found over a much broader bandwidth between 0.5 and 10 kHz (Fig. 2, supplementary video S16, supplementary Fig. S17, supplementary Fig. S18). As the pulses in Fig. 2 are exceptionally strong, the Varroa jolting pulses also stimulate a collection of frequency bands rarely visible on the full collection of spectra.

The maximum acceleration values of the strongest jolts are similar between the honeycomb (0.0032 m/s2) and brood-comb (0.0035 m/s2), with a stronger acceleration for the loudest Petri-dish pulse (0.005 m/s2) (Fig. 2). In spite of this, the trace on the honeycomb spectrogram is noticeably fainter (typically reaching the yellow colour, i.e. approximately 5 times weaker than the signal seen otherwise in the deep red band), due to the signal being spread over a remarkably broad band. All jolting pulses have an exceptionally short time duration, regardless of the substrate. The jolting pulse appears as a single, rapid pulse that takes between 0.05ms and 0.09ms to grow, exponentially, from the start of the signal to maximum acceleration on the honeycomb and Petri-dish substrates, respectively. On the brood-comb, the growing wave front appears gaussian, with a 1ms typical time duration (supplementary Fig. S19).

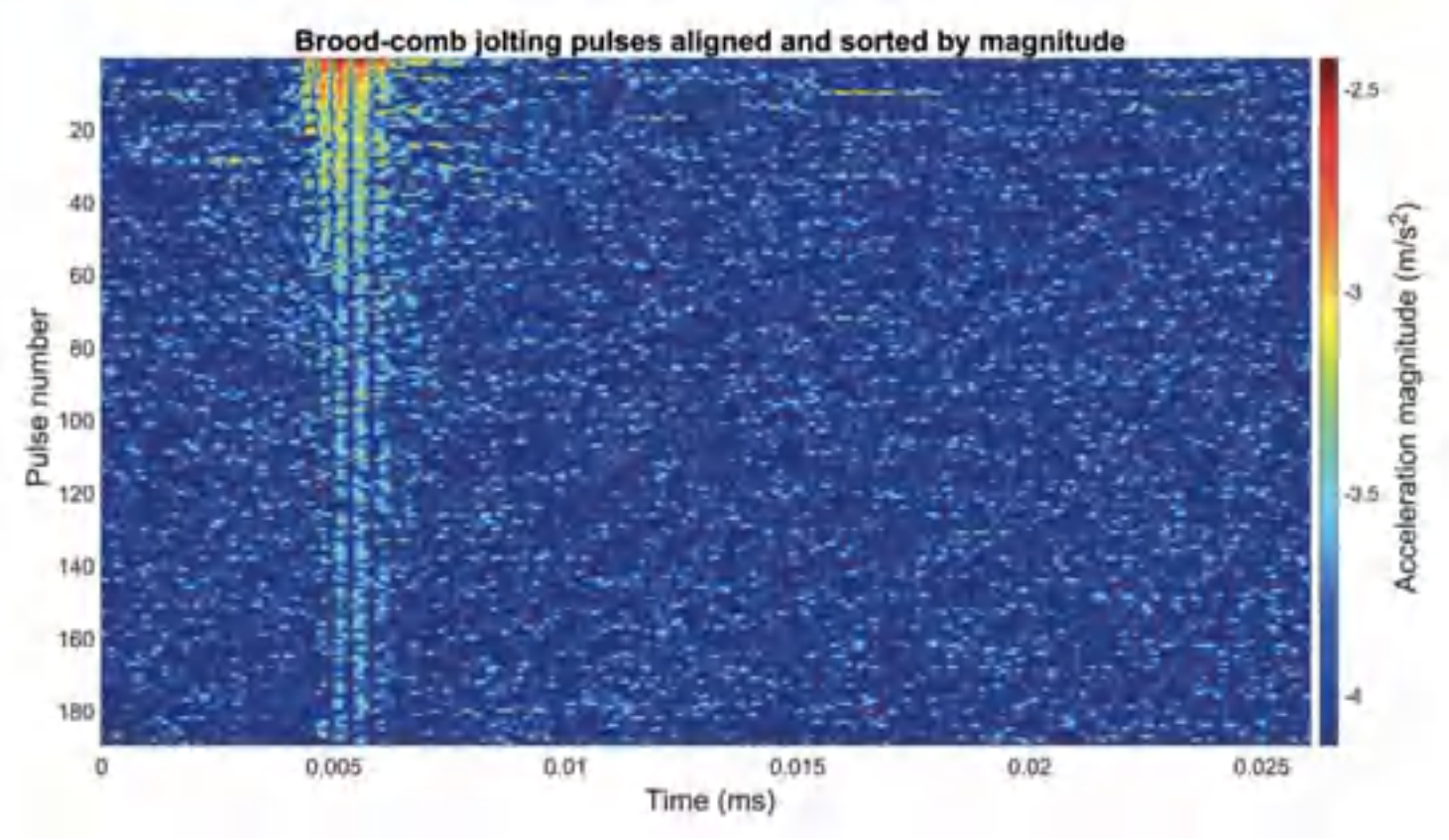


Fig. 3. The collection of all 189 jolting pulses detected in the brood-comb after under-going cross-correlation alignment with the reference pulse (pulse number 1 in this instance), and further sorted by magnitude, in decreasing order. The similarity between pulses observed in this figure is remarkable. The polarity of acceleration has been forced to be positive to allow its magnitude to be shown on a logarithmic (log base 10) scale with dark red showing as the highest magnitude (3.4×10-3 m/s2) and dark blue showing as the minimum magnitude (1/50 of the maximum)

Jolting waveforms registered in both honeycomb and Petri-dish can be described as damped sine waves, with a typical decay constant of 0.1ms and 1.2ms respectively for the strongest pulses (Supplementary Fig. S19). To assess the full pulse collections, average decay constants (λ) were calculated after visual fitting on the clearest individual wave-forms to quantitate the variation in the population of pulses (Petri-dish (n = 20) λ = 1.94ms (mean), 1.03ms (s.d.), honeycomb (n = 5) λ = 0.5ms (mean), 0.37ms (s.d.)).

Separate, repeatable features observed in the pulses detected within the brood-comb substrate prompted us to select the 40 traces with the highest magnitudes, and to average them to showcase the mean accelerometer waveform characteristics. The envelope of the corresponding broodcomb pulse is very well described by a gaussian function (supplementary Fig. S19), with an average full width at halfmaximum of 1.33ms. It is worth noting that there is also a visible, but negligible, exponential decay of the signal.

3.4 Within substrate comparison

The strongest jolting pulses are not necessarily representative of the whole collection of measurements as deviations from pulse to pulse can take place. Amongst the three substrates, jolting pulses detected within the brood-comb exhibit the highest repeatability (Fig. 3 for the waveforms, supplementary Fig. S15 for the corresponding power spectra). All pulses are 4 to 10ms long, with three to five main lobes probably originating from one of the substrate's resonant mode. A few Varroa pulses (3%, based upon visual examination of the spectrograms and wave-

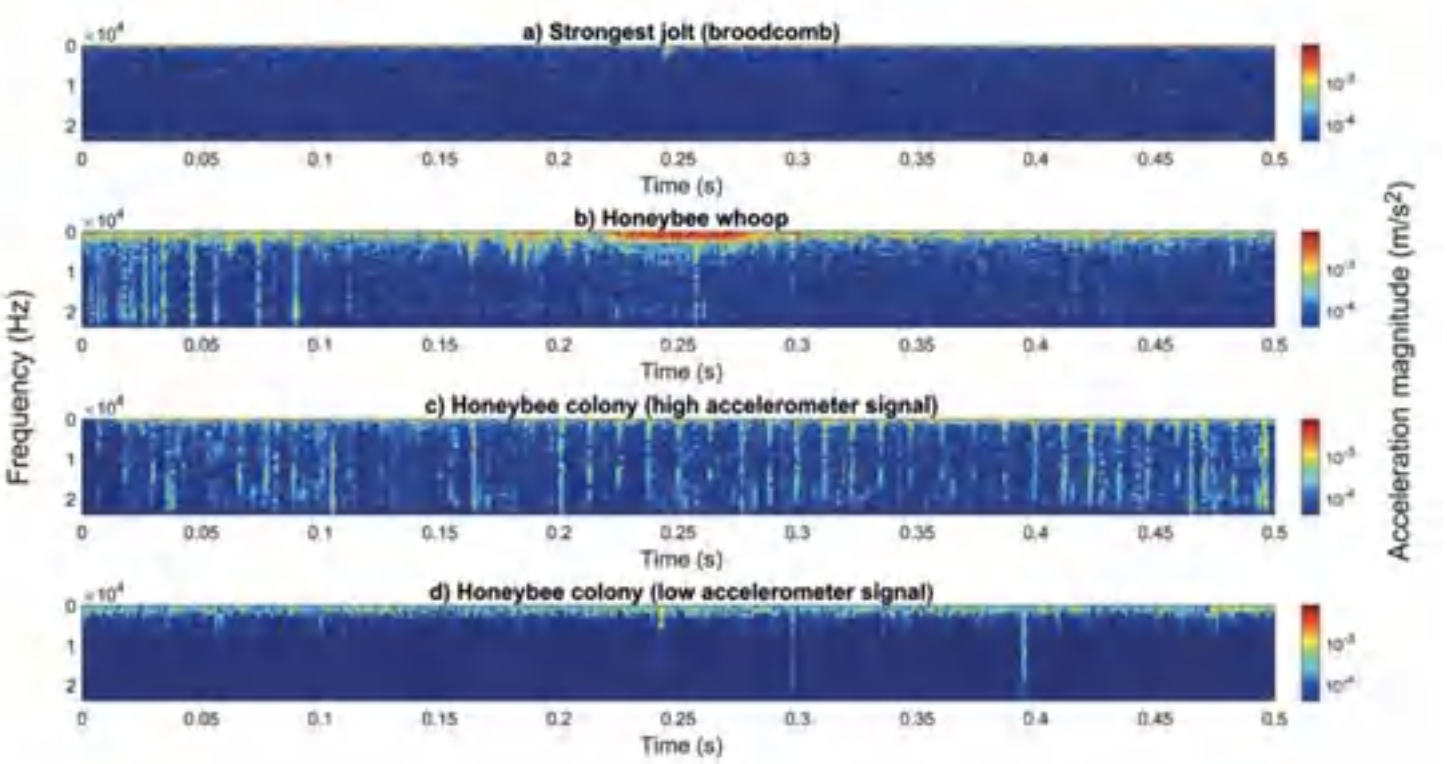


Fig. 4. A series of 0.5s long spectrograms allowing the comparison of the vibration originating from the strongest Varroa jolt on broodcomb (panel a), a honeybee whooping signal that comprises of a single pulse (panel b), and the whole honeybee colony in both low signal (panel c) and high signal periods (panel d). Variation in signal strength between 'c' and 'd' occurs mostly as a result of changes in comb mass density throughout the 21-day period of the brood-cycle. In this figure, the magnitude of the acceleration is logarithmic (to the base 10), where the highest magnitude is dark red (7.5×10-3 m/s2) and the lowest magnitude dark blue (and forced to 1/200 of the maximum). All four panels are scaled identically for ease of comparison. In the top spectrogram, the regular background vibration inherent to the room was calculated and subtracted

forms) exhibit a double peak (e.g. the tenth pulse on Fig. 3), which, upon inspection of the video, appears to be produced by a matching rapid, repeated motion of the Varroa body (supplementary video S20). When slowed down appropriately, jolting pulses in this video can be heard as 'knocking' noises, with the double trace pulses producing two separate knocks.

In the case of the Petri-dish substrate, most of the visible traces in spectrogram format are short, single peaks mostly found at the ultra-high frequency range, again with a few instances exhibiting more than one peak in quick succession (supplementary video S12). However, careful inspection of the original Petri-dish Varroa jolting video does not reveal behaviour changes that could be linked to the production of multiple peaks (supplementary video S9).

Of the 40 strongest jolting pulses on the Petri-dish 25% (n = 10) of the spectra also exhibit lower frequency bands, additional to the ultra-high frequency band (supplementary Fig. S21). These are found around 10.6 to 11.3 kHz, 13.4 to 14.1 kHz, and 15.1 to 15.8 kHz. Of this 25%, seven are found in the top ten strongest of the whole sample.

Varroa jolting pulses registered in the empty honeycomb substrate also exhibit variation. Some of the visible honeycomb pulses comprise of a 'train' of several pulses in quick succession. An exceptional instance of this can be seen (supplementary Fig. S22) with six clear consecutive traces within 10ms of recording. In this case, when observing the mite on the video footage, the expected behaviour is not seen. Instead, the mite jolts its body to the right and then in a forward motion, rather than back to the left (supplementary video S23). This movement has not been seen in other pulses that produce multiple peaks, but still yields vibrational pulses with spec-

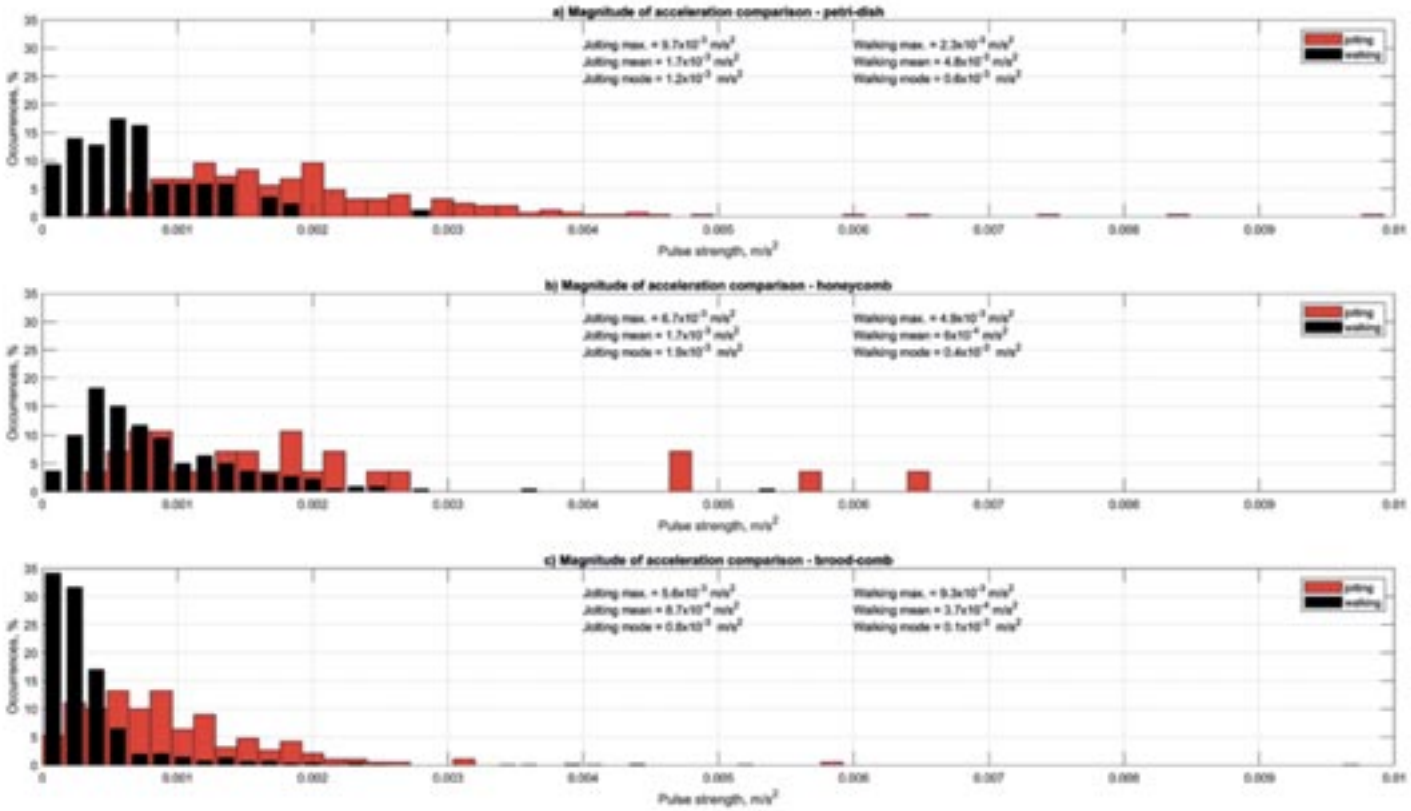


Fig. 5. A series of histograms demonstrating the strength of Varroa jolting against the walking pulses on each substrate. In all three panels the vertical axis is normalised to showcase the distribution probability (Petri-dish jolting/walking pulses n = 250/89, honeycomb jolting/walking pulses n = 28/219, brood-comb jolting/walking pulses n = 189/1272). Histogram bin-width is identical for all plots – but the displayed black walking bars have been narrowed so that the red jolting bars, displayed behind, can be viewed

tral features identical to those of a jolting behaviour.

3.5 Comparison of jolting pulse with other vibrational traces

Here, the Varroa jolting accelerometer waveforms are compared to those originating from (i) the mite walking and (ii) honeybees. We first compare the strongest jolting pulse with the signal captured from a full honeybee colony, using the brood-comb data for the comparison (Fig. 4). The strongest Varroa pulse is compared with a single honeybee 'whooping' signal, and the overall signal originating from the colony during two phases: low accelerometer signal (frame is heavily loaded with brood and/or honey, therefore attenuating vibrational signals) and high accelerometer signal (frame is mostly empty, therefore less attenuation occurs). Despite the large difference in mass and size between an adult honeybee individual (115 ± 7 mg (Schmolz et al. 2005), 15mm) and a Varroa mite (0.42 mg, 1mm), it is most surprising to find that the acceleration magnitude of the strongest jolting pulse is comparable to that of the colony during a period of low signal (jolting pulse = 9.4×10-3 m/s2, low signal colony = 1.9×10-3 m/s2). The jolting pulse is also only five times smaller than (i) an individual pulsed bee signal and (ii) the entire colony when accelerometer signal is high (Fig. 4, supplementary Fig. S24) (jolting pulse = 9.4×10-3 m/s2, whooping signal = 4.5×10-2 m/s2, high signal colony = 4.5×10-2 m/s2). The quoted magnitudes of acceleration represent the integral of all acceleration frequency components for the maximum vertical point in time in each panel (supplementary Fig. S24).

The strongest jolting pulse captured on the empty honeycomb also demonstrates the same

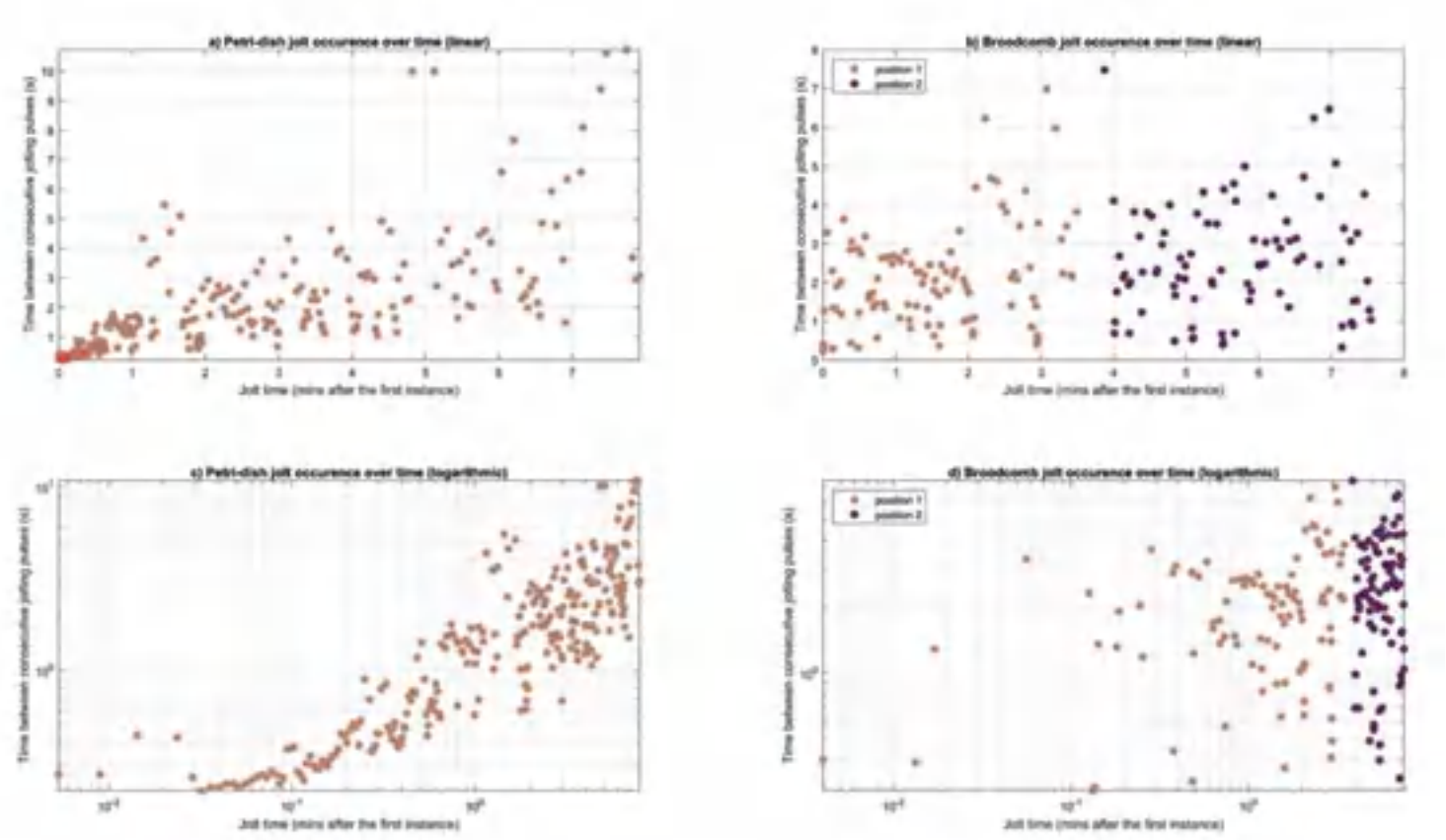


Fig. 6. Jolt occurrence time intervals with respect to time. The data for the mite jolting on Petri-dish (left column) and on brood-comb (right column) are shown here. Jolt occurrence time intervals are showcased in both linear (a, b) and logarithmic (c, d) scales. A change in the colour of the data points is indicative of the mite moving to a new position on the substrate

order of magnitude as the vibrations of the honeybee colony (loudest honeycomb jolting = 9.2×10-3 m/s2 (supplementary Fig. S25), registered from within a normally loaded frame.

The jolting vibrations are then compared to walking mite vibrations, registered on all three substrates (supplementary Fig. S26). On each of the three substrates, the maximum magnitude of acceleration is calculated for every jolting pulse, and a large collection of walking pulses, for comparison. Although for the case of the brood-comb, in rare instances, walking vibrations can be stronger than jolting pulses, the average jolting pulse strength is systematically higher than that of the walking pulses on all substrates (Fig. 5).

The spectra of the walking and jolting pulses are then compared to each other to explore whether they could be discriminated (supplementary Fig. S27). The vibrational pulses originating from a Varroa walking on all three substrates are produced regularly at a frequency ranging from 0.5 to 3 kHz. On the Petri-dish, the 0.5 kHz peak may be a resonance of the Petri-dish, as it is the only frequency clearly observed as a result of stimulation to this substrate (supplementary Fig. S28). On the brood-comb, the vibrations produced by both behaviours share the same frequency band, whereas the Petri-dish and honeycomb demonstrate high discrimination. Both the jolting and walking pulses on honeycomb stimulate the ultra-high frequency bandwidth that seems to originate from a resonant mode inherent to the accelerometer itself (supplementary Fig. S29).

3.6 Estimation of pulse power output

To estimate the power output provided by a mite delivering a jolting pulse, a well-controlled vibration was produced artificially, yielding a vibrational trace similar to that of a Varroa.

The kinetic energy of the bead hitting the Petri-dish (see ‘Methods’) was estimated to be 17.5nJ, which, when delivered within 0.54ms (the

time duration of a jolting pulse to go from 0 to maximum on the Petri-dish (supplementary Fig. S19) results in a power of approximately 32µW. As the mite vibrational pulse is forty times weaker than the artificially produced vibration, this means that the animal must typically deliver approximately forty times less power, i.e. 810nW in each jolting pulse.

3.7 The detectability of jolting pulses using accelerometer sensors

Not all video-evidenced jolting pulses produced a detectable vibrational trace. This must be due to the animal delivering vibrations with varying strength. The fraction of detected pulses decreases with a reduction in the accelerometer's sensitivity, and no jolting vibration was ever detected when using a ten-times less sensitive crystal (4507, Brüel & Kjær, 100 mV/g).

In order to assess the detectability of jolting vibrational pulses on each substrate, the signal to noise ratio (SNR) was estimated for each vibrational trace, and we also independently noted the percentage of pulses that could be picked up by critical listening, to best perceive the collection of jolting pulses. On the brood-comb, 25% of pulses were hearable, corresponding to a SNR boundary of 0.7475. Petri-dish and honeycomb exhibited a higher percentage of audible pulses, 47% (SNR boundary = 0.0142) and 68% (SNR boundary = 0.2265), respectively.

3.8 Periodicity of the behaviour

We now consider all the mite individuals observed for jolting behaviour (supplementary Fig. S8), and describe the periodicity and pattern of the jolting. Mites produced this behaviour for varying lengths of time (from as little as one single jolt up to almost 50 minutes of regular jolting behaviour), often with a few location changes between where jolting was seen to temporarily cease.

Of the mites that were observed jolting (54.5%, n = 30), individuals were categorised based upon the greatest length of time during which the behaviour was regularly produced. We call this a 'bout' of jolting, defined as a period where a mite remains in the same position and jolts with 10 seconds or less between consecutive pulses. If more than 10 seconds elapsed, or mite re-positioning occurred, then the subsequent jolt that followed this was considered to be the start of a new bout. 77% of these mites jolted for periods of less than one minute (supplementary Fig. S8, panel 'b'). Mites producing a jolting bout that lasted more than one minute (13%) were then further scrutinised to identify an overall behaviour pattern during their time spent on the substrate.

The overall duration of jolting varied between individuals, with the longest time-period observed as 49 minutes and 50 seconds. The Petri-dish and brood-comb mites pulsed for approximately 8 minutes each (Fig. 6). The periodicity of the jolting of the mite individual residing on the empty honeycomb is also shown alongside the data for other mites (supplementary Fig. S30) with broadly varying overall pulsing time durations demonstrated. In 50% of cases in this category, mites repositioned during jolting. This was either a minor displacement, where the mite simply stopped pulsing to turn and face another direction, or a larger motion, where it stopped then walked to another area on the substrate before immediately continuing with the behaviour.

For some mites, there appears to be a trend in their pulsing activity, but modest sample size prevents us from drawing interpretive comments. Some individuals jolt rapidly from commencement of the behaviour, with very short time intervals, around 0.5 seconds, between consecutive jolting pulses. As time increases, the temporal density of pulse occurrences decreases (see Fig. 6, panels 'a' and 'c', see also supplementary Fig. S3, panels 'a' to 'd'). Other individuals produce jolting pulses at more regular intervals regardless of time, with a similar rest period between consecutive pulses (Fig. 6, panels 'b' and 'd', supplementary Fig. S30, panels 'e' to 'h'). Where repositioning occurs, identified by a change in colour on the figures (Fig. 6, supplementary Fig. S30) the trends here described appear to continue regardless of the interruption due to movement.

4 Discussion

In this work, we detected vibrational signals originating from individual Varroa mites with accelerometer sensors. As the jolting behaviour is very common, it comes to a great surprise that it has, until now, remained unnoticed. Only one previous study describes 'jolting movements' in Varroa (Piou et al. 2019), however this work instead characterised the reaction of mites to electrostatic substrate discharges. In our study, more than 50% of mites produced jolting behaviour at some stage during our observations, ranging from single jolts to mites that jolted hundreds of times for several minutes.

The synchronicity between video-captured mite movements and accelerometer vibrational traces provides strong evidence that the displacements of the body in left and right directions are features of the same behaviour which also yields a detectable vibration. Critical listening of the audio of the slowed video data also further demonstrates a robust synchronicity where the jolting pulses can be heard as 'knocking', 'clapping' or 'clinking' noises, with a timbre clearly modulated by the nature of the substrate in which the vibration is detected.

On all three substrates investigated, double or multiple peak vibrations were occasionally found in rapid succession. On brood-comb and honeycomb, two rapid, successive body displacements can be seen alongside a double audible 'knock' or 'clap' consistent with the multiple peak vibratory trace seen. For Petri-dish, although visible differences in the jolting vibrational traces are seen (some have single peaks, some multiple peaks), there are no visual or audible differences in the production of both types of pulse when viewing the displacement of the mite. Perhaps the double motion of the body observed on the brood-comb and honeycomb is a result of the uneven comb surface interfering with the flow of the displacement, whereas the Petri-dish is homogenous and flat.

The mechanism behind jolting vibration production can only be speculated presently. The visible motion of the body is approximately 20ms, the vibrational trace itself is only 0.3 to 1ms long, followed by a decay of 1 to 4ms that is likely to result from the resonance of the substrate. This could be indicative of a 'spring' or 'click' mechanism, which is documented in a variety of species that produce ultra-short and ultra-fast movements (Gronenberg 1996; Patek et al. 2011). The relatively slow visual jolting would correspond to the loading of the 'spring', whilst its ultrafast release would cause the remarkably short accelerometer pulse.

4.1 Substrate dependent jolting pulse features

Dependent on the substrate on which it is transmitted, the jolting pulse vibrational trace varies substantially, as would be expected from any vibrational signal captured on a variety of media. In the honeycomb, in particular, there is a remarkably broad band trace that could be described as resulting from a Dirac Delta function (note, also, that for similar accelerometer traces, the broader the spectral band is, the weaker the signal magnitude registered on the spectrum, necessarily, as seen in Fig. 2). This corresponds to an ultra-fast burst of energy delivered to the substrate (Reeping & Reid 2016). A jolting mite delivers a short burst of vibrational energy into the material on which it stands and the shorter this pulse is, the broader the environmental information gathered in the spectrum we register. The pulse growth appears to be the only part of the vibration highly specific to the mite. The oscillations following it, which contribute to the overall time duration of the pulse, must result from the vibrational energy gradually dissipating in the substrate.

The platform on which a vibration is transmitted produces a set of resonance signals that are characteristic of the substrate (Otten et al. 2001), a strong feature that we see in our results. Stronger Petri-dish and honeycomb jolting pulses stimulated an increased number of frequency bandwidths than lower intensity ones, an indication that more resonance information is produced (Otten et al. 2001). Jolting pulses in general stimulate more frequencies than walking pulses, which have a lower frequency bandwidth of 0.5 to 3 kHz, irrespective of the substrate they were delivered on. It is perhaps the result of the natural responding frequencies of the substrate that

cause brood-comb jolting and walking pulses to share a similar bandwidth.

The main resonance of the accelerometer used (17.9 kHz) was coincidentally never stimulated by mite activities. Deviation from signal strength linearity at the moderate resonances of the crystal (11, 15 and 22 kHz) was less than 10% (supplementary Fig. S29), further indicating that the frequency components of the jolting pulses were a genuine result of mite stimulation.

4.2 Successful accelerometer detection of mite signals

Accelerometers have been used successfully by our research group to detect and characterise specific honey bee signals (Bencsik et al. 2015; Ramsey et al. 2018; Ramsey et al. 2020), and we have now demonstrated that the same sensors can also detect the vibrations of a much smaller and lighter organism, V.destructor, on three different substrates. Varroa jolting signal capture is particularly successful on the Petri-dish and honeycomb substrates, but still demonstrates an efficacy of 25% on the denser brood-comb. This is still remarkably good, considering that capped brood-comb is known to be poorer at transmitting vibrational signals in comparison to open, empty cells (Bencsik et al. 2015).

4.3 Application of jolting pulse detection in beehives

Our results suggest that it might be possible to continuously, non-invasively detect live Varroa mites within fully populated hives using accelerometers. We have demonstrated that this signal is highly repeatable, with an identifiable shape, spectrum and time duration, including for Varroa residing on brood-comb, which is the most similar to a real hive substrate.

The average strength of the jolting pulse is well above what would be expected for an animal of such a small size. The vibrational strength of the honeybee colony recording, in which hundreds of bees contribute to the measurement during a period of high signal, is only one order of magnitude higher than that of a single mite jolting pulse. We can see in our data that when there are lulls in bee buzzing, jolting pulses exceed the maximum acceleration of the bee signal, providing a good opportunity for them to be detected. The high signal data strength is, even more surprisingly, comparable to that of the jolting, although there is a limitation to this result: The honeybee vibrations were emanating from an entire hive's frame, which during the low signal period was loaded with brood/honey, whilst the jolting pulse, in comparison, was delivered onto a much smaller section of brood-comb. This perhaps led to the comparable signal strength seen in the low signal data when compared to the jolting pulse. Nonetheless, we have shown that honeycomb pulses are comparable in strength to brood-comb pulses, and these were recorded on a British standard sized frame which, although devoid of contents, was still fully built in terms of the wax cells, i.e close to the expected substrate in the hive. This is especially true for the high signal hive data, as the frame will be closer to a state of emptiness due to a lack of brood e.g. when eggs have just been laid, after a swarm and during winter (Bencsik et al. 2015). Success in detecting Varroa vibrational signals in the hive is therefore expected to vary throughout the year in response to the everchanging periods of high and low signal that occur due to the broodcycle and therefore shifts in frame load (Bencsik et al. 2015).

It is most likely that mites would be successfully detected when within the brood cells, as population modelling predicts that 65% of mites in a honeybee colony will be within the sealed cells at any time (Martin 1998). Varroa have phases where they come into direct contact with the comb in the reproductive phase (Donzé and Guerin 1994), so walking will take place and it is possible that jolting may also occur. We know from our experience that bees fill the cells surrounding the accelerometer with their normal content, including brood. This increases the chances of a mite inhabiting the vicinity of the sensor for many days, improving the chance of its detection. Establishing the function of the jolting pulse may improve mite detection, as it is currently unknown whether it is produced in the brood cells. If we establish when and where the behaviour is likely to occur, we can focus on more

specific search times and locations for its detection. In previous studies, we have placed two accelerometers in a central position on the frame, equidistant from one another and the frame edge. This has worked well for registering frequently occurring honeybee signals (Ramsey et al. 2018) but may not be optimum for mite signal capture, particularly as detection success is likely to be affected by the distance between the mite and accelerometer. In our work so far, mites were always positioned within three to four cm of the sensor, but Varroa could reside everywhere in a real hive. It is likely that an array of accelerometers will be needed for this exploration.

The work discussed here therefore provides a pioneering step towards the detection of mites in a real hive using accelerometers. The features that we have extracted and described can be used to inform a search tool for jolting and walking pulses within a full-sized hive. We can now utilise this breakthrough as the foundation for future endeavours, such as investigating whether the mite vibrational pulses can be detected when originating from the capped brood cells.

Is the jolting pulse functional? – A better understanding of why the jolting pulse is transmitted by the mite may provide additional information on where the behaviour will occur, which may then improve Varroa vibration detection. Some of the features of jolting vibrational trace indicate that it may serve a purpose. Mites lack the necessary anatomical features for detecting airborne sound (Dillier et al. 2006), and they have no eyes (Dillier et al. 2006; Dowling 2015). It is therefore presumed that the other sensory systems guide the species' behaviour, such as the chemosensory and olfactory systems (Dillier et al. 2006; Zeigelmann et al. 2012), and perhaps vibratory information contributes to the successful completion of their life cycle. One feature that we see in our results is the remarkable strength of the jolting pulse. For an animal of its modest size and mass, the jolting vibration is exceptionally strong when compared to the vibrational signals of much larger honeybees. It also requires a large power of 810nW, delivered by the mite. When comparing this to small species that produce functional vibratory signals (leaf cutter ant stridulatory distress signals: output 10–20nW (Markl 1967), organism size 1–10 mg (Roces & Holldobler 1994), 3–16mm; Sehirus impressus (Canthophorus impres sus) communication signal: output 30nW, organism size 25 mg, 6–7mm (Michelsen et al. 1982); Omocestus viridu lus grasshopper courtship signal: output 61nW (Michelsen et al. 1999), organism size 165–310 mg (Kriegbaum 1997), 1–2cm), we can see that Varroa, an animal of a significantly smaller size, produces jolting vibrations that are of a much higher power output. Due to the cost inherent to the high energy requirement of this signal, it would seem unlikely that the vibration we register is simply a by-product of the animals' activity.

Jolting pulses also differ dependent on the substrate on which they are produced, which, as previously discussed, is the result of the frequency response of the substrate. Some species engage in the production of probing pulses that are transmitted into a substrate so that the organism can detect the subsequent reflections and gain environmental information (Wäckers et al. 1998). Parasitoid wasps, mole rats and aye-ayes (Broad & Quicke 2001; Kimchi et al. 2005; Sterling & McCreless 2006) do so in order to identify hosts/ prey or gather knowledge on their surroundings. As jolting pulses vary as a result of the substrate upon which they are transmitted, but walking pulses do not, this could be indicative of a probing function.

We have also established that some mite individuals jolt hundreds of times, continuously, and for prolonged periods. This indicates a large expenditure of accumulated energy, and thereby further suggests that perhaps jolting is beneficial to the animal in some way. The probing pulses of the species mentioned earlier also follow a repetitive action, with parasitoid wasps transmitting vibrations repeatedly until a host is found (Wäckers et al. 1998), mole rats producing 198 ± 15 signals per metre of tunnel that they excavate (Kimchi et al. 2005), and aye-ayes tapping the material containing their prey at a consecutive rate of 97.7 ± 19.9ms over a range of a few seconds (Ramsier & Dominy 2012). Pulse repetition for mites is therefore compliant with the idea of them serving an exploratory purpose, although it remains to be explained why some individuals remain static and jolting for prolonged periods, and why some only jolt a few times.

At this stage, we can only speculate a possible function for the novel jolting behaviour and its corresponding vibrational trace. The results of this work extensively characterise the features of the vibration, but do not explore why the behaviour may be produced. Better understanding of the pulse function will provide exciting avenues to explore, perhaps even allowing further infestation management strategies to be designed, such as non-chemical traps.

The results of this study demonstrate the successful detection of individual Varroa mites using accelerometers on multiple substrates and disclose a behaviour that is new to science, which we refer to as 'jolting'. The features of the associated vibrational pulse strongly indicate that it is possible to detect mites in honeybee hives using accelerometers, and this now requires further work to narrow down the location of pulse production for a more lucrative search. By exploring this, we may also shed light on the function of the pulse, as our early results tantalisingly suggest that the jolting behaviour is beneficial to the animal.

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References

Anderson, D. L., & Trueman, J. W. H. (2000). Varroa jacobsoni (Acari: Varroidae) is more than one species. Experimental & Applied Acarology, 24(3), 165–189. https://doi.org/10.1023/ A:1006456720416

Bąk, B., Wilk, J., Artiemjew, P., Wilde, J., & Siuda, M. (2020). Diagnosis of varroosis based on bee brood samples testing with use of semiconductor gas sensors. Sensors (Basel), 20(14), 4014. https://doi.org/10.3390/s20144014

Barlow, V. M., & Fell, R. D. (2006). Sampling methods for Varroa mites on the domesticated honeybee. Department of Entomology, Virginia Tech., US. Virginia Cooperative Extension. Publication. 444–103, 1–3.

Bencsik, M., Le Conte, Y., Reyes, M., Pioz, M., Whittaker, D., Crauser, D., ... Newton, M. I. (2015). Honeybee colony vibrational measurements to highlight the brood cycle. PLoS One, 10(11), e0141926. https://doi.org/10.1371/journal.pone.0141926

Bienkowska, M., & Konopacka, Z. (2001). Assessment of honeybee colonies infestation by the mite Varroa destructor based on its natural mortality during the summer season. Journal of Apicultural Science, 45, 129–140.

Bilik, S., Kratochvila, L., Ligocki, A., Bostik, O., Zemcik, T., Hybl, M., ... Zalud, L. (2021). Visual Diagnosis of the Varroa Destructor Parasitic Mite in Honeybees Using Object Detector Techniques. Sensors (Basel), 21(8), 2764. https://doi.org/10.3390/s21082764

Bjerge, K., Frigaard, C. E., Mikkelsen, P. H., Nielsen, T. H., Misbih, M., & Kryger, P. (2019). A computer vision system to monitor the infestation level of Varroa destructor in a honeybee colony. Computers and Electronics in Agriculture, 164, 104898. https:// doi.org/10.1016/j.compag.2019.104898

Broad, G. R., & Quicke, D. L. J. (2001). The adaptive significance of host location by vibrational sounding in parasitoid wasps. Proceedings of the Royal Society of London. Series B, Biological Sciences, 286(1484), 2403–2408.

Chazette, L., Becker, M., & Szczerbicka, H. (2016). Basic algorithms for beehive monitoring and laser-based mite control. In 2016 IEEE Symposium Series on Computational Intelligence (SSCI). 1–8.

Chen, Y. P., & Siede, R. (2007). Honeybee viruses. Advances in Virus Research, 70, 33–80. https://doi.org/10.1016/S0065-3527 (07)70002-7

Dietemann, V., Nazzi, F., Martin, S. J., Anderson, D. L., Locke, B., Delaplane, K. S., ... Ellis, J. E. (2013). Standard methods for Varroa research. Journal of Apicultural Research, 52(1), 1–54. https://doi.org/10.3896/IBRA.1.52.1.09

Dillier, F., Fluri, P., & Imdorf, A. (2006). Review of the orientation behaviour of the bee parasitic mite Varroa destructor: Sensory equipment and cell invasion behaviour. Revue Suisse de Zoologie, 113(4), 857–877. https://doi.org/10.5962/bhl.part. 80381

Donzé, G., & Guerin, P. M. (1994). Behavioural attributes and parental care of Varroa mites parasitising honeybee brood. Behavioral Ecology and Sociobiology, 34(5), 305–319. https:// doi.org/10.1007/BF00197001

Dowling, A. (2015). The evolution of parasitism and host association in mites. In S. Morand, B. R. Krasnov, & T. J. Littlewoord (Eds.), Parasite Diversity and Diversification (1st ed., pp. 265– 288). Cambridge: Cambridge University Press. https://doi. org/10.1017/CBO9781139794749.018

Elizondo,V.,Briceno,J.C.,Travieso,C.M.,&Alonso,J.B.(2013). Video monitoring of a mite in honeybee cells. Advanced Materials Research, 664, 1107–1113. https://doi.org/10.4028/ www.scientific.net/AMR.664.1107

Evans, J. D., & Cook, S. C. (2018). Genetics and physiology of Varroa mites. Current Opinion in Insect Science, 26, 130–135. https://doi.org/10.1016/j.cois.2018.02.005

Fakhimzadeh, K., Ellis, J. D., & Hayes, J. W. (2011). Physical control of Varroa mites (Varroa destructor): The effects of various dust materials on Varroa mite fall from adult honeybees (Apis mellifera). Journal of Apicultural Research, 50(3), 203–211. https://doi.org/10.3896/IBRA.1.50.3.04

Frey, E., Odemer, R., Blum, T., & Rosenkranz, P. (2013). Activation and interruption of the reproduction of Varroa destructor is triggered by host signals (Apis mellifera). Journal of Invertebrate Pathology, 113(1), 56–62. https://doi.org/10.1016/j.jip.2013. 01.007

Gregorc, A., Knight, P. R., & Adamczyk, J. (2017). Powdered sugar shake to monitor and oxalic acid treatments to control Varroa mites (Varroa destructor Anderson and Trueman) in honeybee (Apis mellifera) colonies. Journal of Apicultural Research, 56(1), 71–75. https://doi.org/10.1080/00218839.2017.1278912

Gregorc, A., & Sampson, B. (2019). Diagnosis of Varroa mite (Varroa destructor) and sustainable control in honeybee (Apis mellifera) colonies – a review. Diversity (Basel), 11(12), 243. https://doi.org/10.3390/d11120243

Gronenberg, W. (1996). Fast actions in small animals: Springs and click mechanisms. Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology, 178(6), 727–734. https://doi.org/10.1007/BF00225821

Kevan, P. G., Hannan, M., & Ostiguy, A. (2006). A summary of the varroa-virus disease complex in honeybees. American Bee Journal, 146(8), 694–697.

Kimchi, T., Reshef, M., & Terkel, J. (2005). Evidence for the use of reflected self-generated seismic waves for spatial orientation in a blind subterranean mammal. The Journal of Experimental Biology, 208(4), 647–659. https://doi.org/10.1242/jeb.01396

Konig, A. (2021). First results of the be-e-nose on mid-term duration hive air monitoring for Varroa infestation level estimation. Sensors and Transducers, 250(3), 39–41.

Kriegbaum, H. (1997). Grasshopper reproductive strategies measured in the field: A tradeoff between age at maturity and egg production per day. Naturwissenschaften, 84(4), 157–159. https://doi.org/10.1007/s001140050370

Le Conte, Y., Ellis, M., & Ritter, W. (2010). Varroa mites and honeybee health: Can varroa explain part of the colony losses? Apidologie, 41(3), 353–363. https://doi.org/10.1051/apido/ 2010017

Lee, K. V., Moon, R. D., Burkness, E. C., Hutchison, W. D., & Spivak, M. (2010). Practical sampling plans for Varroa destruc tor (Acari: Varroidae) in Apis mellifera (Hymenoptera: Apidae) colonies and apiaries. Journal of Economic Entomology, 103(4), 1039–1050. https://doi.org/10.1603/EC10037

Markl, H. (1967). Die Verständigung durch Stridulationssignale bei Blattschneiderameisen. Zeitschrift für vergleichende Phy siologie, 57(3), 299–330. https://doi.org/10.1007/BF00303001

Martin, S. (1998). A population model for the ectoparasitic mite Varroa jacobsoni inhoneybee (Apis mellifera) colonies. Ecological Modelling, 109(3), 267–281. https://doi.org/10.1016/ S0304-3800(98)00059-3

Martin, S. J. (2004). Acaricide (pyrethroid) resistance in Varroa destructor. Bee World, 85(4), 67–69. https://doi.org/10.1080/00 05772X.2004.11099632

Michelsen, A., & Elsner, N. (1999). Sound emission and the acoustic far field of a signing acridid grasshopper (Omocestus viridu lus L.). The Journal of Experimental Biology, 202(12), 1571–1577. https://doi.org/10.1242/jeb.202.12.1571

Michelsen, A., Fink, F., Gogala, M., & Traue, D. (1982). Plants as transmission channels for insect vibrational songs. Behavioral Ecology and Sociobiology, 11(4), 269–281. https://doi.org/ 10.1007/BF00299304

Milani, N. (1999). The resistance of Varroa jacobsoni Oud. to acaricides. Apidologie, 30(2–3), 229–234. https://doi.org/10.1051/ apido:19990211

Nazzi, F., & Le Conte, Y. (2016). Ecology of Varroa destructor, the major ectoparasite of the western honeybee, Apis mellifera. Annual Review of Entomology, 61(1), 417–432. https://doi. org/10.1146/annurev-ento-010715-023731

Otten, H., Wäckers, F., Battini, M., & Dorn, S. (2001). Efficiency of vibrational sounding in the parasitoid Pimpla turionellae is affected by female size. Animal Behaviour, 61(3), 671–677. https://doi.org/10.1006/anbe.2000.1639

Patek, S. N., Dudek, D. M., & Rosario, M. V. (2011). From bouncy legs to poisoned arrows: Elastic movements in invertebrates. The Journal of Experimental Biology, 214(12), 1973–1980. https://doi.org/10.1242/jeb.038596

Pettis, J. S. (2004). A scientific note on Varroa destructor resistance to coumaphos in the united states. Apidologie, 35(1), 91–92. https://doi.org/10.1051/apido:2003060

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Pietropaoli, M., Tlak Gajger, I., Costa, C., Gerula, D., Wilde, J., Adjlane, N., ... Formato, G. (2021). Evaluation of two commonly used field tests to assess Varroa destructor infestation on honeybee (Apis mellifera) colonies. Applied Sciences (Basel, Switzerland), 11(10), 4458. https://doi.org/10.3390/app11104458

Piou, V., Urrutia, V., Laffont, C., Hemptinne, J., & Vetillard, A. (2019). The nature of the arena surface affects the outcome of host-finding behaviour bioassays in Varroa destructor (Anderson and Trueman). Parasitology Research, 118(10), 2935–2943. https://doi.org/10.1007/s00436-019-06435-2

Ramirez, M., Prendas, J. P., Travieso, C. M., Calderon, R., & Salas, O. (2012). Detection of the mite Varroa destructor in honeybee cells by video sequence processing. In 2012 IEEE 16th International Conference on Intelligent Engineering Systems (INES). 103–108.

Ramsey, M., Bencsik, M., & Newton, M. I. (2018). Extensive vibrational characterisation and long-term monitoring of honeybee dorso-ventral abdominal vibration signals. Scientific Reports, 8, 14571. https://doi.org/10.1038/s41598-018-32931-z

Ramsey, M., Bencsik, M., & Newton, M. I. (2017). Long-term trends in the honeybee whooping signal revealed by automated detection. PLoS One, 12(7), e0181736. https://doi.org/10.1371/ journal.pone.0181736

Ramsey, M., Bencsik, M., Newton, M. I., Reyes, M., Pioz, M., Crauser, D., ... Le Conte, Y. (2020). The prediction of swarming in honeybee colonies using vibrational spectra. Scientific Reports, 10, 9798. https://doi.org/10.1038/s41598-020-66115-5

Ramsey, S. D., Ochoa, R., Bauchan, G., Gulbronson, C., Mowery, J. D., Cohen, A., ... van Engelsdorp, D. (2019). Varroa destruc tor feeds primarily on honeybee fat body tissue and not haemolymph. Proceedings of the National Academy of Sciences of the United States of America, 116(5), 1792–1801. https://doi.org/10.1073/pnas.1818371116

Ramsier, M. A., & Dominy, N. J. (2012). Receiver bias and the acoustic ecology of aye-ayes (Daubentonia madagascariensis). Communicative & Integrative Biology, 5(6), 637–640. https:// doi.org/10.4161/cib.21509

Reeping, D., Reid, K. (2016). Introductory Engineering Mathematics. Momentum Press. 149–152.

Roces, F., & Holldobler, B. (1994). Leaf density and a trade-off between loadsize selection and recruitment behaviour in the ant Atta cephalotes. Oecologia, 97, 1–8. https://doi.org/10.1007/ BF00317902

Rosenkranz, P., Aumeier, P., & Ziegelmann, B. (2010). Biology and control of Varroa destructor. Journal of Invertebrate Pathology, 103, S96. https://doi.org/10.1016/j.jip.2009.07.016

Schmolz, E., Kosece, F., & Lamprecht, I. (2005). Energetics of honeybee development. Thermochimica Acta, 437(1–2), 39–47. https://doi.org/10.1016/j.tca.2005.06.001

Sterling, E. J., & McCreless, E. E. (2006). Adaptations in the ayeaye: a review. Lemurs. 159–184.

Szczurek, A., Maciejewska, M., Bąk, B., Wilk, J., Wilde, J., & Siuda, M. (2020a). Gas sensor array and classifiers as a means of varroosis detection. Sensors (Basel), 20(1), 117. https://doi. org/10.3390/s20010117

Szczurek, A., Maciejewska, M., Zajiczek, Z., Bąk, B., Wilk, J., Wilde, J., & Siuda, M. (2020b). The effectiveness of Varroa destructor infestation classification using an e-nose depending

on the time of day. Sensors (Basel), 20(9), 2532. https://doi.

org/10.3390/s20092532

Tihelka, E. (2018). Effects of synthetic and organic acaricides on

honeybee health: A review. Slovenian Veterinary Research,

55(3), 119–140. https://doi.org/10.26873/SVR-422-2017 Wäckers, F. L., Mitter, E., & Dorn, S. (1998). Vibrational sounding by the pupal parasitoid Pimpla (Coccygomimus) turionellae: An additional solution to the reliability-detectability problem. Biological Control, 11(2), 141–146. https://doi.org/10.1006/

bcon.1997.0592

Ziegelmann, B., Lindenmayer, A., Steidle, J., & Rosenkranz, P.

(2012). The mating behaviour of Varroa destructor is triggered by a female sex pheromone. Apidologie, 44(3), 314–323. https:// doi.org/10.1007/s13592-012-0182-5

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BEES LIFE

SPINNER FIRST, THEN THE COLONY?

I will always remember a conversation with now the late grandpa Mihailo Kulinčević from Rađeno Selo near Valjevo. He was a very respected man. His endless faith in God, ethics, courage, wisdom and unselfishness always made him a desirable adviser and man to talk to. Grandpa Mihailo lived for 98 years and died on Christmas 2000. In a moment of the conversation, this great optimist and enthusiast seemed as if he had become suspicious. To my question whether young beekeepers still address him for help and advices, he said with wistfulness: “Son, today many children start to breed bees, some of them love them, and the other buy a spinner and honey pots first, and then acquire a colony. It is not likely that they will become good beekeepers. This, my son, does not relate to you, for my Jova (Jovan Kulinčević, PhD.) says you are a very hard working person and if you keep learning and endure, you can achieve a lot in beekeeping”.

I was very flattered. The words of grandpa Mihailo still live inside of me. I have been trying to work both more and better. Whenever I wish to write an article about beekeeping, I think of how nobody will need my findings, of course you should have a “spinner and honey pots”. I hope that there are much more those who are interested in knowledge and experiences, because only through holding an abundance of information, and by your own selection, you can build a personal attitude and approach, because there are no many occupations as individual as beekeeping. I always have in mind that faith and love in any kind of job open the door of success.

How to achieve a success in the black locust and the following nectar flows?

The main prerequisite for considering this topic is certainly in previously provided conditions

for good wintering, and therewith a successful wintering of all bee colonies. It would not be sufficient to once more remind of these conditions; although we are maybe late for this winter, we are certainly on time for the next one. Well, which are the requirements that need to be met:

- to have at least 15-20 thousand healthy long-lived bees in the winter cluster;
- to have a young, favourable selected, queen in the cluster
- to have provided and correctly arranged reserve of abut 20 kg of good quality honey;
- to have at least one kilo of stored pollen in the hive;
- to have a correct warming and ventilation of beehives provided;
- to have the apiary protected against cold winds, and still have it moderately aired;
- to fence the apiary in order to prevent the access of domestic and wild animals;
- to protect the hive entrances against mice and shrews;
- to have other conditions provided that could be specific for the locality at the which the apiary is situated

If any of these requirements has not been met, you can not calmly and peacefully wait for the spring and the approaching beekeeping season. It is certain that the majority of professional, conscientious, experienced and responsible beekeepers have provided all these conditions on time, thus let's see what kind of spring development can be expected with them and with what kind of bee colonies we will enter the black-locust, but also all the following nectar flows. For the estimate of possible spring development in 2005, we will use data from 2004, collected at the Apicentre.

The size of brood was determined in 87 bee colonies in LR hives. The date of the first measuring was April 1st, 2004. The average total brood surface per a colony was approximately 80.5 dm2, or 4.6 frames completely full with brood on both sides, meaning that from March 11th to April 1st (21 days) the queen laid approximately (32,200 cells laid in 21 days) about 1,533 eggs a day. On the day of the measuring, there were about 6.6 frames in the hives thickly occupied by bees, plus forages, which makes nearly 18,000 bees.

The date of the second measuring was May 1st, 2004. The average brood surface per one colony was approximately 129.5 dm2, translated into frames, 7.4 fully laid frames on both sides. The conclusion is that (129.5 dm2 of brood × 400 cells = 51 800 laid cells) from April 10th to May 1st (21 days) the queen approximately laid (51,800 brood cells : 21 days) about 2,466 eggs a day. On the day of the measuring, the hives had approximately 10 frames thick with bees, plus foragers on the outside, which makes nearly 30,000 of bees in total.

The average inflow made for 3 days of black locust nectar flow measured in the period from May 12th to May 15th was 21.5 kg, reminding that in 2004 the majority of beekeepers did not make this kind of inflow even in two black locust nectar flows. We should especially emphasize the fact that none of these bee colonies swarmed. It is interesting to state the data that in 44 colonies the queens were from 2003, and the rest of them from 2002, and that an insignificantly higher crop was achieved in the colonies which were in the second testing year, while in the previous years it was a much more common occurrence. We can approximately estimate or calculate with what number of bees these colonies participated in the black locust forage. If on May 1st, these colonies had the average of 30 thousand bees and over 50 thousand brood cells, then every next day we could expect the additional 1,500 bees. These are the bees laid after April 10th, when the average laying ability of the queen was about 2,000 eggs a day. At the same time, we can expect that the mortality is nearly 500 bees a day, and these are bees laid in the period from February 20th till March 10th, when the average laying ability of the queen was approximately the same.

With this kind of colony growth, by May 6th, (St George's day), there will be about 40,000 bees in a hive. In that period, in the large area of Serbia, the opening of first black locust blossoms start, but there are still about 7 more days till the full blossoming and nectar flow. If about May 6th, when both of brood supers of the LR hive are full with bees, we reverse the brood supers (due to arches of honey that will now be in the lower super) and add a honey supper, we have completely freed the passage for bees from the brood

to the honey super. By this activity, without extracting the frames from the brood super into the honey super, we enabled the bees not overcrowd in the brood super and undisturbedly store the honey above it. Now we already have an expanded space of the hive, which will be filled with bees the number of which will by the full black locust flow be increased for about 10 thousand (7 days x 1,500 = 10,500).

By blocking the brood gradually, due to intensive inflow of nectar, the comb surface with the young brood will become smaller, due to what several thousand bees will become free, thus the power of the bee colony will reach the absolute biological maximum of nearly 60,000 bees, and with timely adding of bee supers, it is possible to achieve maximum crops allowed by natural conditions in that period. After the black-locust forage and removal of honey supers, in the brood super, in addition to the remaining frames with brood, we will leave enough honey and empty frames for the queen to lay in order to maintain the biological power of the colony and successful forage for the following intensive nectar flows (linden and sunflower).

By the linden and sunflower nectar flow, a queen excluder and a honey super should be put on the brood super, because there is always the possibility of a not-so-intensive forage, and this kind of forages can provide conditions for the queen laying in the honey super, which would be quite irrational.

In the linden nectar flow, and especially sunflower nectar flow, queen excluders can decrease the inflow even for 20%, and at the same time provide enough space for the queen laying, which could influence the weakening of the bee colonies and the inability to breed enough bees, the main job of which would be to, after the return from the forage in the late summer, raise the sufficient number of long-lived bees with which the bee colony will enter the approaching winter. At the end of this article, I would like to thank Prof. Jovan Kulinčević, PhD, and his associates for

data about the performed measuring that I presented in this text.

The fact is that the queens, the selection and testing of which are done at the “Apicentre”, are reared upon the same technology and conditions as all the queens from the Apicentre meant for the market. Due to that, in the majority of the bee colonies where the queens from this program has been installed, should achieve approximately the same result, but it obviously is not like that. The causes should certainly bee looked for in the reasons such as:

- beekeeping with insufficiently functional beehives, in which the brood space cannot be expanded, if needed, and therewith create conditions for a full biological development of the bee colony;
- the unnatural and complicated technology of beekeeping;
- bad quality, insufficient or too abundant nutrition of the bee colony;
- unprofessional diagnostics of the diseases in bees and brood, and therewith the inadequate usage of medicines;
- insufficient care, omitting of needed actions and disharmony with the time, nectar flow and other environmental conditions, and similar.

As many other, for several years I have been looking for the most optimal and most profitable way of beekeeping. During all this time, I have done the beekeeping with various types of hives: LR, DB, DB of ten and long hives. I have applied almost all beekeeping techniques I have heard about at lectures or found in beekeeping literature. I dealt with double, two-queen, or beekeeping with several queens and with all other activities aiming the creation of some kind of phantom colonies that were supposed to bring tones and tones of honey. Finally, when I became the member of the “Apicentre” and gained some insight in their technology, I became aware of the uselessness of making these unnatural, disorganized and unstable artificial colonies that were the result of imagination, but with very weak backup of natural laws upon which bees behave and have been surviving for centuries. Finally, I realized that bees “do not read books” and make the beekeeper’s wishes true, but behave upon their own instincts.

Many of us have had to pass a very long way in order to finally find what has been found a long time ago. I guess this is normal for people who do not know how much they know, but now, when we know how much we do not know, we can finally by the spinner and many more honey pots.

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MYSTERY OF SWARMING

FROM UNKNOWN DEPTHS OF BEEHIVE

Beekeepers familiar with my series of works could notice that the work methods I apply in my apiaries fit into general principles of beekeeping, but are used in general and without a recipe upon which beekeeping “should” be done. That is why the flexibility of my beekeeping methods leaves space for individual knowledge and intuition of each beekeeper to, in his/her own conditions and upon his/her own abilities, manage his/her colonies in the most rational way. That is the point and all the charm and the key of success of a modern beekeeper.

The beekeeper is the only one knowing the biology of the bees, who feels the “spirit” of the colony and who can, in various situations, predict their behavior and react appropriately, with a big possibility of achieving the purpose he does the beekeeping for. All the others expecting to get everything in writing or said about how to do a successful beekeeping, almost never do it. That is why I will use one more opportunity to, in my own way, answer the question asked by several beekeepers from the middle of May till the middle of June. Why the bees swarm, although they have undertaken all the measures for preventing swarms – in the way the books read?

It is nice and useful for beekeepers to read and learn from other people's experience, but beekeeping is not a read book, because unknown "depths" of beehive have kept the secrets of bee colonies for centuries. One of them is natural swarming, which is especially important to beekeepers, therefore they do everything they can to reveal it. Many scientists have been examining the pre-swarming and swarming stage of colonies and have come to diverse theories. Gerstung (1891) suggested a theory presenting a colony with maximum population of nursing bees, which make the critical factor of swarms, because they produce the surplus of food for proportionally reduced area of young brood. Demuth (1921) thought that the overcrowding of the hive's brood area by brood and bees is the main cause of swarming. Colin Butler (1952) established a theory upon which queens produce the substance known as Queens Mandibular Pheromones (Q.M.P.). When this substance is available for worker bees in sufficient quantities, the bees do not build queen cells. On contrary, when they get the substance in insufficient quantities, swarming occurs. This theory has been proved experimentally. I accepted it personally and have been successfully using it in the forming of nurse colonies with a queen. I limit the queen in the bottom super, above the excluder I place two honey supers with frames full of open honey. Above them there is a rearranged brood super full of young nurse bees. The Queen substance can not reach them or is below the needed threshold. The result of that is the acceptance of grafted larvae and the breeding of queen cells. Derek J. Gue (1998) published his new theory about causes of bee swarming. He claims: "Pheromone of a swarm is produced by nurse bees in a numerous, healthy colony during the swarming season and this pheromone is stronger than all the other factors".

Many recognized beekeeping practitioners stated their opinions about the causes of natural bee swarming, but it still remains the mystery in the unknown depths of beehive! In my modest opinion, bee swarming is a law of nature and bees swarm because they want to swarm! They swarm because the reproduction of the species is the most sublime goal of their existence. There is not only one cause of this ancient urge, but is necessary to, in certain circumpstances, fulfill several favorable conditions.

Fist of all, it is necessary that the bee colony reaches its full development according to its genetic features, but also in accordance with the size of the habitat they live in. Nature arranged that the maximum development of bees fits into the period when at certain area there are enough honey plants blossoming, at which both the swarm and the parent colony will provide sufficient food reserves for their survival.

The bees will not miss this kind of concordance of conditions, since the crown of their hard work, for centuries adjusted to many factors, are finished preparations and the act of swarming itself! Behind the stage in a crowded habitat, swarming bees take the reserve food from the comb that they will soon leave. When this happens, there is nothing else to wait for! With their mother queen, a crowd of bees burst out of the entrance of the hive and rush into the endless blue of a sunny day and the beginning of a new life together.

The beekeeper watches them and realizes that, in front of him, in the crowd of agitated, almost in a trans, bees, something extraordinary, something sublime happens. He needs to understand their joy and endless happiness charming them in their circling flight, by which they end the

ancient wish of all the generations - to survive as long as there is sunlight and eternity!

And how can a beekeeper hold out, if by this fascinating swarms going out he loses his entire yearly profit from beekeeping? If you don't make mistakes, you don't make anything, but we can also learn from our mistakes and they should not be repeated. Therefore, a beekeeper for his future work needs to learn more about the life and rules of bee colony and to use his own and the experiences of other beekeepers. In addition to this, a modern beekeeper needs to know that corrections are necessary in beekeeping, even in the beekeeping "truths", always in accordance with the time and always changeable conditions he does the beekeeping in. Beekeeping is a craft and art in breeding bees and their management, and the channeling of the laws of bee nature and subjecting them to the interests of beekeepers is not an easy job at all.

But when a beekeeper learns how to bee happy for his success and to bear bad beekeeping years, his omissions and disappointments, then he is already a real beekeeper, and the real beekeeper cannot be shaken by the mystery of swarms from the unknown depths of beehive!

A special attention needs to be paid to each of the periods of the colony development. The works need to be adjusted to current circumstances we do the beekeeping in, because every beekeeping year is a story in itself. In the years like this one, when favorable time and nectar flow conditions accelerate the spring development of bees and maximize it before the main flow, for maintaining their working mood, the usual works such as expanding of the brood super, making young bees busy with building combs and adding of honey supers, are not enough. Demaree's method can for a while postpone the threatening swarming urge, but these are obviously extremely swarming years and it will not be a guarantee that the bees will not start the nursing of queen larvae.

The beekeeping literature describes many methods of using nectar flows and preventing of natural bee swarming. The most frequently rec-

ommended is that, before the beginning of the nectar flow, the queen needs to be limited, which is supposed to provide a rational usage of the nectar flow and prevention of natural swarming. In my opinion, it is completely wrong and, in modern beekeeping, irrational. My beekeeping practice convinced me that before the beginning of the main nectar flow (black locust, meadow, lime-tree…) the queen does not need to be limited but provided with space!

About twenty days before the expected nectar flow, the first main flow, bee colonies that have been developing successfully have almost or entirely reached the maximum in the brood. Starting from then, the surface of young brood becomes decreased any way, without any kind of our intervention, therefore the mass of just emerged bees is left without employment. The brood super becomes too tight for them, and in the honey super without nectar there is no more work for them. Therefore they usually stand still in clusters in the space between the frames and the floorboard, waiting for the maturing of the queen cells and the call of nature to go on with the prolongation of the species. There won't be long until a swarm appears "out of the season"!

Instead of limiting the queen and feeding the bees with syrup (about twenty days before the expected nectar flow) I provide the queen with a space for expanding the brood, and in bees I simulate the swarms they want.

On the previously modified brood super (in my case consisting of a standard LR and one Farrar's super), I add one additional super with well built empty comb and one frame of young brood, in order to have the bees with the queen occupy it as soon as possible. When this happens, I divide the former brood super and newly added super in which the queen already lays eggs by a queen excluder. I keep this kind of stage for the following 5-6 days, to provide the bees in the lower part of the quenelles brood super with a close contact with their queen and that the young brood they are nursing become

too mature for the possible breeding of queen cells. Then, above the former brood super, and below the excluder and the added super in which the queen is intensively laying eggs, I add a honey super. With its volume and young comb it definitely divides the two brood supers. The colony is practically divided, without any kind of barrier, except for one queen excluder. Bees in the lower quenelles brood super cover and keep the mature brood warm, and the young bees coming out of it are recruited for the nursing of the bigger and bigger young brood, with their queen, but in "the other house". This kind of directing of the work of bees in a strong colony activates all the bees and keeps them in a maximum working mood. In case, in swarming years like this one, the bees "get crazy" and decide to start swarming and nurse queen larvae any way, they can do it only in the upper brood super where there is the queen and the young brood, i.e. where, upon my wish, they crated the base for the future young colony.

This kind of, most frequently, good quality swarming queen cells in the newly-formed brood super can be used, because, by the beginning of the nectar flow, I divide the newly formed swarm from their parent colony. I return the queen from the swarm to the former brood super of the production colony, the bees of which accept it as a savior and with incredible working enthusiasm they start the renovation of the colony and using of the nectar flow.

Beekeepers and my students familiar with my working technique most frequently ask: How much honey will the weakened parent colony bring after the removal of a swarm in the main nectar flow? My answer is that it can never be predicted for sure, because the inflow of nectar depends, first of all, on weather conditions during the blossoming of honey plants. There is the possibility of a bad summer flow, thus there might be no enough inflow even for the bees themselves. In that case, by removing of a swarm from a strong colony before the (never secure) nectar flow, we get something after all. In the years when the nectar flow is successful, my production colonies that have given a swarm will bring inside somewhat less honey than the others in full power and working mood, but the given swarm and the complete control over colonies in their swarming urge are sufficient compensation for maybe somewhat smaller honey crop from the main flow. In addition to that, if I later put the early swarm with a young queen together with the parent colony, this kind of colonies can bring extremely high crops in summer nectar flows and they enter the winter period very strong and with sufficient food reserves.

This does not mean that this is the only way it should be done. I myself always adjust my working technique to the circumstances of any kind I do my beekeeping in, thus, I would like to suggest everybody to adjust his/her beekeeping to the conditions and abilities. But, regardless all the differences, the dilemma remains about how to use intensive nectar flows with strong colonies in the most efficient way, and still keep the swarms under control, because, if there is no swarm control – the beekeeping lessons will be quite expensive!

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EXPLORING THE RISK OF MICROPLASTICS TO POLLINATORS: FOCUSING ON HONEY BEES

Abstract

The rapid increase in global plastic production and usage has led to global environmental contamination, with microplastics (MPs) emerging as a significant concern. Pollinators provide a crucial ecological service, while bee populations have been declining in recent years, and MPs have been recognized as a new risk factor contributing to their losses. Despite the pervasive distribution and persistence of MPs, understanding their risks to honey bees remains a critical knowledge gap. This review summarizes recent studies that investigate the toxicity of MPs on honey bee health from different perspectives. The findings revealed diverse and material-/size-/dosage-dependent outcomes, emphasizing the need for comprehensive assessments in the follow-up studies. MPs have been detected in honey and in bees' organs (e.g., gut and brain), posing potential threats to bee fitness, including altered behavior, cognitive abilities, compromised immunity, and dysfunction of the gut microbiota. It should be noticed that despite several laboratory studies suggesting the aforementioned adverse effects of MPs, field/semi-field experiments are still warranted. The synergistic toxicity of MPs with other environmental contaminants (pesticides, antibiotics, fungicides, heavy metals, etc.) still requires further investigation. Our review highlights the critical need to understand the relationships between MPs, pollinators, and the ecosystem to mitigate potential risks and ensure the sustainability of vital services provided by honey bees.

Keywords Plastic pollution · Bees · Pollinators · Ecotoxicity · Bee decline

Introduction

The deterioration of habitats due to human-caused environmental pollution is one of the main factors that is currently threatening biodiversity (Jaureguiberry et al. 2022). There is a great deal of interest in studies that show how pollutants affect an organism's life cycle, including longevity, success in reproduction, developmental rates, underlying physiological and genomic effects, and feedback loop effects on population dynamics (Anetor et al. 2022; Harmon et al. 2024).

These investigations deepen our knowledge of the ecological consequences of pollution in different habitats (Rai et al. 2023; Rangel-Buitrago

et al. 2024). Industrial plastic output has quickly expanded, reaching over 400 Mt (million tons)/year globally (Lampitt et al. 2023). Because of its low cost, plastic has become one of the most used materials, particularly in the packaging industry, and is now an important component of municipal garbage. Global plastic particle production is estimated to have reached 19–23 Mt in 2016, and some of these particles found their way into aquatic habitats. By 2030, up to 53 Mt particles are predicted to be produced annually (Borrelle et al. 2020).

The quantity of plastic waste created annually per person varies from 221 kg in the USA and 114 kg in OECD (Organization for Economic Cooperation and Development) countries in Europe to an average of 69 kg in Japan and Korea (OECD 2022).

Most plastic pollution results from improperly collecting and disposing of larger plastic debris or macroplastics. However, there is also significant concern over the leakage of microplastics (MPs), which are synthetic polymers smaller than 5 mm in diameter and are further classified into two subgroups, i.e., primary and secondary MPs. Primary MPs are produced directly as tiny materials and are frequently designed for use in consumer products such as cosmetics, detergents, and cleaning solutions. Secondary MPs are formed when bigger plastic materials degrade (break down) in the atmospheric or aquatic environment due to natural weathering processes. Items such as road markings, synthetic textiles, industrial plastic pellets, tire wear, and municipal sewage sludge have all been identified as potentially significant contributors to environmental microplastics (Rolsky et al. 2020; Järlskog et al. 2020).

Given their widespread distribution and persistent nature, MPs have become a serious environmental danger, drawing the attention of ecotoxicologists concerned about their potential toxicity (Wright et al. 2013; Ghosh et al. 2023). Exposure to MPs in aquatic biota has been shown to induce various toxicological effects (Du et al. 2021). Furthermore, recent research has demonstrated that MPs and nanoplastics (NPs; synthetic polymers with dimensions ranging from 1 nm to 1 µm) are found in typical matrices such as soil, sediment, and freshwater inland, where their abundance is greater than in the marine environment (Li et al. 2024). As a result, these materials interact with terrestrial organisms, including invertebrates, terrestrial fungi, and ani-

mal pollinators, which play a crucial role in mediating important ecosystem services and functions (de Souza Machado et al. 2018; Al Naggar et al. 2021; Dissanayake et al. 2022; Rai et al. 2023).

Insects (class Insecta), with more than one million described species, constitute more than 2/3 of all known animal species (Stork 2018). Insects are important components of virtually all terrestrial ecosystems and have a significant effect on the survival of all life, including humans. In addition to being food for other plants and animals, insects also serve as pollinators, decomposers, phytophagous, predators, and parasites in ecosystems (Scudder 2017). This implies that MPs originating from anthropogenic contributions have a high potential for interacting with insects because their life cycles involve numerous environmental media, such as soil and water bodies (Al Naggar et al. 2021; El Kholy and Al Naggar 2023; Haavik and Stephen 2023; Li et al. 2024), as well as diversity of biological characteristics among different insect orders.

Insect pollinators, in particular honey bees, are integral to both the natural world and contemporary food production. They support a resilient, genetically diverse plant ecosystem, are necessary for pollinating food crops, and are thus vital to the security of food for numerous human populations worldwide. MPs are everywhere and have been detected in various plant species foraged by bees, resulting in honey (Liebezeit and Liebezeit 2013, 2015; DiazBasantes et al. 2020). In addition, it has been identified in apiaries in China, and honey bees have been used as samplers for MPs in Denmark's urban and suburban areas (Deng et al. 2021; Edo et al. 2021). Furthermore, a recent study showed that MPs integrated by honey bees from their diet are transported to honey, wax, and larvae, raising concerns about their potentially harmful effects on bees and pollination services (Fig. 1) (Alma et al. 2023). In addition, the use of microfiber sheets to trap the small hive beetle (SBH) (Aethina tumida) (Coleoptera: Nitidulidae) results in the integration of microfibers into honey as well as the gut and cuticle of bees, providing further worries about textile microfiber contamination because bees can breakdown and fragment them (Buteler et al. 2023).

In 2021, we asked if the widespread occurrence of MPs would have unforeseen detrimental impacts on the health and fitness of honey bees,

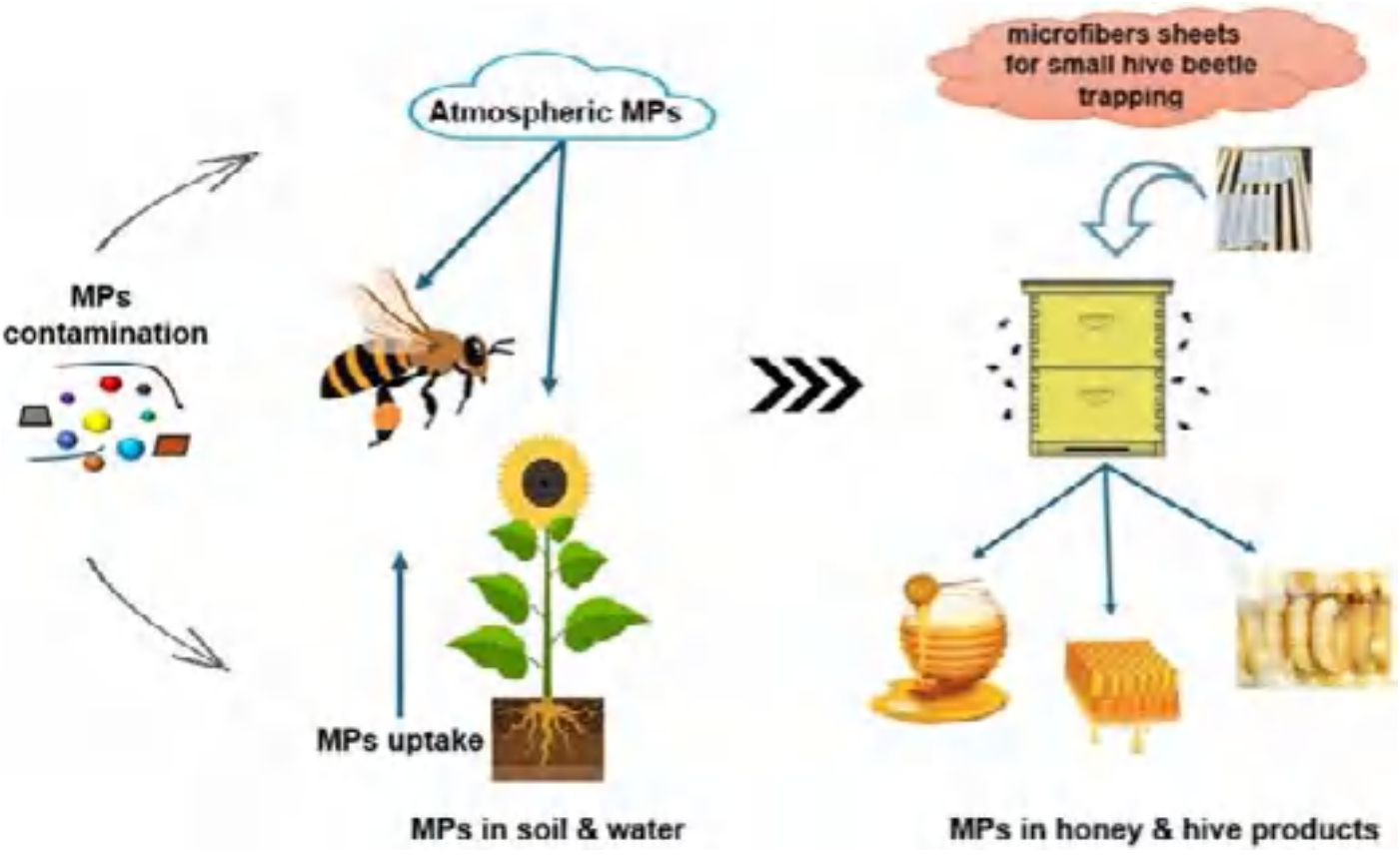

Fig. 1 Diagram depicting how honey bees are exposed to MPs and transition to honey and other hive product transition

as well as to bring the scientific community's attention to the probable risks of MPs to honey bee fitness (Al Naggar et al. 2021). There was only one published study; therefore, we posed a number of critical research questions that need to be answered before MPs can be considered a potential hazard to bees. Here, we reviewed the Web of Science (WOS) database literature to April 2024, using keywords (microplastics and pollinators). We found that nine studies evaluated the effects of MPs on honey bee health (Deng et al. 2021; Wang et al. 2021, 2022; Buteler et al. 2022; Balzani et al. 2022; Al Naggar et al. 2023; Pasquini et al. 2024; Zhu et al. 2024; Ferrante et al. 2024). As a result, this review aims to summarize available research on whether MPs endanger bees and pollination services, as well as to identify knowledge gaps.

Toxicity of MPs on honey bee health

Effects on survival

Determining the survival of honey bees after exposure to any environmental toxicant is crucial for risk assessment, because any stressor that affects the health of individual honey bees, dramatically reducing their survival, could be reproduced at the colony level, eventually leading to colony collapse. The effects of MPs on honey bee survival have so far been proven to be quite limited and concentration-, shape-, and polymer-dependent (Table 1). In every experiment, bees were exposed to MPs in sugar syrup, with chronic exposure ranging from 7 to 21 days in seven studies and acute exposure in two (48 h). Furthermore, in seven studies (Deng et al. 2021; Wang et al. 2021, 2022; Balzani et al. 2022; Pasquini et al. 2024; Zhu et al. 2024; Ferrante et al. 2024), bees were exposed to spherical MPs, whereas in just two studies (Buteler et al. 2022; Al Naggar et al. 2023), they were exposed to fibers or irregularly shaped MPs.

Out of the four studies that utilized polystyrene (PS) spheres (Deng et al. 2021; Wang et al. 2021, 2022; Al Naggar et al. 2023), only one demonstrated a lower survival rate (~ 25%) of bees exposed to a mixture of PS-MP spheres of varied sizes (0.5, 5, 50 μm) at 100 mg/L and also reported hair loss and body color change (Deng et al. 2021). Furthermore, there was no effect on survival when bees were either acutely exposed for 48 h to PS fibers (0.04–0.39–0.25 µm) (Buteler et al. 2022) or chronically exposed for 14 days to irregularly shaped PS-MPs (27–93 μm) (Al Naggar et al. 2023). Bee survival was found to decrease after 7 days of exposure to only higher concentrations (50 mg/L) of polyethylene (PE) MP spheres (lethal time (LT) at 7th day, 52.5% PE bees vs. 33.3% control bees; LT at 9th day, 74.2% PE bees vs. 62.5% control bees) (Balzani et al. 2022). Additionally, oral exposure to 100-µm PE-MP spheres increased mortality (~ 40%) and susceptibility to infections (Zhu et al. 2024).

Interestingly, exposing bees to different concentrations (0.5–50 mg L–1) of PS (4.8–5.8 μm in size) or polymethylmethacrylate/plexiglass (PMMA) (40 μm) for 10 days separately had no adverse effects on bee survival. However, at medium (5 mg/L) and high (50 mg/L) concentrations of PS and PMMA, an additive negative effect on bee survival (20–25%) decrease was noted (Ferrante et al. 2024), raising concerns about the deleterious interactive effects of MPs on honey bee health because various MP polymers with varying sizes and shapes were found in honey and on the bodies of bees (Diaz-Basantes et al. 2020; Deng et al. 2021; Edo et al. 2021).

Table 1 Summary of the effects of MPs on honey bee health

Polymer	Shape	Size	Conc	Type of exposure and duration	Effect	References
Polystyrene (PS)	Spheres	25 µm	0.5, 5, and 50 mg/L	Oral exposure for 14 days in sugar syrup	Survival not affected. Food intake decreased. Body weight not affected. Gut microbiome: changed. antioxidative gene (cat): affected. Detoxification genes: altered (CypQ1 and Gst53). Gut immune system–related coding genes: altered	Wang et al. (2021)
		0.5, 5, 50 µm	0.1, 1, 10, 100 mg/L	Oral exposure for 21 days in sugar syrup	Survival: decreased. Food intake not affected. Midgut tissue damaged. PS transferred to the hemolymph, trachea, and malpighian tubules. The susceptibility of bees to Israeli acute paralysis virus infection increased	Deng et al. (2021)
		100 nm, 1 µm, and 10 µm	10^8 particles/mL	Oral exposure for 15 days in sugar syrup	Survival not affected. Food intake not affected. Body weight decreased. Intestinal dysplasia occurred. Stimulated immune inhibitory genes and depressed genes related to detoxification and energy balance. Susceptibility to the pathogenic bacteria *Hafnia alvei* increased leading to a five times higher mortality rate	Wang et al. (2022)
	Fiber	0.04–0.39–0.25µm	10, 100 mg MP/L	Oral exposure for 48 h in sugar syrup	Survival not affected. Bees consumed MP-free solutions faster than solutions with 10 and 100 mg MPs/L. Foraging behavior not affected	Butzler et al. (2022)
	Irregular	Small (27 ± 17 µm) or large (93 ± 25 µm)	1, 10, 100 µg/mL	Oral exposure for 14 days in sugar syrup	Survival not affected. Food consumption decreased. Body weight decreased	Al Naggar et al. (2023)
Polystyrene (PS) or/and plexiglass (poly(methyl methacrylate) (PMMA))	Spheres	PS 4.8–5.8 µm and PMMA 1–40 µm	0.5, 5, and 50 mg L^{-1}	Oral exposure for 48 h in sugar syrup	PS reduced sucrose responsiveness, while PMMA had no significant effect. Combination had a marked negative effect on sucrose responsiveness. PMMA, PS, and MIX impaired bee learning and memory in bees. 1–5 µm thermoset amino formaldehyde MPs penetrated and accumulated in the brain after only 3 days of oral exposure	Pasquini et al. (2024)
		PS 4.8–5.8 µm and PMMA 1–40 µm	0.5, 5, and 50 mg L^{-1}	Oral exposure for 10 days in sugar syrup	An additive negative effect of PS and PMMA on bee survival and immune response. A reduction in the abundance of several cuticular compounds. Hive entry guards did not show increased inspection or aggressive behavior toward exposed foragers, allowing them to enter the colony without being treated differently from uncontaminated foragers	Ferrante et al. (2024)
Polyethylene (PE)	Spheres	0.2–9.9 µm	0.5, 5, 50 mg/L	Oral exposure for 7 days in sugar syrup	Survival decreased at the higher conc. Food intake increased at lower conc. No effect on sucrose sensitivity, habituation to sucrose, and appetitive olfactory learning and memory. PE was found to affect only bees' ability to respond consistently to sucrose	Balzani et al. (2022)
		1, 10, 100 µm	10^3, 10^4, and 10^5 particles/mL	Oral exposure for 15 days in sugar syrup	Survival decreased. Body weight not affected. Gut physical damage and microbiota dysbiosis occurred. Honeybee gut *Snodgrassella alvi* selectively enriched on PE-MPs' surface. PE-MPs make honeybees more susceptible to opportunistic pathogens *Hafnia alvei*	Zhu et al. (2024)

Effects on food consumption and body weight

A decrease in food intake can result in reduced energy reserves and a decrease in body weight. This can compromise the overall health and resilience of individual bees (Oliveira et al. 2019). There is no clear pattern for the effects of MPs on food intake and body weight, and the results are opposed (Table 1). For example, exposure to 25 μm PS-MP spheres reduced food intake at only a lower concentration (0.5 mg/L) without having a detrimental effect on body weight (Wang et al. 2021); in contrast, exposure to 100-nm PS spheres in size led to a significant decrease to 91.67% of the original weight of bees (Wang et al. 2022). In contrast, when bees were exposed to irregularly shaped PS fragments, both food intake and body weight were reduced by around 16% and 18.5%, respectively, which was independent of size and concentration (Al Naggar et al. 2023). The authors explained the later results by pointing to the high specific area and irregular shape of PS-MP fragments, which may impede honey bees' ability to digest food and hinder their digestive tracts. Other long-term effects were also proposed, like stomach lining damage or localized ulcerations that may cause a false sense of fullness, as shown in marine animals exposed to plastics (Cole et al. 2011; de Barros et al. 2020).

On the other hand, PE-MPs affected feeding behavior in a concentration-dependent manner, with bees consuming more food than controls when exposed to low concentration PE (0.5 mg/L); however, in bees exposed to a higher PE amount (50 mg/L), no effect on body weight was noted, despite a reduced survival, as indicated in the "Effects on food consumption and body weight" section (Balzani et al. 2022). Similarly, although a decrease in survival rate was observed, no effects on body weight were seen in bees exposed to PE-MPs of various sizes and concentrations (Zhu et al. 2024).

Effects on behavior & cognition

Honey bee behavior and cognition are crucial for the growth and success of a bee colony. The sophisticated social structure and division of labor within a honey bee colony are based on multiple tasks performed by individual bees, and their cognitive abilities are vital for the effective functioning of the entire colony (Klein et al. 2017). There are just four studies (Buteler et al. 2022; Balzani et al. 2022; Pasquini et al. 2024; Ferrante et al. 2024) that investigated the potential effects of MPs on honey bee behavior and cognition (Table 1). Buteler et al. (2022) investigated whether honey bee foragers visited resources and water solutions more frequently with or without MPs. They discovered that bees absorbed PS microfibers supplied in water and sucrose solutions indiscriminately, and no impacts on foraging behavior were detected, though bees consumed PS-free solutions quicker than solutions with 10 and 100 mg PS microfibers/L (Buteler et al. 2022). Whereas exposure to a higher concentration of PE-MP spheres (50 mg/L) has been shown to only alter the bees' capability to consistently respond to sucrose, not their sensitivity to it, their ability to get habituated to it, or their ability for learning and memory (Balzani et al. 2022).

Recently, the effects of PS or/and PMMA spheres, either singly or mixed, on the cognitive and behavioral performance of bees were studied (Pasquini et al. 2024). Using the proboscis extension reflex (PER) assay, the researchers found that PS lowered sucrose responsiveness by around 10%, while PMMA had no significant effect; however, the combination had a significant negative effect, with around 30% drop in sucrose response. PS alone at the highest concentration (50 mg/L) had a significant impact on the learning phase, with a 40% decline in PER response, while PS and PMMA either individually or combined impaired memory of bees both mid-term specific memory (MTM) and early long-term specific memory (eLTM) by around 25–35%. Interestingly, using 3D brain imaging, the authors were also able to confirm the translocation of MPs to bee brain tissues within 3 days of oral treatment; nevertheless, a causal relationship between the reported cognitive deficiencies was not established (Pasquini et al. 2024).

Surprisingly, changes in bee cuticle profiles were identified when exposed to PS or/and PMMA, with PMMA being the primary cause. De-

spite these chemical changes, nest guards showed no intensified inspection or aggressive behavior toward exposed foragers, allowing them to enter the colony undisturbed (Ferrante et al. 2024).

Translocation, accumulation, and histopathological effects

Translocation and accumulation of MPs in tissues can have several deleterious impacts (D'Costa 2022; Zeng et al. 2023). MPs have been shown to interact with the gut lumen in the fruit fly (Drosophila melanogaster) (Diptera: Drosophilidae), be taken up by gut enterocytes, pass through the intestinal barrier to the hemolymph, be absorbed by hemocytes and malpighian tubules, and even translocate to flies' compound eyes and ocelli (Lee and Jang 2014; El Kholy and Al Naggar 2023). Similarly, MPs have been shown to be ingested and accumulated in the midgut and hindgut, as well as transported to the trachea and malpighian tubules (Deng et al. 2021; Wang et al. 2021) as well as brain tissues (Pasquini et al. 2024). Histological investigation showed that the intestinal tracts of bees exposed to PS-MP spheres (0.5 and 5 μm in size) had larger lumens, wider intercellular spaces that eventually disrupted the microvilli, compromised peritrophic membrane integrity that increased PM shedding, and nearly completely broken basement membranes (Deng et al. 2021). Similar to this, after PS-NP spheres were administered, the bee gut basement membrane showed raptures, the cytoplasm showed signs of vacuolization and lysing without any visible nuclei, and the PS-100 nm group's crypt depth/intestinal wall thickness ratio value was noticeably higher than that of the control and 1-µm PE-MP treatment groups (Wang et al. 2022). For bees treated with 1-µm and 10-µm PE-MP spheres, fewer layers of cells were found in midgut tissues, as evidenced by reduced intestinal wall thicknesses. Conversely, the peritrophic membrane, dense layers of columnar cells, and basement membrane were damaged in bees subjected to 100-µm PE-MPs, leading to a decrease in the thickness of the intestinal wall and the dispersion of midgut contents into the alimentary canal lumen (Zhu et al. 2024).

Effects on immunity and gut microbiota

The gut microbiota in honey bees plays a crucial role in maintaining their health and general well-being (Zheng et al. 2017; Raymann and Moran 2018; Jones et al. 2018; Steele et al. 2021; Dosch et al. 2021). However, exposure of bees to different environmental toxicants alters the diversity and composition of their gut microbiome as well as their immunity, resulting in major consequences for bee health (Motta et al. 2018; Rothman et al. 2019; El-Seedi et al. 2022; Al Naggar et al. 2022; Al Naggar and Wubet 2024).

There are only three studies that have investigated the consequences of exposure to MPs on the gut microbiota (Table 1). In the first study, bees were exposed to PS-MP spheres for 14 days, which reduced the diversity of bacteria in the gut, producing profound abnormalities in their gut microbiomes, as well as changes in gene expression related to oxidative damage, detoxification, and immunity, although there was no decrease in bee survival (Wang et al. 2021). The second study found that administering 100-nm PS spheres reduced the relative abundance of Bifidobacterium and Lactobacillus in the guts. This, in turn, activated immune inhibitory genes and suppressed genes linked to energy balance and detoxification, increasing the susceptibility of bees to the pathogenic bacteria Hafnia alvei and resulting in mortality of 92% of bees, which was 5 and 1.5 times higher than those from the "control" and the "H. alvei" groups, respectively (Wang et al. 2022). In the third study, honey bee mortality is linked to both gut physical damage and dysbiosis of the microbiota (Zhu et al. 2024). Snodgrassella alvi, a core functional gut bacterium, was selectively enriched on the surface of PE-MPs, which perturbed the gut microbial communities in honey bees and made bees more susceptible to the pathogenic bacteria

Hafnia alvei, resulting in around 30 and 40% higher mortality in bees co-exposed to 100-µm PE-MPs and H. alvei, compared to healthy bees exposed to 100-µm PE-MPs and those challenged with H. alvei, respectively (Zhu et al. 2024). A crucial mechanism underpinning gut microbial dysbiosis in the later study appeared to

be the colonization of Snodgrassella from the intestinal wall to the surface of PE-MPs, which reduced its function as a protective barrier for the intestinal wall of honey bees. Given that a healthy and balanced gut microbiome is critical for maintaining healthy colonies (Raymann and Moran 2018), these MPinduced effects pose a potential risk to bee fitness and performance, but they must be validated by field and in-hive experiments.

Furthermore, ingestion of PS-MP spheres resulted in a significant change in gene expression linked to membrane lipid metabolism, immune response, detoxification, and the respiratory system (Deng et al. 2021). Subsequently, the authors investigated whether PS could make honey bees more susceptible to Israeli acute paralysis virus (IAPV) infection and discovered that the IAPV titer of IAPV was much greater in the PS with the IAPV group than that in IAPV, but no significant difference in survival rate was found.

Discussion

Honey bees visit every environmental compartment when collecting nectar, honeydew, pollen, and other plant exudates. If MPs pollute surrounding compartments, they will eventually be introduced into honey bees and hive products (Al Naggar et al. 2021). Therefore, MPs were detected in honey and in the bee body. As a result, nine studies have been conducted under controlled laboratory conditions to investigate the potentially harmful effects of MPs on honey bee health (Deng et al. 2021; Wang et al. 2021, 2022; Buteler et al. 2022; Balzani et al. 2022; Al Naggar et al. 2023; Pasquini et al. 2024; Zhu et al. 2024; Ferrante et al. 2024). Some data indicated detrimental effects on bee mortality, feeding rate, behavior, immunity, and gut microbiota, as described above and summarized in (Fig. 2).

However, a single semi-field in-hive experiment was carried out, and it was discovered that adult bees that consumed sugar solutions contaminated with 50 mg PS microfibers for 1 month dispersed the fibers throughout their colonies. Treated colonies did not demonstrate differences with control hives in honey reserves, adult or brood populations (Alma et al. 2023). Given that this is the only in-hive experiment to look into the potential impacts of MPs on honey bee health at the colony level, it is unclear whether the non-observed negative effects are due to honey bee population dynamics or other causes.

For example, one could argue that while some bees have died because of PS microfibers, this has been compensated by the enormous number of individuals in the hive. Or the number of PS microfibers translocated to bees through feeding was insufficient to cause detrimental ef-

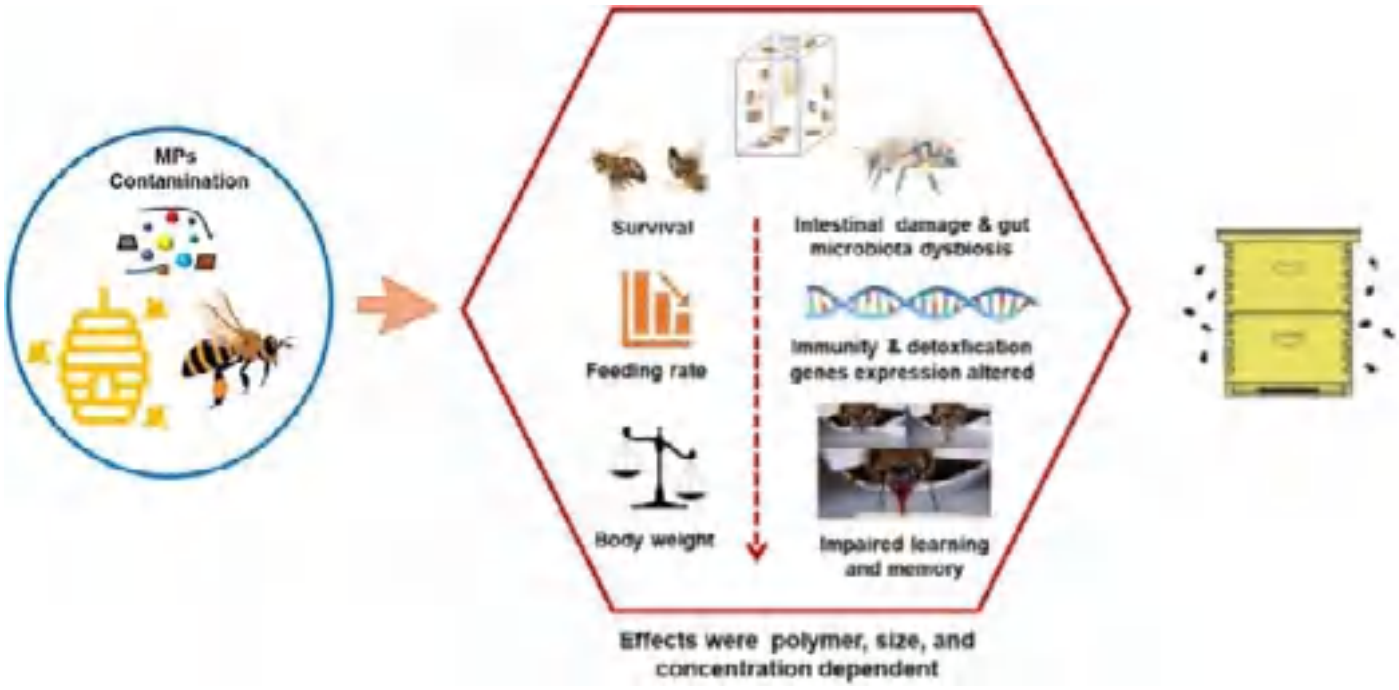

Fig. 2 A graphical summary of the effects of MPs on honey bee health so far

fects at the colony level. On the other hand, this scenario might look different if bees were exposed to different polymers, either separately or in combination, given that several polymers of varied forms, sizes, and concentrations were found in honey and on the bodies of bees (Deng et al. 2021; Edo et al. 2021). Therefore, more research is required to test these hypotheses.

Despite our emphasis on the importance of investigating the combined impacts of MPs and other environmental contaminants such as heavy metals, pesticides, and nanomaterials, as well as parasites and pathogens (Al Naggar et al. 2021), there have not been any studies conducted yet. Given that numerous studies have found severe interactions between MPs and pesticides on the health of living organisms (Wang et al. 2020, 2023; Albendín et al. 2023; Guru et al. 2023), their combined effects are particularly important for bees, which are often exposed to agrochemicals commonly used for pest control, as well as in-hive miticides used to control hive pests and parasites. Furthermore, the impacts of PS, PET-MPs, and titanium dioxide (TiO2) nanoparticles were evaluated using the tropical pollinator Partamona helleri (Apidae: Apinae: Meliponini). Changes in hemocyte counts (a shift in the proportion of plasmatocytes and prohemocytes in treated individuals) and walking behavior (average speed (cm/s)), meanders (°/cm; the absolute sum of the angle the individual turned divided by the distance the individual covered), resting time (s), and interactions (considered interaction when the bees approached a distance ≤ 0.68 cm) were identified (Viana et al. 2023).

On the other hand, communication between flowers and pollinators is a crucial component of sexual reproduction in flowering plants and is mutually beneficial to both (Wester and Lunau 2017; Shah et al. 2023). For instance, plants require compatible pollen distribution and receipt at low costs, whereas pollinators need floral rewards that may be taken as fast and efficiently as feasible. Regretfully, a recent study discovered that polypropylene (PP)-MP fragment deposits negatively affected seed production and the quantity of pollen tubes that reached the ovaries (Carvallo and Muñoz-Michea 2023). MPs and NPs have also been shown to affect plant phytohormonal and metabolic profiles, potentially affecting nectar production and synthesis (Li et al. 2021; Bouaicha et al. 2022). These documented NP and MP-induced changes may affect the biology of pollination, most likely by impeding or postponing interactions between plants and pollinators. Thus, more investigation is required to validate these hypotheses.

Conclusion and future directions

The data demonstrate a wide range of material-/size-/ dosage-dependent effects, underscoring the importance of comprehensive evaluations in future investigations. MPs have been found in honey and in bee organs (for example, the gut and brain), presenting potential hazards to bee fitness such as altered behavior, cognitive capacities, reduced immunity, and gut microbiome dysfunction.

Even though multiple laboratory studies have demonstrated the negative effects mentioned above, field and semi-field investigations are still recommended to look into the potential negative effects of MPs on brood pattern, queen laying, drone vitality, and colony vigor and whether or not it depends on MP type, size, shape, and concentrations.

Further research needs to be conducted to determine how MPs interact with various honey bee diseases and other environmental contaminants, including pesticides, antibiotics, fungicides, heavy metals, and others.

Additionally, the widespread existence of MPs as an increasing environmental contaminant highlights the necessity of acknowledging the significant impacts of ensuing gut dysbiosis on the physiological condition of the bees. Taken together, our review indicates the significance of knowing the interactions between MPs, pollinators, and the ecosystem to avoid potential threats and preserve the sustainability of critical services provided by honey bees.

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References

Al Naggar Y, Brinkmann M, Sayes CM et al (2021) Are honey bees at risk from microplastics? Toxics 9:109. https://doi.org/10.3390/ toxics9050109

Al Naggar Y, Sayes CM, Collom C, et al (2023) Chronic exposure to polystyrene microplastic fragments has no effect on honey bee survival, but reduces feeding rate and body weight. Toxics 11. https://doi.org/10.3390/toxics11020100

Al Naggar Y, Singavarapu B, Paxton RJ, Wubet T (2022) Bees under interactive stressors: the novel insecticides flupyradifurone and sulfoxaflor along with the fungicide azoxystrobin disrupt the gut microbiota of honey bees and increase opportunistic bacterial

pathogens. Sci Total Environ 849:157941. https://doi.org/10.1016/j.scitotenv.2022.157941

Al Naggar Y, Wubet T (2024) Chronic exposure to pesticides disrupts the bacterial and fungal co-existence and the cross-kingdom network characteristics of honey bee gut microbiome. Sci Total Environ 906:167530. https://doi.org/10.1016/j.scitotenv. 2023.167530

Albendín MG, Alarcón I, Coello MD et al (2023) The effects of exposing Solea senegalensis to microbeads with and without pesticides. Water, Air, Soil Pollut 234:132. https://doi.org/10.1007/ s11270-023-06096-z

Alma AM, de Groot GS, Buteler M (2023) Microplastics incorporated by honeybees from food are transferred to honey, wax and larvae. Environ Pollut 320:121078. https://doi.org/10.1016/j.envpol.2023. 121078

Anetor GO, Nwobi NL, Igharo GO, et al (2022) Environmental pollutants and oxidative stress in terrestrial and aquatic organisms: examination of the total picture and implications for human health. Front Physiol 13. https://doi.org/10.3389/fphys.2022.931386

Balzani P, Galeotti G, Scheggi S et al (2022) Acute and chronic ingestion of polyethylene (PE) microplastics has mild effects on honey bee health and cognition. Environ Pollut 305:119318. https://doi. org/10.1016/j.envpol.2022.119318

Borrelle SB, Ringma J, Law KL et al (2020) Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. Science 369:1515–1518. https://doi.org/10.1126/science.aba3656

Bouaicha O, Tiziani R, Maver M et al (2022) Plant species-specific impact of polyethylene microspheres on seedling growth and the metabolome. Sci Total Environ 840:156678. https://doi.org/10. 1016/j.scitotenv.2022.156678

Buteler M, Alma AM, Stadler T, et al (2022) Acute toxicity of microplastic fibers to honeybees and effects on foraging behavior. Sci Total Environ 822. https://doi.org/10.1016/j.scitotenv.2022. 153320

Buteler M, Villalobos E, Alma AM et al (2023) Management practice for small hive beetle as a source of microplastic contamination in honey and honeybee colonies. Environ Pollut 334:122151. https:// doi.org/10.1016/j.envpol.2023.122151

Carvallo GO, Muñoz-Michea V (2023) Microplastics clog reproduction in a monkeyflower species. BioRxiv 46:101611

Cole M, Lindeque P, Halsband C, Galloway TS (2011) Microplastics as contaminants in the marine environment: a review. Mar Pollut Bull 62:2588–2597. https://doi.org/10.1016/j.marpolbul.2011.

09.025

D'Costa AH (2022) Microplastics in decapod crustaceans: accumulation, toxicity and impacts, a review. Sci Total Environ 832:154963. https://doi.org/10.1016/j.scitotenv.2022.154963

de Barros MSF, dos Santos Calado TC, de Sá Leitão Câmara de Araújo M (2020) Plastic ingestion lead to reduced body condition and modified diet patterns in the rocky shore crab Pachygrapsus transversus (Gibbes, 1850) (Brachyura: Grapsidae). Mar Pollut Bull 156:111249. https://doi.org/10.1016/j.marpolbul.2020.111249

de Souza Machado AA, Kloas W, Zarfl C et al (2018) Microplastics as an emerging threat to terrestrial ecosystems. Glob Chang Biol 24:1405–1416. https://doi.org/10.1111/gcb.14020

Deng Y, Jiang X, Zhao H et al (2021) Microplastic polystyrene ingestion promotes the susceptibility of honeybee to viral infection. Environ Sci Technol 55:11680–11692. https://doi.org/10.1021/ acs.est.1c01619

Diaz-Basantes MF, Conesa JA, Fullana A (2020) Microplastics in honey, beer, milk and refreshments in ecuador as emerging contaminants. Sustainability 12:5514. https://doi.org/10.3390/su121 45514

Dissanayake PD, Kim S, Sarkar B et al (2022) Effects of microplastics on the terrestrial environment: a critical review. Environ Res 209:112734. https://doi.org/10.1016/j.envres.2022.112734

Dosch C, Manigk A, Streicher T et al (2021) The gut microbiota can provide viral tolerance in the honey bee. Microorganisms 9:871. https://doi.org/10.3390/microorganisms9040871

Du S, Zhu R, Cai Y et al (2021) Environmental fate and impacts of microplastics in aquatic ecosystems: a review. RSC Adv 11:15762–15784. https://doi.org/10.1039/D1RA00880C

Edo C, Fernández-Alba AR, Vejsnæs F et al (2021) Honeybees as active samplers for microplastics. Sci Total Environ 767:144481. https://doi.org/10.1016/j.scitotenv.2020.144481

El-Seedi HR, Ahmed HR, El-Wahed AAA et al (2022) Bee stressors from an immunological perspective and strategies to improve bee health. Vet Sci 9:199. https://doi.org/10.3390/vetsci9050 199

El Kholy S, Al Naggar Y (2023) Exposure to polystyrene microplastic beads causes sex-specific toxic effects in the model insect Drosophila melanogaster. Sci Rep 13:1–11. https://doi.org/10. 1038/s41598-022-27284-7

Ferrante F, Pasquini E, Cappa F et al (2024) Unravelling the microplastic menace: different polymers additively increase bee vulnerability. Environ Pollut 352:124087. https://doi.org/10.1016/j. envpol.2024.124087

Ghosh S, Sinha JK, Ghosh S et al (2023) Microplastics as an emerging threat to the global environment and human health. Sustainability 15:10821. https://doi.org/10.3390/su151410821

Guru A, Rady A, Darwish NM et al (2023) Synergetic effects of polyethylene microplastic and abamectin pesticides on the eyes of zebrafish larvae and adults through activation of apoptosis signaling pathways. Environ Toxicol Pharmacol 102:104215. https://doi.org/10.1016/j.etap.2023.104215

Haavik LJ, Stephen FM (2023) Insect ecology. Forest entomology and pathology. Springer International Publishing, Cham, pp 91–113

Harmon SM, Chen Q, Ma C, et al (2024) The effects of microplastic pollution on aquatic organisms. In: Microplastic contamination in aquatic environments. Elsevier, pp 355–379. https://doi.org/ 10.1016/B978-0-443-15332-7.00001-6

Järlskog I, Strömvall A-M, Magnusson K et al (2020) Occurrence of tire and bitumen wear microplastics on urban streets and in sweepsand and washwater. Sci Total Environ 729:138950. https://doi. org/10.1016/j.scitotenv.2020.138950

Jaureguiberry P, Titeux N, Wiemers M, et al (2022) The direct drivers of recent global anthropogenic biodiversity loss. Sci Adv 8. https://doi.org/10.1126/sciadv.abm9982

Jones JC, Fruciano C, Marchant J et al (2018) The gut microbiome is associated with behavioural task in honey bees. Insectes Soc 65:419–429. https://doi.org/10.1007/s00040-018-0624-9

Klein S, Cabirol A, Devaud J-M et al (2017) Why bees are so vulnerable to environmental stressors. Trends Ecol Evol 32:268–278. https://doi.org/10.1016/j.tree.2016.12.009

Lampitt RS, Fletcher S, Cole M et al (2023) Stakeholder alliances are essential to reduce the scourge of plastic pollution. Nat Commun 14:2849. https://doi.org/10.1038/s41467-023-38613-3

Lee KP, Jang T (2014) Exploring the nutritional basis of starvation resistance in Drosophila melanogaster. Funct Ecol 28:1144–1155. https://doi.org/10.1111/1365-2435.12247

Li J-Y, Yu Y, Craig NJ et al (2024) Interactions between microplastics and insects in terrestrial ecosystems—a systematic review and meta-analysis. J Hazard Mater 462:132783. https://doi.org/10. 1016/j.jhazmat.2023.132783

Li S, Wang T, Guo J et al (2021) Polystyrene microplastics disturb the redox homeostasis, carbohydrate metabolism and phytohormone regulatory network in barley. J Hazard Mater 415:125614. https:// doi.org/10.1016/j.jhazmat.2021.125614

Liebezeit G, Liebezeit E (2015) Origin of synthetic particles in honeys. Polish J Food Nutr Sci 65:143–147. https://doi.org/10.1515/ pjfns-2015-0025

Liebezeit G, Liebezeit E (2013) Non-pollen particulates in honey and sugar. Food Addit Contam Part A 30:2136–2140. https://doi.org/10.1080/19440049.2013.843025

Motta E, Raymann K, Moran NA (2018) Glyphosate perturbs the gut microbiota of honey bees. Proc Natl Acad Sci 115:10305–10310. https://doi.org/10.1073/pnas.1803880115

OECD (2022) OECD. https://www.oecd.org/environment/plastic-pollu tion-is-growing-relentlessly-as-waste-management-and-recyclingfall-short.htm. Accessed 01-06-2024

Oliveira M, Ameixa OMCC, Soares AMVM (2019) Are ecosystem services provided by insects "bugged" by micro (nano)plastics? TrAC Trends Anal Chem 113:317–320. https://doi.org/10. 1016/j.trac.2019.02.018

Pasquini E, Ferrante F, Passaponti L et al (2024) Microplastics reach the brain and interfere with honey bee cognition. Sci Total Environ 912:169362. https://doi.org/10.1016/j.scitotenv.2023.169362

Rai M, Pant G, Pant K et al (2023) Microplastic pollution in terrestrial ecosystems and its interaction with other soil pollutants: a potential threat to soil ecosystem sustainability. Resources 12:67. https://doi.org/10.3390/resources12060067

Rangel-Buitrago N, Ben-Haddad M, Nicoll K et al (2024) Marine plastic pollution in the Anthropocene: a linguistic toolkit for holistic understanding and action. Ocean Coast Manag 248:106967. https://doi.org/10.1016/j.ocecoaman.2023.106967

Raymann K, Moran NA (2018) The role of the gut microbiome in health and disease of adult honey bee workers. Curr Opin Insect Sci 26:97–104

Rolsky C, Kelkar V, Driver E, Halden RU (2020) Municipal sewage sludge as a source of microplastics in the environment. Curr Opin Environ Sci Heal 14:16–22. https://doi.org/10.1016/j.coesh.2019. 12.001

Rothman JA, Leger L, Kirkwood JS, McFrederick QS (2019) Cadmium and selenate exposure affects the honey bee microbiome and metabolome, and bee-associated bacteria show potential for bioaccumulation. Appl Environ Microbiol 85. https://doi.org/10. 1128/AEM.01411-19

Scudder GGE (2017) The importance of insects. In: Insect Biodiversity. Wiley, pp 9–43. https://doi.org/10.1002/9781118945568.ch2

Shah S, Ilyas M, Li R et al (2023) Microplastics and nanoplastics effects on plant-pollinator interaction and pollination biology. Environ Sci Technol 57:6415–6424. https://doi.org/10.1021/acs. est.2c07733

Steele MI, Motta EVS, Gattu T, et al (2021) The gut microbiota protects bees from invasion by a bacterial pathogen. Microbiol Spectr

9. https://doi.org/10.1128/spectrum.00394-21

Stork NE (2018) How many species of insects and other terrestrial arthropods are there on Earth? Annu Rev Entomol 63:31–45. https://doi.org/10.1146/annurev-ento-020117-043348

Viana TA, Botina LL, Bernardes RC et al (2023) Ingesting microplastics or nanometals during development harms the tropical pollinator Partamona helleri (Apinae: Meliponini). Sci Total Environ 893:164790. https://doi.org/10.1016/j.scitotenv.2023.164790

Wang K, Li J, Zhao L et al (2021) Gut microbiota protects honey bees (Apis

mellifera L.) against polystyrene microplastics exposure risks. J Hazard Mater 402:123828. https://doi.org/10.1016/j.jhazm at.2020.123828

Wang K, Zhu L, Rao L et al (2022) Nanoand micro-polystyrene plastics disturb gut microbiota and intestinal immune system in honeybee. Sci Total Environ 842:156819. https://doi.org/10.1016/j. scitotenv.2022.156819

Wang T, Yu C, Chu Q et al (2020) Adsorption behavior and mechanism of five pesticides on microplastics from agricultural polyethylene films. Chemosphere 244:125491. https://doi.org/10.1016/j.chemo sphere.2019.125491

Wang Y, Zhao Y, Liang H et al (2023) Single and combined effects of polyethylene microplastics and acetochlor on accumulation and intestinal toxicity of zebrafish (Danio rerio). Environ Pollut 333:122089. https://doi.org/10.1016/j.envpol.2023.122089

Wester P, Lunau K (2017) Plant–pollinator communication. pp 225–257. https://doi.org/10.1016/bs.abr.2016.10.004

Wright SL, Thompson RC, Galloway TS (2013) The physical impacts of microplastics on marine organisms: a review. Environ Pollut 178:483–492. https://doi.org/10.1016/j.envpol.2013.02.031

Zeng Y, Deng B, Kang Z et al (2023) Tissue accumulation of polystyrene microplastics causes oxidative stress, hepatopancreatic injury and metabolome alterations in Litopenaeus vannamei. Ecotoxicol Environ Saf 256:114871. https://doi.org/10.1016/j.ecoenv.2023. 114871

Zheng H, Powell JE, Steele MI et al (2017) Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. Proc Natl Acad Sci 114:4775–4780. https://doi.org/10. 1073/pnas.1701819114

Zhu L, Wang K, Wu X et al (2024) Association of specific gut microbiota with polyethylene microplastics caused gut dysbiosis and increased susceptibility to opportunistic pathogens in honeybees Science of the Total Environment Association of specific gut microbiota with polyethylene micr. Sci Total Environ 918:170642. https://doi.org/10.1016/j.scitotenv.2024.170642

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DETERMINATION OF RESIDUES IN HONEY AFTER TREATMENTS WITH FORMIC AND OXALIC ACID UNDER FIELD CONDITIONS

Abstract

Formic acid and oxalic acid field trials for control of Varroa destructor were carried out in autumn according to the Swiss prescriptions during three successive years in different apiaries in Switzerland. The following parameters were determined in honey that was harvested the year after treatment: formic acid, oxalic acid and free acidity. The following range of values were found in honeys of untreated colonies: formic acid, from 17 to 284 mg/kg, n = 34; oxalic acid, from 11 to 119 mg/kg, n = 33. There was a small, but unproblematic increase in formic acid levels in comparison to the levels in the controls; average: 46 mg/kg, maximum: 139 mg/kg. No increase in formic acid was found with increasing number of treatment years. If emergency formic acid treatments were carried out in spring, the residue levels were much higher: average increase of 193 mg/kg, maximum 417 mg/kg. The oxalic acid content remained unchanged, even after two successive treatments during the same autumn. No rise of free acidity was encountered after a combined treatment with formic and oxalic acid during the three trial years.

1. INTRODUCTION

Presently synthetic acaricides are used regularly for the control of Varroa destructor Anderson and Truman, (formerly Va rroa Jacobsoni Oudemans). However, due to their lipophilic and persistent nature, they accumulate in wax, and to a smaller extent in honey (Bogdanov et al., 1998b; Wallner, 1999). Recently, acaricide resistant mites have appeared in many countries of the world (Milani, 1999). Because of these problems, natural, non-toxic substances such as different organic acids (Liebig, 1997; Imdorf and Charrière, 1998) and essential oils (Imdorf et al., 1999) were developed for the control of V. destructor. These substances are used increasingly throughout Europe.

Oxalic and formic acids are natural constituents of honey. They are allowed for use in biological beekeeping (EU Council Regulation, 1999). According to the EU residue regulation (1995) formic acid has a GRAS status (Generally Recognised As Safe) and the establishment of a MRL (Maximum Residue Limit) is not necessary.

Until now residue studies have been carried out under controlled experimental conditions after

single treatments with formic acid (Stoya et al., 1986; Hansen and Guldborg, 1988; Krämer, 1994; Barbattini et al., 1994; Capolongo et al., 1996; Radke and Hedtke, 1998). There are several short communications on residues after single treatments with oxalic acid (Radezki, 1994; Mutinelli et al., 1997; Del Nozal et al., 2000; Bernardini and Gardi, 2001), but they do not allow for a detailed assessment of eventual residue problems. To date, no long-term field trials have been carried out measuring the possible residues after repeated use of formic and oxalic acid in practical beekeeping. Indeed, such studies are necessary for the assessment of possible long-term effects of acaricide residues on the quality of bee products. Field trials on the elucidation of long-term effects of the repeated application of thymol (Bogdanov et al., 1998a) and synthetic acaricides (Bogdanov et al., 1998b) on honey and wax quality have been conducted. As organic acids are hydrophilic substances which do not accumulate in wax, only honey studies are necessary. The purpose of the present study was to determine honey residue levels following formic and oxalic acid treatments under practical Swiss beekeeping conditions.

2. MATERIALS AND METHODS

2.1. Treatments with organic acids

Three trials were carried out in apiaries, situated in various parts of Switzerland during 1996, 1997 and 1998.

The number of apiaries involved in the trials each year varied over the three trial years (see Tabs. III to VI).

The average number of hives per apiary was 12 (minimum 9). In these apiaries the beekeepers followed a definite treatment protocol.

Two formic acid treatments were carried out in August–September, followed by one oxalic acid treatment during the broodless late autumn period between November and December. Honey samples from neighbouring apiaries, treated with Apistan were taken as controls (see also Sect. 2.2).

2.1.1. Normal treatments with formic acid in autumn

Formic acid treatments were applied twice in autumn, usually in August and September by different application devices, mostly by the Liebefeld dispenser (Charrière et al., 1998a). Two applications with 130 ml 70% formic acid per colony were applied by evaporation over seven days.

2.1.2. Emergency treatments with formic acid in spring

Emergency treatments with formic acid in spring were carried out during 1997 and 1999 in 7 different apiaries which did not take part in the trials indicated above (Tab. IV). The emergency treatments had to be carried out because of high

mite infestation pressure that endangered colony survival. One treatment with formic acid was carried out just after the spring honey harvest during one week, in the period between the end of May and the beginning of June, as described above for the normal treatment. The honey supers were taken off before the treatment.

2.1.3. Oxalic acid treatments

Oxalic acid treatments by spraying were carried out during the broodless period between beginning of November and end of December. Spraying was carried out by applying a solution of 30 g oxalic acid dihydrate/L water according to Charrière et al. (1998b).

In 1998, trickling was carried out in some apiaries before spraying in separate trials to test the efficacy of different methods of application of oxalic acid. In these cases, 50 ml of 60 g oxalic acid dihydrate/L water:sucrose (1:1) solution was trickled per bee colony according to Charrière et al. (1998b) before the spray treatment.

2.2. Honey sampling

Most trials samples were taken from the first honey harvested in spring. However under some circumstances (e.g. lack of a sufficient spring honey flow) beekeepers in Switzerland harvest the first honey in summer. For that reason we had to include summer honeys in the trial.

Honey yields were not measured, as we wanted to test the residues under practical conditions independent of the honey yield. The honey yields are taken from the official regional harvest reports of the Swiss Beekeeping Associations (averages and range of variation):

1996: 4.1, 1.7–7.3 1997: 6.9, 2.0–12.6

1998: 12.9, 7.9–15.6 1999: 9.9–12.4.

As the control and trial samples were taken from neighbouring apiaries, the yields of the control and trial honeys were similar.

Control and treatment samples were divided into two groups according to their electrical conductivity (see Sect. 2.3). The hypothesis that the electrical conductivity for a given date did not differ among samples from untreated and treated colonies was tested by the Student's t-Test (Microsoft Excel 1997).

2.2.1. Trial honey samples

After the autumn treatments, honey samples were collected from the first honey harvest in spring or in summer of the following year. After the spring treatments, the samples were collected from the summer harvest of the same year. In each apiary three honey samples were collected during the filling of honey from the honey tank at the beginning, middle and the end of the filling process. The average of the measurements of these three samples was used for the trial evaluation.

2.2.2. Control honey samples

Control honey samples were collected in the same way as the trial samples from apiaries that were in the neighbourhood of the treated apiaries to guarantee that the control honeys were as similar as possible to the trial honeys. The control apiaries were treated with Apistan.

2.3. Honey analysis

The honey samples were stored at room temperature until analysis within 6 months after the honey harvest. Formic and oxalic acid were assayed by the Böhringer oxalic acid kit, by which both acids are determined in the same sample (Böhringer Mannheim, 1997).

The stability of formic and oxalic acids was tested by measuring these acids in two honeys, stored at room temperature (20–25 o C) for six months. The honeys had the following initial concentrations:

honey 1: formic acid 71.6 mg/kg and oxalic acid 45.0 mg/kg;

honey 2: formic acid 224.0 mg/kg and oxalic acid 89.0 mg/kg.

Measurements after 3 and 6 months showed that the acid concentrations remained at their initial level (all measurements fell within the precision limits of the method, see Tab. I).

For determination of recovery, honey was spiked with 150 and 300 mg formic acid/kg and with 50 and 100 mg oxalic acid/kg (n = 8 for each concentration level). The recoveries for the two concentration levels were the same, thus, the re-

covery is presented here as an average of both concentration levels:

formic acid: 93% (sd = 4%, n = 16) for honey spiked with 150 and 300 mg/kg;

oxalic acid: 84% (sd = 24%, n =16) for honey spiked with 50 and 100 mg/kg.

The precision of the measurement, defined by the coefficient of variation CV %, was determined for three different concentration levels (Tab. I). One of the honeys measured was an "unspiked" blank, the others were the same sample of honey, spiked with 150 and 300 mg/kg formic acid and 50 and 100 mg/kg oxalic acid. The precision of the formic acid measurement was somewhat better than that of the oxalic acid determination. The limit of detection of the two enzymatic methods fell between 3 to 5 mg/kg.

Free acidity (in milliequivalents (meq) per kg) and electrical conductivity (in millisiemens (mS) per cm) were determined according to the harmonised methods of the International Honey Commission (Bogdanov et al., 1997). Honeys with less than 0.8 mS/cm belonged to the group consisting of floral honeys and blends of floral and honeydew honey, while those having more than 0.8 mS were assigned to the honeydew type (Bogdanov et al., 1999b). As the content of formic and oxalic acid depends on the honey type (Tab. II), the trial and the control honeys belonged to the same honey group, which, in all but one trial (see Sect. 3.1) belonged to the group consisting of blossom and blends of blossom and honeydew honeys. There was no statistically significant difference between the electrical conductivities of the trial and the control honeys. No unifloral honeys were present in the control and the treatment honey groups as tested by sensorial analysis.

3. RESULTS

3.1. Natural content of formic acid, oxalic acid and free acidity

Table II summarises the measurements of total acidity, and formic and oxalic acid in all control honeys during the three trial years. The natural formic acid content varied between 17 and 284 mg/kg, while the oxalic acid content varied between 8 and 119 mg/kg. The average content

Table I. Precision and repeatability of the enzymatic determination of formic and oxalic acid. Values in mg/kg.

	Formic acid			Oxalic acid		
average	74.1	214.9	353.9	47.9	86.7	134.1
st. deviation	3.3	7.7	10.3	6.4	16.0	22.7
CV %	4.4	3.6	2.9	13.4	18.4	16.9
n (samples)	11	8	10	9	8	12

Table II. Formic acid, oxalic acid and free acidity in untreated honeys. Data expressed as: conductivity (Co) in mS/cm; formic acid (FA) and oxalic acid (OA) in mg/kg; free acidity (FAc) in meq/kg.

	blossom, blossom-honeydew blends				honeydew honeys			
	Co	FA	OA	FAc	Co	FA	OA	FAc
average	0.41	41	25	17	0.98	93	70	27
st. deviation	0.14	17	11	5	0.14	68	25	6
min-max	0.15–0.69	17–85	8–51	8–26	0.85–1.3	42–284	38–119	19–37
n (samples)	23	24	23	21	10	10	10	8
P (*t*-Test)					0.000	0.002	0.000	0.000

of both acids and of the free acidity in honeydew honey was significantly higher than that of the group consisting of blossom honey and blends of blossom and honeydew honey. On average, honeydew honeys contained twice the amount of formic acid, almost three times more oxalic acid, and 60% more free acidity.

3.2. Effects of the treatments on formic and oxalic acid content and on free acidity

3.2.1. Normal treatments with formic acid in autumn

The effects of the formic acid treatments on honey residues over in three consecutive years are summarised in Table III. There was a significant increase in formic acid in the honeys from apiaries which had been treated during the preceding autumn. The overall average increase of formic acid content during the three year trial was 46 mg/kg, range 0–139 mg/kg.

In three different apiaries, formic acid treatments were carried out over three successive years. No increase of the formic acid content of honey after the repeated treatments was observed (Fig. 1). The lack of increase stems from the large natural variation in formic acid, so that small differences between the honeys harvested in the apiaries during the three years could not be detected. Originally we had planned to carry out formic acid accumulation experiments in seven apiaries. However, in four of them honeydew harvest was encountered in one of the trial years. As only blossom and blossom-honeydew honeys were considered for our study, the results from these four apiaries could not be taken into account.

3.2.2. Emergency treatments with formic acid in spring

As described in Section 2.1.2, emergency treatments due to high Varroa infestation rate had to be applied in seven apiaries in Switzerland. The results of the trials are summarised in Table IV. The average increase in the formic acid content was 193, (range 38 mg/kg–417 mg/kg). In some cases the increase of formic acid could changed of in honey taste (see Sect. 4.5.2). On the other hand, these urgent treatments caused only a slight, statistically insignificant increase in free acidity; the 40 meq/kg limit was never exceeded.

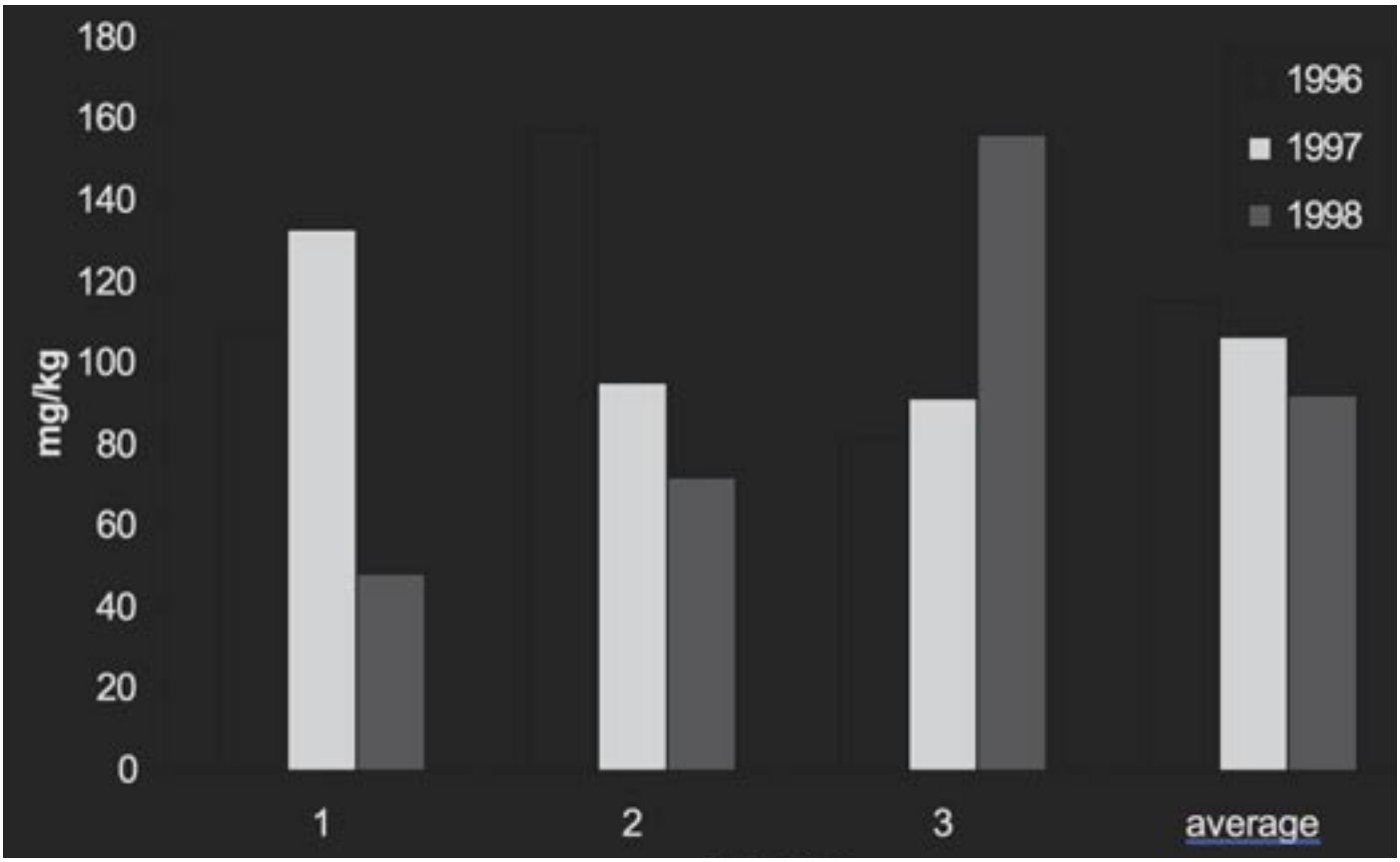
3.2.3. Treatments with oxalic acid

The effects of the oxalic acid treatments on honey residues, over three consecutive years are summarised in Table V. There was no increase of oxalic acid in the honeys from apiaries which had been treated during the preceding autumn; both control and trial honeys had similar values. A combined treatment with spraying and trickling of

Table III. Influence of a formic acid treatment in autumn, carried out over three consecutive years, on the formic acid content of honey during the following year in different Swiss apiaries. Values in mg/kg. The honeys were of blossom and mixed blossom-honeydew type (see Materials and methods).

	1996		1997		1998	
	control	treatment	control	treatment	control	treatment
average	45	94	31	91	41	71
st. deviation	18	49	6	27	21	33
min-max	20–80	17–157	20–40	66–133	17–85	42–156
n (apiaries)	10	10	5	6	9	11
P (*t*-Test)		0.008		0.001		0.030

Figure 1. Residues in honey after repeated long-term treatment with formic acid in autumn during three consecutive years. The trials were carried out in three Swiss apiaries. The honeys were of blossom and mixed blossom-honeydew type. (Further details, see Materials and methods.)



oxalic acid in autumn also did not lead to a significant increase of the oxalic acid concentration in honey.

3.2.4. Influence of a combined treatment with formic and oxalic acid on honey acidity

In most apiaries both formic and oxalic acid were applied during the three trial years. The effect of these combined treatments on honey acidity is summarised in Table VI. The free acidity of the control and treatment samples was practically the same. Only one honey, with 46 meq/kg slightly exceeded the present limit of 40 meq/kg for free acidity.

4. DISCUSSION

4.1. Determination methods

Enzymatic assays for the determination of organic acids in honey have been used mostly as they are very specific and do not need expensive instrumentation. Our results showed that the measurements with the Böhringer oxalic acid kit has acceptable precision and recovery. In this test, both oxalic and formic acid can be determined in the same sample solution, which is very convenient. Alternatively, the Sigma company oxalic acid determination kit can be used (Mutinelli et al., 1997). HPLC (Del Nozal et al., 1998, 2000) and GC-MS (Unterweger et al., 2001) are valuable alternatives, that have been used for organic acid determination in honey, especially when more then one organic acid is determined. The comparability of the enzymatic and the HPLC measurements has not been strictly established, but the values found by both methods are similar. Indeed, Del Nozal et al. (1998 and 2000) measured formic and oxalic acid by HPLC and found formic and oxalic acid amounts, similar to the findings of all other researchers, who used enzymatic measurement (see Sect. 4.2).

4.2. Natural content of organic acids

Table IV. Influence of a formic acid treatment, carried out in spring, on the formic acid content and free acidity of honey, harvested during the summer of the same year. Formic acid (FA) values in mg/kg, free acidity (FAc) in meq/kg. Ap.: apiary number.

Ap.	Trial year, honey type	Control FA (FAc)	Treatment FA (FAc)	Increase of FA (FAc)
	1997			
1	honeydew	127 (27)	403 (30)	276 (3)
2	honeydew 1999	89 (18)	506 (30)	417 (12)
3	blossom, blossom-honeydew blends	20 (14)	58 (13)	38 (-)
4	blossom, blossom-honeydew blends	81 (21)	285 (22)	204 (1)
5	blossom and blossom-honeydew blends	42 (23)	261 (26)	219 (3)
6	blossom and blossom-honeydew blends	23 (25)	103 (26)	80 (1)
7	honeydew	47 (28)	166 (26)	119 (-)
	average	61 (22)	254 (25)	193 (3)

Formic and oxalic acid are natural honey constituents. It is not clear to what extent they originate from bees or from the nectar, but it has been reported that oxalic acid is added to honey by the bees (Echigo and Takenaka, 1974). The formic acid content of honey has been determined by many researchers. Values from 9 to 1229 mg/kg have been found, the highest values being those of chestnut honey (Stoya et al., 1986; Kary, 1987; Talpay, 1989; Sabatini et al., 1994; Capolongo et al., 1996; Del Nozal et al., 1998). Weakly aromatic honey like acacia, rhododendron, and citrus have lower values than strongly flavoured ones like erica, honeydew, and chest-

Table V. Influence of an oxalic acid spray treatment in autumn, carried out over three consecutive years, on the oxalic acid content of honey, harvested during the following year in different Swiss apiaries. In 1998 trickling was carried out in addition to spraying. Values in mg/kg. The honeys were of blossom and mixed blossom-honeydew type (see Materials and methods).
s: spraying; s+t: spraying+trickling.

	1996		1997		1998		
	control	treatment	control	treatment	control	treatment s	treatment s+t
average	41	33	22	18	19	19	26
st. deviation	12	18	9	9	5	10	12
min–max	18–79	16–51	8–30	6–27	10–30	9–36	15–44
n (apiaries)	9	9	5	6	9	4	7
P (*t*-Test)		0.149		0.152		0.969	0.145

nut honeys (Talpay, 1989; Sabatini et al., 1994; Capolongo et al., 1996), which coincides with our results.

There are few measurements of the natural content of oxalic acid in honey (Kary, 1987; Mutinelli et al., 1997; Del Nozal et al., 2000; Bernardini and Garda, 2001). Values from 8 to 300 mg/kg have been found in different honeys. Light honeys, such as rosemary and lavender, have a lower oxalic acid content than strong flavoured dark ones like honeydew and heather (Del Nozal et al., 2000), which also coincides with our results.

4.3. Residues after treatment with formic acid

In other publications, the formic acid residues after a long-term, 7-day formic acid treatments were measured in the sugar feed (Stoya et al., 1986; Krämer, 1994; Capolongo et al., 1996). In two studies (Stoya et al., 1986; Capolongo et al., 1996) a kinetic follow-up of residues after the treatment in autumn was carried out. The residues were measured before and after the treatment and were followed until spring. After a strong initial rise of formic acid in autumn, the levels in spring diminished to reach the values before treatment. In all of these studies it was not tested if the formic acid content of honey was significantly influenced. Our results show, that a 7 day treatment with formic acid leads to a small, but significant increase of formic acid in honey. This rise is not problematic, as it does not influence the quality of the honey (see Sect. 4.5). The increases in formic acid content were similar during all trial years, although the honey yields during these years were different. We think that that formic acid that remains in the sugar feed in the brood combs is the main reason for these residues. Such feed remains are common in Swiss type hives. Under beekeeping conditions in magazine hives, as used in other countries, smaller residues are expected.

In emergency cases with high mite infestation rate in spring, a treatment with formic is often necessary. Our results show that a treatment with formic acid in spring might lead to an increase of formic acid in summer honey that is near the taste threshold of this acid in honey (see Sect. 4.5.2). Thus, under moderate climate conditions, formic acid should be used generally during the period outside the honey flow, mostly just after the honey harvest in late summer.

4.4. Residues after treatments with oxalic acid

Other researchers (Radezki, 1994; Mutinelli et al., 1997; Del Nozal et al., 2000; Bernardini and Gardi, 2001) did residue studies after single oxalic acid treatments and could not detect residues in the sugar feed, compared to the levels found before the treatments. Our studies, carried out with honey, lead to the same conclusions. Even two oxalic acid treatments in autumn did not cause an increase of the oxalic acid concentration in honey of the next year.

Table VI. Influence of consecutive treatments with formic acid (long-term) and oxalic acid (spraying), carried out in autumn in different Swiss apiaries and harvested during the following year, on free acidity of honey. Values in meq/kg honey. The honeys were of blossom and mixed blossom-honeydew type (see Materials and methods).

	1996		1997		1998	
	control	treatment	control	treatment	control	treatment
average	22	24	17	18	13	14
st. deviation	5	9	1	5	3	8
min–max	13–26	13–46	16–18	14–28	8–17	6–34
n (apiaries)	11	7	5	6	9	10
P (*t*-Test)		0.671		0.525		0.694

4.5. Residues and honey regulations

4.5.1. Acidity

According to the present EU honey regulation (2002) and also according to the Codex Alimentarius draft for a new honey standard (2000), there is a maximum limit for free acidity of honey of 50 milliequivalents per kg honey.

Our results, as those of Stoya et al. (1986), show that long-term formic acid treatment in autumn according to the prescriptions will not increase honey acidity above the required limit.

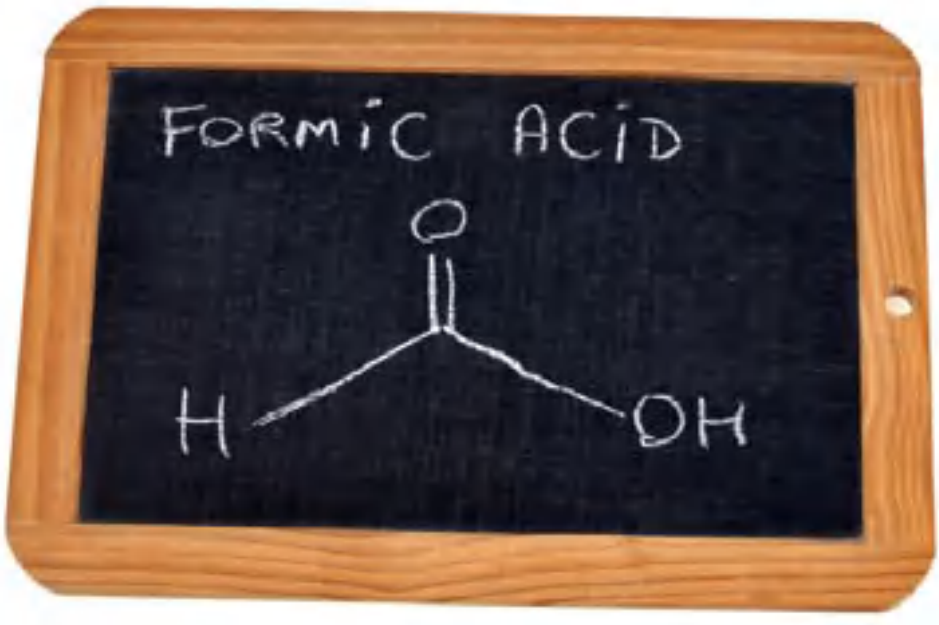
4.5.2. Taste

According to the existing honey standards no substances are allowed to be added to honey to change its natural taste. The taste threshold for formic acid added to honey was determined for mildy flavoured blossom honeys and wac found to be around 300 mg/kg (Capolongo et al., 1996; Bogdanov et al., 1999a). For stronger flavoured honeys like honeydew and chestnut honey it falls between 600 and 800 mg/kg (Capolongo et al., 1996; Bogdanov et al., 1999a). The residues after normal formic acid treatments in autumn are much lower than these thresholds, hence there is no risk for a change of honey taste due to an increase of formic acid concentration. However, according to our results, when emergency treatments with formic acid are carried out in spring, the formic acid residues in the summer honey might be close to the taste threshold of this acid. Therefore, this type of treatment should be avoided. It should be taken into consideration, that the honey yields in Switzerland are relatively modest (see Sect. 2.2) and in the Swiss type honey hive, there are often some small remains of autumn sugar feeds. In countries with greater honey harvests and different honey types the residues of formic acid should be theoretically smaller.

The honey taste will be changed only if about 400 mg/kg of oxalic acid is added to blossom honey or if 900 mg/kg is added to honeydew honey (Bogdanov et al., 1999a). Treatments with oxalic acid do not cause oxalic acid residues, therefore there is absolutely no danger for a change of honey taste due to oxalic acid treatments.

4.5.3. Maximum residue limits

In an EU regulation, formic acid and components of essential oils like thymol and menthol are defined as GRAS (Generally Recognised As Safe) substances, therefore it is not necessary to fix a MRL (EU Regulation 2796, 1995). Oxalic acid is a natural constituent of most vegetables and its content lies between 300 and 17 000 mg/kg, the highest content being that of parsley (Agricultural Handbook, 1984). Thus, most vegetables contain much higher amounts of oxalic acid than honey. Considering the small daily intake of honey, its contribution to the total daily intake of oxalic acid is negligible. From a nutritional point of view, oxalic acid, like formic acid, also



should have a GRAS status. Moreover, no significant residues are expected after oxalic acid treatments.

4.5.4. Registration of oxalic acid for the control of V. destructor

The main registration problem of oxalic acid treatments seems to be the toxicity of oxalic acid for the person applying the treatment. Provided the necessary precautions are met, there is no risk for the user when spraying and trickling of this acid (Knuti, 1996). On the other hand, the user toxicity of the recently developed oxalic acid sublimation treatment (Radetzki and Bärmann, 2001) has not been evaluated.

No oxalic acid residues are to be expected after repeated field spraying and trickling use of this acid, therefore there are no objective arguments against the registration of these treatment modes for the control of V. destructor.

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References

Agricultural Handbook (1984) No. 8–11, Vegetables and Vegetable Products, in: Haytowitz D., Matthews R. (Eds.), Human Nutrition Information Service, Nutrition Monitoring Division, USDA, Washington DC, 502 p.

Barbattini R., Greatti M., D'Agaro M., Sabatini A.G., Colombo R., Marcazzan G.L. (1994) Utilizzo dell'acido formico nella lotta contro Varroa jacobsoni: verifica dell'efficacia e dei residui nel miele, L'Ape nostra amica 16, 4–9.

Bernardini M., Gardi T. (2001) Influence of acaricide treatments for varroa control on the quality of honey and beeswax, Apitalia 28, 21–24.

Bogdanov S., Martin P., Lüllmann C. (1997) Harmonised methods of the European honey commission, Apidologie (extra issue), 1–59.

Bogdanov S., Imdorf A., Kilchenmann V. (1998a) Residues in wax and honey after Apilife VAR treatment, Apidologie 29, 513–524.

Bogdanov S., Kilchenmann V., Imdorf A. (1998b) Acaricide residues in some bee products, J. Apic. Res. 3, 57–67.

Bogdanov S., Kilchenmann V., Fluri P., Bühler U., Lavanchy P. (1999a) Influence of organic acids and components of essential oils on honey taste, Am. Bee J. 139, 61–63.

Bogdanov S. and 21 other members of the IHC (1999b) Honey quality, methods of analysis and international regulatory standards: review of the work of the International Honey Comimssion, Mitt. Lebensm. Hyg. 90, 108–125.

Böhringer Mannheim (1997) Methods of Enzymatic BioAnalysis and Food Analysis, Determination of oxalic acid, pp. 114–117.

Capolongo F., Baggio A., Piro R., Schivo A., Mutinelli F., Sabatini A.G., Colombo R., Marcazzan G.L., Massi S., Nanetti A. (1996) Trattamento della varroasi con acido formico: accumulo nel miele e influenza sulle sue caratteristiche, L'Ape nostra Amica 18, 4–11.

Charrière J.D., Imdorf A., Bachofen B. (1998a) Fünf Ameisensäure-Dispenser im Vergleich, Schweiz. Bienen-Zeitung 121, 363–367.

Charrière J.D., Imdorf A., Fluri P. (1998b) Was kann von der Anwendung der Oxalsäure gegen die Varroa erwartet werden?, Schweiz. Bienen-Zeitung 121, 503–506.

Codex Alimentarius (2000) Alinorm 01/25, Draft revised standard for honey at step 8 of the Codex procedure.

Del Nozal M.J., Bernal J.L., Marinero P., Diego J.C., Frechilla J.I., Higes M., Llorente J. (1998) High performance liquid chromatographic determination of organic acids in honeys from different botanical origin, J. Liq. Chromatogr. Relat. Technol. 21, 3197–3214.

Del Nozal M.J., Bernal J.L., Diego J.C., Gomez L.A., Ruiz J.M., Higes M. (2000) Determination of oxalate, sulfate and nitrate in honey and honeydew by ion-chromatography, J. Chromatogr. A 881, 629–638.

Echigo T., Takenaka T. (1974) Production of organic acids in honey by honeybees, J. Agric. Chem. Soc. Jap. 48, 225–230.

EU Council (2002) Council Directive 2001/110/EC of 20 December 2001 relating to honey. Official Journal of the European Communities L10, pp. 47–52. Available at: http: //europa.eu.int/eur-lex/pri/en/oj/dat/2002/l_010/l_01020020112en00470052.pdf (verified on 24 May 2002).

EU (1995) Regulation of the commission 2796, concerning the establishments of MRL values of veterinary drugs, Official Journal of the EU No. 290, Bruxelles, Belgium.

EU (1999) Council Regulation No. 1804 on organic farming, Chapter Beekeeping and Beekeeping Products. Official Journal of the European Communities of 19 July 1999, L 222, C. Bruxelles, Belgium. Available at: http: //europa.eu.int/eur-lex/pri/en/oj/dat/1999/l_222/ l_22219990824en00010028.pdf (verified on 24 May 2002).

Hansen H., Guldborg M. (1988) Residues in honey and wax after treatment of bee colonies with formic acid, Tidsskr. Planteavl. 92, 7–10.

Kary I. (1987) Untersuchungen zur Rückstandsproblematik in Bienenhonig im Rahmen der Varroatosebekämpfung, Dissertation, Giessen, Deutschland.

Knutti R. (1996) Oxalsäure zur Bekämpfung der Varroa Eine Gefahr für den Imker?, Schweiz. Bienen-Zeitung 119, 508.

Krämer K. (1994) Varroabekämpfung in Bienenvölkern unter Verwendung von 85 % iger Ameisensäure, Bienenwelt 36, 214–218.

Imdorf A., Charrière J.D. (1998) Wie können die resistenten Varroamilben unter der Schadensschwelle gehalten werden?, Schweiz. Bienen-Zeitung 121, 287–291.

Imdorf A., Bogdanov S., Ibanez Ochoa R., Calderone N. (1999) Use of essential oils for the control of Varroa jacobsoni (Oud.) in honey bee colonies, Apidologie 30, 209–228.

Liebig G. (1997) Alternative Varroabekämpfung. Mit organischen Säuren aus der Krise?, Bienenwelt 39, 289–297.

Milani N. (1999) The resistance of Varroa jacobsoni
Oudemans to acaricides, Apidologie 30, 229–234.

Mutinelli F., Baggio A., Capolongo F., Piro R., Prandin L., Biaison L. (1997) A scientific note on oxalic acid by topical application for the control of varroosis, Apidologie 28, 461–462.

Nanetti A., Stradi G. (1997) Oxalsäure-Zuckerlösung zur Varroabekämpfung, Allg. Dtsch. Imkerztg. 31, 9–11.

Radetzki T. (1994) Oxalsäure, eine weitere organische Säure zur Varroabehandlung, Allg. Dtsch. Imkerztg. 28, 11–15.

Radetzki T., Bärmann M. (2001) Verdampfungsverfahren mit Oxalsäure. Feldversuch mit 1509 Völkern im Jahr 2000, Allg. Dtsch. Imkerztg. 35, 20–23.

Radtke J., Hedtke C. (1998) Gehalt an Ameisensäure und freien Säuren im Honig nach Sommerbehandlung mit Ameisensäure, Apidologie 29, 404–406.

Sabatini A.G., Marcazzan G.L., Colombo R., Garagnani
M. (1994) Applicazione di un metodo enzimatico per la determinazione dell'acido formico e dell'acido lattico presenti nel miele, Apicoltura 9, 135–145.

Stoya W., Wachendörfer G., Kary I., Siebentritt P., Kaiser
E. (1986) Ameisensäure als Therapeutikum gegen Varroatose und ihre Auswirkungen auf den Honig, Deutsche Lebensmittel-Rundsch. 82, 217–221.

Talpay B. (1989) Inhaltsstoffe des Honigs-Ameisensäure (Formiat), Deutsche Lebensmittel-Rundsch. 85, 143–147.

Unterweger H., Wacha C., Bandion F. (2001) Bestimmung von Oxalsäure in Honig mittels GC MS (SIM), Ernährung 25, 111–115.

Wallner K. (1999) Varroacides and their residues in bee products, Apidologie 30, 235–248.

CLIMATE CHANGE
AND ITS IMPACT ON THE PRODUCTION OF HONEY IN SLOVENIA

As evident, the temperature has been rising in Slovenia in the last decade. We, beekeepers, have been noticing premature vegetation and blossoming of plants and longer periods of no grazing for the bees during the summertime, as a result of milder winters with minimal to no/zero snowfall.

Plants that have been frostbitten sustain immediate damage, which can be seen a few days after the frost with the death of the flower shoots followed by poorer flowering and no grazing for the bees.

The consequences of climate change are also reflected in the altered precipitation patterns in the country.

Slovenia has been experiencing longer periods of rainfall, especially during the best months for honey production - May and June.

These prevent the bees from leaving their hives during the rain to collect pollen from the nature.

The shortage of pollen results in weaker development of the bee colonies and ultimately lower production of honey during the subsequent grazing period.

Frosts in the month of April can also cause great damage to honeydew agents, especially Physokermes piceae Schrank on Picea abies. Low temperatures do not harm them when they are dormant, but with mild winters and higher average temperatures they start to develop prematurely resulting in hypothermia and subsequent damage. Forests play an important role in Slovenia, as they cover more than 58% of its surface.

Therefore, they also play a vital role in beekeeping. Honey producing plants in Slovenia, Picea abies and Abies alba, can provide excellent grazing for the bees during the favourable year/s.

Climate change and global warming have been having adverse effects in our forests. In addition to more frequent natural disasters (ice rains, windstorms, forest fires), we have been recording Picea abies with an extensive pest infestation, reducing the proportion of conifers.

The same scenario is also to be predicted/expected in the future where the climatic conditions will be more favourable mainly for the growth of leafy species of plants, which unfortunately do not offer anything worth mentioning to the bees.

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CHARACTERISATION OF BEE PRODUCTS IN SLOVENIA

Slovenia is known for its rich plant diversity, providing beekeepers with access to various bee pastures that enable the production of different types of honey. Each type of honey exhibits unique physico-chemical, microscopic, and sensory properties. These characteristics are influenced by the sources of nectar and honeydew, as well as the climatic and regional conditions in which the honey is produced.

Recently, environmental changes have slightly altered the properties of honey, leading to difficulties in accurately determining the type of honey. As a result, it has become essential to regularly update the descriptions of the sensory, physico-chemical and microscopic properties of the various types of honey produced in Slovenia. Additionally, new types of honey have emerged recently, and their characteristics are yet to be fully described. The aim of the “Characterisation of bee products” project, carried out by the Slovenian Beekeepers’ Association in collaboration

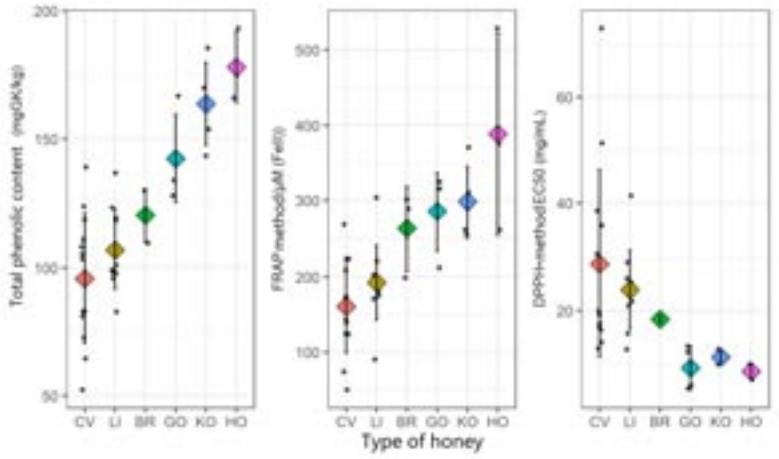

Figure 1: Results of analyses of the total phenolic compounds content and antioxidant activity using the FRAP and DPPH• methods in honey samples of Slovenian origin in the programme year 2024. Black dots are the results of individual samples, and the coloured diamonds with handles illustrate the average with standard deviation for each type of honey. Legend: CV-multifloral, LI-linden, BR-ivy, GO-honeydew, KO-chestnut, HO-fir

with University of Ljubljana and Jožef Stefan Institute, is therefore to establish a comprehensive database of typical characteristics for not only honey, but also other bee products of Slovenian origin, including propolis, royal jelly, bee pollen, and bee venom. Data collection began in 2014 for honey, in 2017 for propolis, royal jelly, and bee pollen, and in 2023 for bee venom. Each year, up to 40 samples of each bee product are analysed.

In honey, we collect data on its basic physicochemical properties, antioxidant and antimicrobial activity. We also conduct palynological analysis and sensory analysis to identify the type of honey. Slovenian beekeepers produce a wide variety of honeys, with acacia, linden, multifloral, fir, spruce, chestnut, and honeydew honeys being the most common. Over the years, data has also been gathered on some rarer types of honey in Slovenia, such as rapeseed, buckwheat, wild cherry, and ivy honey. Our results confirm a known connection between the honey colour and antioxidant activity. We found that darker honey types (e.g. fir, chestnut, honeydew) exhibit higher antioxidant and antimicrobial activity than lighter

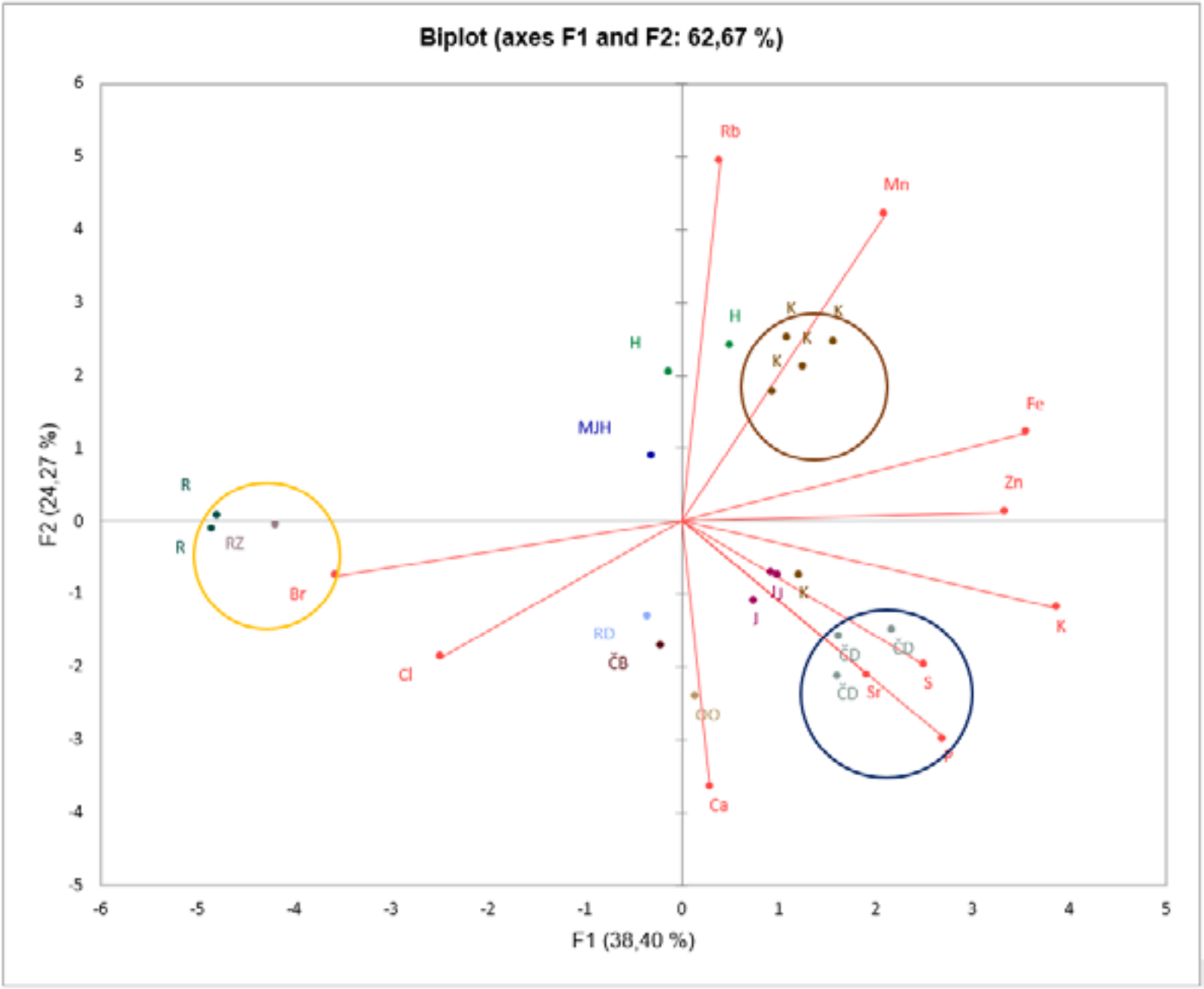

Figure 2:
Distribution and association of element vectors according to the botanical origin of bee pollen in programme year 2024. Legend: K-chestnut, OO-rapeseed, R-dandelion, RD-common dogwood, MJH-small ash and oak, J-maple, A-buckwheat, H-oak, ČD-red clover, RZ-dandelion and buttercups, ČB-figworts

Figure 3:
Expert committee during sensory analysis of royal jelly samples from programme year 2024.

honey types (e.g. acacia and linden) (Figure 1).

For monofloral bee pollen, we collect data on the botanical origin (through palynological analysis), antioxidant and antimicrobial activity, as well as the content of macro(K, P, S, Ca, Cl) and microelements (Fe, Mn, Zn, Rb, Br, Sr), along with sensory properties. The most common monofloral bee pollens in our database are from chestnut, dandelion, maple, and rapeseed. Chestnut bee pollen stands out for having the highest antioxidant activity. The data on element content in monofloral bee pollen (Figure 2) is particularly valuable to consumers seeking natural sources of nutrients as an alternative to synthetic supplements.

For royal jelly, data is gathered on its physico-chemical properties (water, protein, and free acid content, pH), antioxidant activity and sensory properties (Figure 3). The characteristics of Slovenian royal jelly samples are typically well within ISO standards. In most of the samples chestnut pollen is present, similar to Slovenian honey, where 97% of samples contain chestnut pollen. Seasonal and environmental conditions are reflected in the sensory properties of the royal jelly, such as floral aroma, acidity and astringency.

In propolis, we collect data on phenolic content and sensory properties. While the total phenolic content is relatively low, the diversity of phenolic compounds is high, reflecting the broad spectrum of natural sources for propolis used by bees.

For bee venom, data is collected on melittin, total protein, and dry matter content, as well as visual properties (colour and purity level). Although the number of samples analysed so far is limited, a significant correlation has already been observed between the visual assessment of purity and melittin content. The quality of bee venom is most influenced by the production technology and storage.

The results of the project demonstrate that the bee products composition is influenced by the diversity of natural sources, environmental conditions, and production technology. As the consumption of bee products is increasing each year, so does the risk of encountering products of

questionable quality and origin. Therefore, the Slovenian bee product database, which is updated annually, plays a critical role in comparing the characteristics and quality of bee products over time. Annual reports (available on SBA website, in Slovenian language), and thus publicly available data serve as a vital resource for ensuring the authenticity and quality of Slovenian bee products, benefiting beekeepers, researchers, and consumers alike.

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References

Debelak A., Janžekovič A., Ratajc U., Samec T. 2023. Poročilo aplikativne raziskave Karakterizacija čebeljih pridelkov za leto 2023. Lukovica, Čebelarska zveza Slovenije, 124 str.

Kandolf Borovšak A., Lilek, N., Samec, Debelak, A., Janžekovič A. 2022. Končno poročilo aplikativne raziskave Karakterizacija čebeljih pridelkov. Lukovica, Čebelarska zveza Slovenije, 193 str.

Kandolf Borovšak A., Lilek, N., Samec, Bertoncelj, J., Korošec, M., Klemenčič Štrukelj, N. 2019. Končno poročilo aplikativne raziskave Karakterizacija čebeljih pridelkov in vpliv postopkov obdelave in shranjevanja cvetnega prahu na njegovo kemijsko in mikrobiološko sestavo. Lukovica, Čebelarska zveza Slovenije, 129 str.

Korošec, M., Kandolf Borovšak, A., Božič, J., Bertoncelj, J., Justinek, J., Lilek, N., Kozmus, P. 2016. Končno poročilo projekta Karakterizacija slovenskega medu. Lukovica in Ljubljana, Čebelarska zveza Slovenije in Biotehniška fakulteta (UL), 62 str.

Ratajc U., Samec T., Debelak, A., Golob, S. 2024. Poročilo aplikativne raziskave Karakterizacija čebeljih pridelkov za leto 2024. Lukovica, Čebelarska zveza Slovenije, 124 str.

ANTON JANŠA BEEKEEPING SCHOOL:

NEW OPPORTUNITIES FOR BEEKEEPING EDUCATION AND PRESERVATION OF TRADITION

Slovenia has a rich beekeeping heritage deeply embedded in our culture. The Anton Janša Beekeeping School has started to take shape under the Slovenian Beekeepers' Association, bearing the name of Anton Janša, a pioneer of modern beekeeping. The school aims to continue his rich legacy through the education of future generations of beekeepers and aims to spread knowledge and preserve our beekeeping traditions.

Although the school is still in its early stages of development, it already has a clear mission – to combine and transfer the rich knowledge of Slovenian beekeeping to the youngest through beekeeping clubs and beginner beekeeper courses, as well as to more experienced beekeepers through professional lectures and workshops.

The school is currently working intensively on assembling its teaching staff, who will provide high-quality education in various areas of beekeeping in the future. The programs will be designed to be accessible and relevant to all participants, allowing each individual to find the type of education that best suits their needs.

Some of the school's programs are already based on educational activities that have proven to be successful in the past under the Slovenian Beekeepers' Association.

These include beekeeping clubs aimed at young learners, beginner beekeeping courses, where participants learn the basics of beekeeping

ANTON JANŠA
BEEKEEPING SCHOOL

and a series of expert lectures covering topics such as queen bee breeding, apitherapy, beekeeping tourism, melliferous plants and others. Additionally, the school will offer practical workshops and seminars, where participants can acquire practical skills essential for successful beekeeping in the modern world.

Another significant part of our work will be the training of lecturers. We want our lecturers to not only excel in sharing knowledge but also to continually improve their competencies and follow of new trends. We have already successfully hosted an event known as Rihar's Day, dedicated to Dr. Jože Rihar, a distinguished beekeeper and lecturer who contributed greatly to the development of beekeeping in Slovenia. His dedication and passion for beekeeping and sharing knowledge serve as an inspiration to all of us in this field. We aim to make Rihar's Day a traditional event that will annually bring together lecturers and experts to exchange knowledge and experiences, thereby preserving his legacy. We understand that high-quality education is achieved when lecturers are well-prepared, motivated and ready for the challenges of the future.

At this moment, the Anton Janša Beekeeping School is inviting Slovenian experienced lecturers and experts in the field of beekeeping to join our team. The opportunity to participate in shaping and implementing programs at the new school is unique, offering a chance to co-create the future of Slovenian and eventually, global beekeeping.

We will connect with various beekeeping organizations, experts, and research institutions (both locally and internationally), providing opportunities for continuous exchange of knowledge, experiences, and new ideas. This will contribute to a better understanding and development of beekeeping. Together, we can create an educational center that meets the highest standards and becomes a source of knowledge for beekeepers.

By joining forces, we can further contribute to the development of beekeeping, preserving our rich heritage, and protecting bees and biodiversity.

Although the school is initially focused primarily on Slovenia, our ambitions are high. We look optimistically toward the future, where we will combine tradition with new ideas. Our goal is to create sustainable and modern beekeeping that will inspire beekeepers not only in Slovenia but also around the world.

We look forward to new challenges, opportunities, and possibilities that the future will bring, and we are open to new ideas and suggestions that will help us achieve our goals.

Boris Potočnik
Advisor for Education and Training
at the Slovenian Beekeepers' Association

A new book

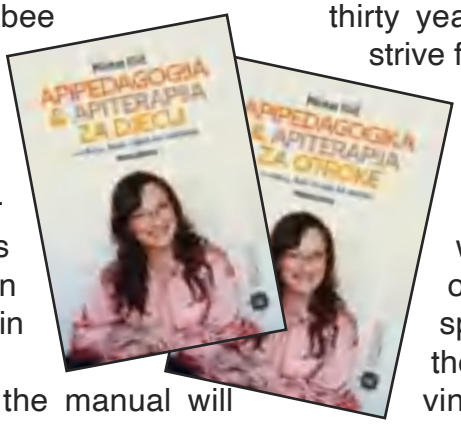
APIPEDAGOGY & APITHERAPY FOR CHILDREN

in kindergarten, school, and school for parents

Manual by Nina Ilič in which, in a professional, correct, and interesting way, the reader is introduced to a very complex issue related to introducing children (of kindergarten, school, and older age) to the wonderful world of bees. The author conveys her rich experience, knowledge, and love to the reader with incredible ease and introduces him to all the necessary facts related to the basics of apitherapy and apipedagogy, healthy nutrition, selection of bee products, honey massage, and various other activities; presents experiences related to experiential learning and learning with bees intended for children of different ages. In addition to the above, the author seriously, responsibly, patiently, and extremely clearly conveys important advice related to responsibility when children visit apiaries, worked out to the smallest detail. Aware that children and their health are the greatest treasure for parents, she clearly outlines all the steps and defines the responsibility of all entities working with the youngest, which until now has been insufficiently known and cared about until now. The basic question that anyone who wants to do this very noble and specific calling responsibly and correctly should ask himself is: Who is

responsible if something goes wrong at some point? Do I have the right, out of ignorance, best intentions, self-promotion, profit, or any other motive, to endanger the visitors of my apiary, especially the youngest ones? Are my competencies for working with children sufficient because I am a parent/grandfather, or do I still need more knowledge, expertise, and responsibility? Can I provide first aid if needed and do I have everything I need for it?

This manual tells so much clearly and interestingly. I sincerely hope that all those who want to work conscientiously and professionally with children and young people will have it in their home library. The manual is intended for a wider readership, parents who want to learn the basics of proper nutrition for the development of their children, as well as the basics of apitherapy. It is intended for teachers, professors and educators who want to present the wonderful world of bees to children in a new and interesting dimension. It is certainly intended for apitherapists and beekeepers who need to learn a lot in order to be able to responsibly and professionally deal with the challenges they often set themselves by bringing children to their apiaries to whom they want to show the beauty of this noble profession and introduce them to bee products. You should always remember that the bee is the only poisonous animal that lives with humans, and with our love for these little creatures comes a great responsibility when we want to host someone in our apiary.



I sincerely hope that the manual will stimulate not only significant changes in society and the local community but also in the work of state institutions that should emulate our Slovenian colleagues and support the development of apipedagogy, apitherapy, apiculture and beekeeping in such a way as to find models of financial support for professional training of educators, teachers and pedagogues who, with the support of local apitherapists and beekeepers, would participate in the implementation of apipedagogical activities in the community. We have the opportunity to follow the innovative concept in Croatia and in all the countries of the region precisely thanks to the author who was understanding of the interest she aroused in her work and made it possible for the manual to be made available in Croatian and English in addition to the Slovenian language, for which I am especially grateful. The manual that we have in front of us sets clear guidelines and gives practical instructions on how to practically realize a whole series of socially beneficial activities.

By sharing her practical experiences, the author opens the door to a wonderful world of new knowledge related to children and bees, apitherapy, democracy and children's rights, and a completely innovative concept of apipedagogy, which was developed precisely thanks to Mrs. Nina Ilič, a certified apitherapist who, dare I say, started an api (r)evolution in working with children. With love and special attention, I will recommend the manual to everyone who wants good for our children, society, and community. Only with a lot of hard work and love can the new generation build a more beautiful and better world that we all aspire to, and we should start with the youngest in this endeavor.

Mark Winston, a Canadian biologist, in his book Bee Time: Lessons from the Hive shares his thirty years of experience studying bees: We strive for altruism, work ethic, flexibility, teamwork and communication, which are the highlights of life in a bee colony, but we often fail. . achieve what bees do with ease. I believe that this manual will serve as a basis for the development of our youngest into smart, responsible, and empathetic members of the society we want to live in. I am convinced that by applying the knowledge pres-ented in this manual, children from rural, but more importantly, urban areas will have a beautiful and carefree childhood connected to nature, plants, and animals where they feel safe and happy; to possess the ability to accept all the challenges that life puts before them and be the foundation of a more humane and beautiful society.

Gordana Hegić
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