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NO BEES LIFE

EBA MAGAZINE

29 COUNTRIES

FROM WHICH EBA HAS MEMBERS

(48 beekeeping organizations)

In order of confirmation of the Statute of EBA

403.585 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
Prishtina
Portugal
Spain
Slovakia
Austria

GENERAL SPONSOR

OF THE
EUROPEAN BEEKEEPING ASSOCIATION

SAVA
INSURANCE
GROUP

AMONG GOOD PEOPLE

SAVA
ZAVAROVALNA
SKUPINA

V DRUŽBI DOBRIH LJUDI

SILVER SPONSOR

OF THE
EUROPEAN BEEKEEPING ASSOCIATION

SILVER SPONSOR

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BRONZE SPONSOR

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COMMISSIONER CHRISTOPHE HANSEN IS COMING TO SLOVENIA

On May 24th, the EU Commissioner responsible for beekeeping will be at the World Bee Day celebration! At the invitation of Boštjan Noč, President of the Slovenian Beekeeping Association and the European Beekeeping Association, the European Commissioner for Agriculture and Food, Christophe Hansen, is coming to Slovenia.

As part of his visit, he will meet with the leadership of the Slovenian Beekeeping Association and the European Beekeeping Association and will participate as a keynote speaker at the World Bee Day celebration, which will take place on May 24th in the Municipality of Kranj in Britof pri Predoslje.

ANNOUNCEMENT OF EBA MEETING

The EBA will meet with Commissioner Ms. Jessika Roswall in May.

We thank the esteemed Commissioner for her time to listen to the EBA leadership. Among other things, the Commissioner is responsible for biodiversity and contributes to the new climate change adaptation plan and the vision for agriculture and nutrition. The goal is for bees to become part of the measures also from environmental funds.

EBA WILL CONDUCT WEBINARS

What is new in the work of EBA is the constant upgrading and strengthening of our capacities. EBA will conduct webinars starting in April 2025.Lecturers and content for the webinars are proposed by Dr. Urška Ratajc, who coordinates them in the scientific committees as she is present at the meetings of the two committees. Webinars are announced in the EBA Magazine “NO BEES, NO LIFE”. and on the EBA website at least one month before the event.

Follow the work of the EBA in 2025, many innovations await us.

NEW DIRECTOR-GENERAL FOR AGRICULTURE, FISHERIES, SOCIAL AFFAIRS AND HEALTH APPOINTED IN THE COUNCIL'S GENERAL SECRETARIAT

The Council has appointed Mr. David Brozina as the new Director-General of the Directorate-General for Agriculture, Fisheries, Social Affairs and Health (DG LIFE). He will assume his new role as from 1 April 2025.

A Slovenian national, David Brozina currently serves as Ambassador, Deputy Permanent Representative of Slovenia to the EU.

EBA President Mr. Boštjan Noč sent a congratulatory letter to Mr. David Brozina what did Mr. David Brozina thanked him wholeheartedly.

"On behalf of the Beekeeping Association of Slovenia and the European Beekeeping Association, I congratulate you on your appointment as Director General of the Directorate for Agriculture, Fisheries, Social Affairs and Health of the Council of the EU.

As you know, beekeeping is an important part of agriculture and I believe that you will help us as much as possible in your new job.

I wish you successful work!"

"Dear Mr. Boštjan Noč,

Thanks for the congratulations. Of course I will take care of beekeeping. I will help when needed. I wish you a successful work,

David Brozina"

THE EUROPEAN SYMPOSIUM "SCIENCE AGAINST COUNTERFEITERS" WAS HELD IN SERBIA

The European Symposium "Science against counterfeiters" was held in Serbia, at the 16th State Beekeeping Fair in the organization of SPOS - SFBO (Serbian Federation of Beekeeping Organizations) with representatives of the laboratories Intertek from Germany, Celvia from Estonia ana Analab from Serbia, in which the President of the EBA also participated. The Symposium "SCIENCE AGAINST COUNTERFEITERS" brought together leading European laboratories for the detection of counterfeit honey, which today threaten beekeepers and beekeeping like never before, leading it into a bottomless abyss, since honest beekeepers and businessmen can no longer make a living, because they are completely defeated by unfair competi-

tion. Symposium participants:

1) Boštjan Noch
President of the European Beekeeping Association (EBA)

2) Uwe Karassek, Philip Krafzig
INTERTEK Laboratory, Bremen, Germany

3) Kaarel Kryutshkov
CELVIA Laboratory, Tartu, Estonia

4) Ivan Smajlović
Laboratory ANA LAB, Pančevo, Serbia

The beekeepers present expressed great concern about the state of the honey market. Laboratory representatives tried to provide their views on solving this problem through existing and innovative honey analysis methods.

A recording of the meeting will be published on the EBA website in the coming days.

EBA PRESIDENT VISITS SERBIA

13th Regional Beekeepers' Conference of the Braničevo District, entitled "World-European-Serbian Modern Beekeeping".

A large number of beekeepers from all over Serbia, Slovenia and Romania gathered in Kostolac on February 16th for the 13th Regional Beekeepers' Conference of the Braničevo District, entitled "World-European-Serbian Modern Beekeeping".

"With us is the President of the European Beekeeping Association and the President of the Beekeeping Association of Slovenia, Mr. Boštjan Noč, a man who fights for the rights of half a million European beekeepers, who, through the European Union, fights with his colleagues for 750 million European citizens to enjoy real honey and all beekeeping products from real honey producers, and not counterfeiters, who currently sell around 80 percent, and in some countries even more than 100 percent, of fake honey in Europe. He is the main initiator of the establishment of the World Bee Day before the General Assembly of the United Nations, which is celebrated every year on May 20.

He is also one of the initiators of the European Beekeeping Association, which was founded in Belgrade last year.

He is also a great friend of our association" said the President of the Beekeepers' Association "Požarevac", Mr. Stefanović.

The meeting was also attended by the President of the SerbianFederation of Beekeeping Organizations, Mr. Rodoljub Živadinović, whom President Mr. Stefanović wholeheartedly thanked, saying: "We thank the President of the Serbian Federation of Beekeeping Organizations and one of the main assistants and Vice President of the EBA, Mr. Rodoljub Živadinović, for their presence."

"Where there are bees, there is health. Honey nourishes and protects against many diseases.

Whoever is protected by a honey pharmacy, does not need another medicine. Therefore, let's protect our flying pharmacists," said Mr. Stefanović.

The topics of the Conference were of interest to all beekeepers, with special attention paid to the topics "Working technology in changing climate conditions" – lecturer Mr. Boštjan Noč and "Global problems of beekeeping – causes and possible solutions" – Mr. Rodoljub Živadinović.

All those gathered agreed that it is important to foster such gatherings, because it is an opportunity to discuss problems, concerns, and to exchange experiences and knowledge that is important for quality bee care and for the sustainability and development of beekeeping.

EBA SCIENTIFIC COMMITTEE FOR THE CONSERVATION OF INDIGENOUS HONEY BEES ESTABLISHED

At EBA Executive Board meeting, the Scientific Committee was established, consisting of:

Daniil Brant – Estonia;
Dr. Aleksandar Uzunov – North Macedonia;
Ratko Pavlović – Serbia;
Michael Rubinigg – Austria;
Alexandra Valentine – Ireland.

Congratulations to all the selected candidates, who will elect their leadership at future meetings.

HEAD OF EBA SCIENTIFIC COMMITTEES DR. URŠKA RATAJC RECEIVES HIGH RECOGNITION!

The European Beekeeping Association is honored that Dr. Urška Ratajc received the Miroslav Zei Award on February 12, 2025 – the highest recognition of the institute for research achievements in the fields of the National Institute of Biology (NIB).

She received the award for outstanding doctoral work in the field of NIB activities!

Dr. Urška Ratajc, on behalf of the European Beekeeping Association, we sincerely congratulate you!

THE REQUIREMENT FOR A TRACEABILITY SYSTEM TO COMBAT HONEY FRAUD

AND THE IMPLEMENTATION OF THE COMMITTEE'S DECISION TO LIST THE COUNTRIES OF HARVESTED

According to the amended directive, it is mandatory to indicate the country or countries of origin on the label of honey mixtures in descending order, along with the percentage of each origin. This will help distinguish imported honey from domestic honey and provide consumers, in accordance with Regulation 1169/2022, with the right to know the geographical origin of all foods.

However, there is significant concern regarding this legislative requirement, as there is currently no laboratory method to determine the percentage of honey listed on the label. Only strict administrative control can provide a solution.

However, to implement proper administrative control, a traceability system is required.

The amended directive states that the Commission may issue an implementing act by June 14, 2029, detailing the methods and criteria for determining the place of honey harvest and traceability requirements at the Union level. Until then, there will be no traceability system for imported adulterated honeys, which has serious implications for the EU honey market.

The need for a reliable traceability system was emphasized by the Commission in its first coordinated research conducted in 2015-2017. We have now reached 2025, and the gap remains until 2029. This means that the Commission will have taken 12 years to propose a traceability system!

We believe that this issue is of utmost importance and should already be under discussion, with proposals being made and a feasible, harmonized system being developed as soon as possible. A year has already passed since May 2024, when Directive 2024/1438 was issued, yet the honey platform has focused on less pressing matters.

For this reason, the EBA's Scientific Committee on Safety and Quality of Bee Products has decided to discuss this issue and propose the following measures, which would link the reporting of harvest countries and their percentages to a traceability system. These measures aim to provide a foundation for discussion and accelerate the implementation of a reliable control system:

Proposed Measures

1. **Implementation of a Honey Inventory Balance**: Monitor the inflows and outflows of honey in the warehouses of distributors, beekeepers, and traders. This practice involves tracking the quantities of honey received and dispatched to maintain accurate records and ensure quality control.
2. **Enforcement of Directive 90/675/EEC**: Apply the principles governing the organization of veterinary checks on products entering the EU from third countries.
3. **Mandatory Inspections at Border Posts**: Conduct inspections for honey originating not only from third countries but also from other EU member states.
4. **Immediate Electronic Reporting of Honey Imports**: The Honey Inventory Balance

should be updated electronically for each honey shipment upon arrival at border stations. Sampling should also be conducted at the delivery site by Veterinary Services.

5. **Creation of an Imported Honey Database**: Establish a reference laboratory in each member state, with the participation of the General Chemistry Laboratory, to monitor imported honey.

6. **Development of a Pollen Atlas for Imported Honey**: Utilize sampling data in advanced analytical methods such as NMR, FTIR, SNIFFING, and GC-MS.

7. **Obligation for Honey Importers to Declare Mixing Percentages**: Importers must declare the mixing percentages and total quantity of honey per batch. They should provide purchase invoices from the countries listed on the label, and their stock records must align with the remaining balance after each mixing batch.

8. **Strict Administrative Control**: Verify the authenticity of domestic honey purchase invoices from import traders. The Electronic Registry of Beekeepers should be connected with the Honey Inventory Balance for accurate monitoring.

9. **Inspection of Large-Scale Beekeepers**: Beekeepers with more than 150 hives should be subject to inspections and relevant sampling upon delivery by Veterinary Services when supplying honey to traders, packers, or for export. This information is necessary to support the mandatory traceability system.

10. **Establishment of a Minimum Total Pollen Count**: Set minimum pollen content requirements not only for baker's honey but also for monofloral and polyfloral honeys.

11. **Mandatory Blockchain-Based Traceability System:** Implement a digital ledger system (such as blockchain technology) to record all honey transactions, from production to import, storage, and retail. This system would ensure tamper-proof documentation of honey origins, blending processes, and movement across supply chains.

Blockchain-based traceability enables secured information sharing, facilitates product quality monitoring and control, allows real-time data acquisition, and ensures transparency and visibility throughout the supply chain.

12. **Unique Identification for Each Batch**: Require a unique traceability code (QR code, barcode, or RFID tag) on each batch, allowing consumers and regulatory bodies to access detailed information about the honey's origin and movement history.

13. **Mandatory Analytical Testing for High-Risk Imports**: Establish a risk-based approach where honey from high-risk countries (with a history of fraud or contamination) undergoes mandatory laboratory testing before being released onto the EU market.

14. **Prohibition of High-Risk Honey Blends**: Restrict the blending of honey from countries that persist in exporting fraudulent honey to the EU, preventing dilution of quality.

15. **Regular Audits and Unannounced Inspections**: Conduct random on-site audits of importers, traders, and packers to ensure compliance with the Honey Inventory Balance system. Implement unannounced inspections at storage facilities and processing plants.

16. **Certification for Third-Country Suppliers**: Require non-EU honey exporters to be pre-certified by recognized EU-approved auditing bodies to guarantee compliance with European quality and authenticity standards.

17. **Consumer Transparency Initiative**: Launch an EU-wide public database where consumers can verify honey batch origins and access laboratory test results through a QR code system.

Opinion

If the Committee is willing to implement the above measures, it should prioritize discussions on these matters, as time is limited until the target date of June 14, 2029. Addressing these issues will be far more effective in combating honey fraud than focusing on less significant matters, such as additional criteria for overheated honey.

Andreas Thrasyvoulou
Emeritus Professor
Aristotle University
of Thessaloniki, Greece
Member of the EBA Scientific
Committee for the Safety and
Quality of Bee Products

SERBIA IS THE FIRST EUROPEAN COUNTRY WITH FULL CONTROL OF IMPORTED HONEY AT THE BORDER!

In the previous issue of EBA magazine, on page 28, we already informed you that Serbia has become the first European country with an innovative accredited method that can determine all types of foreign sugars added to honey. In agreement with the state, its full implementation has now begun!

HOW DID WE ACHIEVE THIS VICTORY?

THE SERBIAN GOVERNMENT HELD AN IMPORTANT MEETING ON HONEY

A high-level multidisciplinary meeting was organized on January 23, 2025, and was scheduled due to major problems with counterfeit, defective, falsely declared, and often harmful

honey on the Serbian market, which comes from unscrupulous honey packaging companies, and has flooded our market, similar to Europe.

As for beekeeping in Serbia, the situation is on the edge of an abyss, because we all know that the euro has devalued by about 100% in the last ten years, and the current prices of honey, which are miserable, are simply half the prices they used to be. And how could it not be, when according to the latest research from the end of last year, 79.13% of honey substitutes are present in the largest markets in Serbia (in September 2023, there were 88%).

The problem, of course, lies in companies that practically produce fake honey, or add various types of sugar to minor quantities of honey purchased from beekeepers, and no one regularly controls this.

The Serbian Federation of Beekeeping Organizations (SFBO / SPOS in Serbian) was represented at the meeting by the President of the Association, MD Rodoljub Živadinović.

On behalf of the currently most advanced Serbian laboratory for determining economic fraud in food and beverages, i.e. their authenticity, the meeting was attended by M.Sc. in Technology Ivan Smajlović, Director of the Laboratory ANA LAB DOO PANČEVO.

The President of SPOS expressed his displeasure because exactly 18 months and 3 days have passed since he first asked the previous Minister of Agriculture to solve the problem of counterfeit honey with our proposed solution. After that, the new Minister, Dr. Aleksandar Martinović, came in, with whom the talks continued, then he delegated the State Secretary to work with us operationally on establishing a final solution, and on November 13, 2024, the Minister gave his consent to implement the agreed solution, but the first deadline for implementation was missed 6 days before this meeting.

Now he has again requested that the state take the necessary measures to solve the problem of the massive appearance of surrogate (counterfeit) honey in stores in Serbia and offered a way to do so. The Chief of Staff of the Prime Minister very openly expressed the views of the state that there is a desire to meet us, it was agreed with him and the Minister of Agriculture how and when to do so, and he wished that we would have more trust in the state in the future. The President of SPOS thanked him for the agreement, but also said that we really want to have trust, but that the beekeepers have unfortunately lost it for several reasons and are not interested in any words, but only in actions. The President of SPOS told the Chief of Staff of the Prime Minister that he personally, based on his obviously extremely sincere discussion and the promises made, certainly sees reasons for trust, but that the beekeepers are only interested in actions, and that trust will develop again when the promises are implemented within the agreed deadlines.

MEETING WITH THE PRESIDENT OF SERBIA

On February 7, 2025, the President of SPOS spoke with the President of Serbia Aleksandar Vučić and the Minister of Agriculture Dr. Aleksandar Martinović at the apiary of a member of our Association (Saša Pustinjak from Novi Kneževac) about ways to combat counterfeit honey and received a promise that all agreements would be

urgently implemented. Already on February 13, the ANA LAB laboratory became authorized for official controls, as we stated in the previous issue of the EBA magazine.

QUALITY CONTROL OF IMPORTED HONEY STARTED ON FEBRUARY 13, 2025.

First, the action to control the authenticity of imported honey was launched, and sampling from all import contingents began, as promised!

In the first 8 days alone, about 100 tons of honey were attempted to be imported, samples were taken from each contingent. The first analysis results from the ANA LAB laboratory have already started to arrive on the day of writing this text, and the results are negative! The barrels contain honey with added foreign sugars!

A day later, on February 14, 2025, the official control of honey in Serbian markets also began, but the results have not yet arrived on the day of writing this text.

THE SERBIAN GOVERNMENT HAS ADOPTED A **CONCLUSION** ON THE FIGHT AGAINST COUNTERFEITS

On February 7, the President of Serbia and the Minister of Agriculture promised that the Government would formalize the fight against all counterfeits using a new laboratory method, which the Government of the Republic of Serbia implemented at its session on February 20, 2025, when it adopted Conclusion 05 No. 330-1473/2025, which also represents the official beginning of the fight against counterfeit honey, milk and dairy products, wine and spirits, as well as illegal plant protection products.

Here is the full Conclusion published in the Official Gazette of the Republic of Serbia, No. 15/2025:

Based on Article 61 of the Law on State Administration ("Official Gazette of the Republic of Serbia", No. 79/05, 101/07, 95/10, 99/14, 30/18 – other laws and 47/18) and Article 43, paragraph 3 of the Law on the Government ("Official Gazette of RS", no. 55/05, 71/05 - correction, 101/07, 65/08, 16/11, 68/12 - US, 72/12, 7/14 - US, 44/14 and 30/18 - other law), on the proposal of the Ministry of Agriculture, Forestry and Water Management,

The Government brings

CONCLUSION

1. The Ministry of Agriculture, Forestry and Water Management and the Ministry of Internal and Foreign Trade, responsible for placing on the market and controlling the quality and safety of food, namely honey, milk and milk products, wine and strong alcoholic beverages, are ordered to take urgent measures through their inspections to increase market control, as well as control at the border of the Republic of Serbia, in order to suppress fraud and prevent the placing on the market of these products whose qualitative and chemical indicators indicate misleading production and distribution practice.

2. The ministries from point 1 of this conclusion are ordered to, in cooperation with all competent state bodies and institutions, as well as in cooperation with the Ministry of Internal Affairs, implement continuous measures related to food products from point 1 of this conclusion, which include:

1) increased inspection supervision on the market of the Republic of Serbia;

2) increased inspection supervision of shipments at border crossings during import and export;

3) application of the most modern accredited methods of quality control and product authenticity;

4) engagement of domestic accredited laboratories in the procedure of verification of authenticity and other frauds;

5) exchange of data with international institutions in order to prevent cross-border traffic of food products from point 1 of this conclusion, as well as illegal means of plant protection that are applied to fruits, vegetables, industrial plants and food products and hinder their traffic in the Republic of Serbia and other countries.

3. The ministries referred to in point 1 of this conclusion are required to submit to the Government a special report on the measures taken and the results achieved within 30 days from the date of publication of this conclusion, and after that they will submit regular reports to the Government every three months.

4. Publish this conclusion in the "Official Gazette of the Republic of Serbia".

05 number 330-1473/2025
In Belgrade, February 20, 2025
Government
President,
Miloš Vucevic, s.r.

AN IMPORTANT MEETING WAS HELD ON FEBRUARY 22 WITH THE PRESIDENT OF SERBIA, THE MINISTER OF AGRICULTURE, THE MINISTER OF ECONOMY, INTERNAL AND FOREIGN TRADE, AND THE ADVISOR TO THE PRESIDENT OF SERBIA FOR AGRICULTURE

The President of Serbia, Aleksandar Vučić, the Minister of Agriculture, Dr. Aleksandar Martinović, the Minister of Economy, Internal and Foreign Trade, Adrijana Mesarović, and the Advisor to the President of Serbia for Agriculture, Prof. Dr. Dragan Glamočić, visited the more than exemplary agricultural farm of SPOS member Dejan Milošević from Drmno near Požarevac on February 22, 2025, and used the opportunity to discuss beekeeping issues. The meeting was held after a tour of the farm.

The President of SPOS criticized the pace of taking honey samples from markets, as only 11 samples had been taken the previous day, and requested that the cause be discovered, and that the action be intensified in scope, as only a small number of markets have been covered so far.

What is very good about this occasion is that for the first time in post-socialist Serbia, the market inspection has been included in the whole story, for which the President of SPOS was particularly grateful, because in recent years the market inspection has persistently avoided working on food.

This act will change everything fundamentally, especially when, in accordance with the Government Conclusion, the Ministry of Internal Affairs is also involved, because this is about serious fraud and deception of both a financial nature, tax evasion (honey in Serbia has a preferential VAT rate of only 10%) and much more.

IT WILL NOT BE EASY

The Serbian Federation of Beekeeping Organizations is aware that it will not be easy to overcome the numerous lobbies of honey packers in the coming period, who, we are aware of this, have their own corrupt people in the inspections, but we will not give up the fight. Now, great steps forward have been made and the fight has been maximally institutionalized. We thank all the authorities for finally understanding our problems and for starting to solve them together in a serious way.

Thanks also to the ANA LAB laboratory, which is the perfect tool in this fight and without which we would not have been able to find a suitable method in the world that can so effectively detect not only C4, but also C3 foreign sugars in honey, as well as added oligosaccharides of all kinds. EBA magazine wrote about the method in the issue.

EIM - IRMS SPIDER WEB FOR COUNTERFEITERS

ASSESSING VIRULENCE OF VARROA DESTRUCTOR MITES FROM DIFFERENT HONEY BEE MANAGEMENT REGIMES

Abstract

The mite Varroa destructor is an important honey bee parasite that causes substantial losses of honey bee colonies worldwide. Evolutionary theory suggests that the high densities at which honey bees are managed in large-scale beekeeping settings will likely select for mites with greater growth and virulence, thereby potentially explaining the major damage done by these mites. We tested this hypothesis by collecting mites from feral bee colonies, “lightly” managed colonies (those from small-scale sedentary operations), and “heavily” managed colonies (those from large-scale operations that move thousands of colonies across the US on a yearly basis). We established 8 apiaries, each consisting of 11 colonies from a standardized lightly managed bee

background that were cleared of mites, and artificially infested each apiary with controlled numbers of mites from feral, lightly managed, or heavily managed bees or left uninoculated as negative control. We monitored the colonies for more than 2 years for mite levels, colony strength (adult bee population, brood coverage, and honey storage), and survival. As predicted by evolutionary theory, we found that colonies inoculated with mites from managed backgrounds had increased V. destructor mite levels relative to those with mites from feral colonies or negative controls. However, we did not see a difference between heavily and lightly managed colonies, and these higher mite burdens did not translate into greater virulence, as measured by reductions in colony strength and survival. Our results suggest that human management of honey bee colonies may favor the increased population growth rate of V. destructor , but that a range of potential confounders (including viral infections and genotype-by-genotype interactions) likely contribute to the relationship between mite reproduction and virulence.

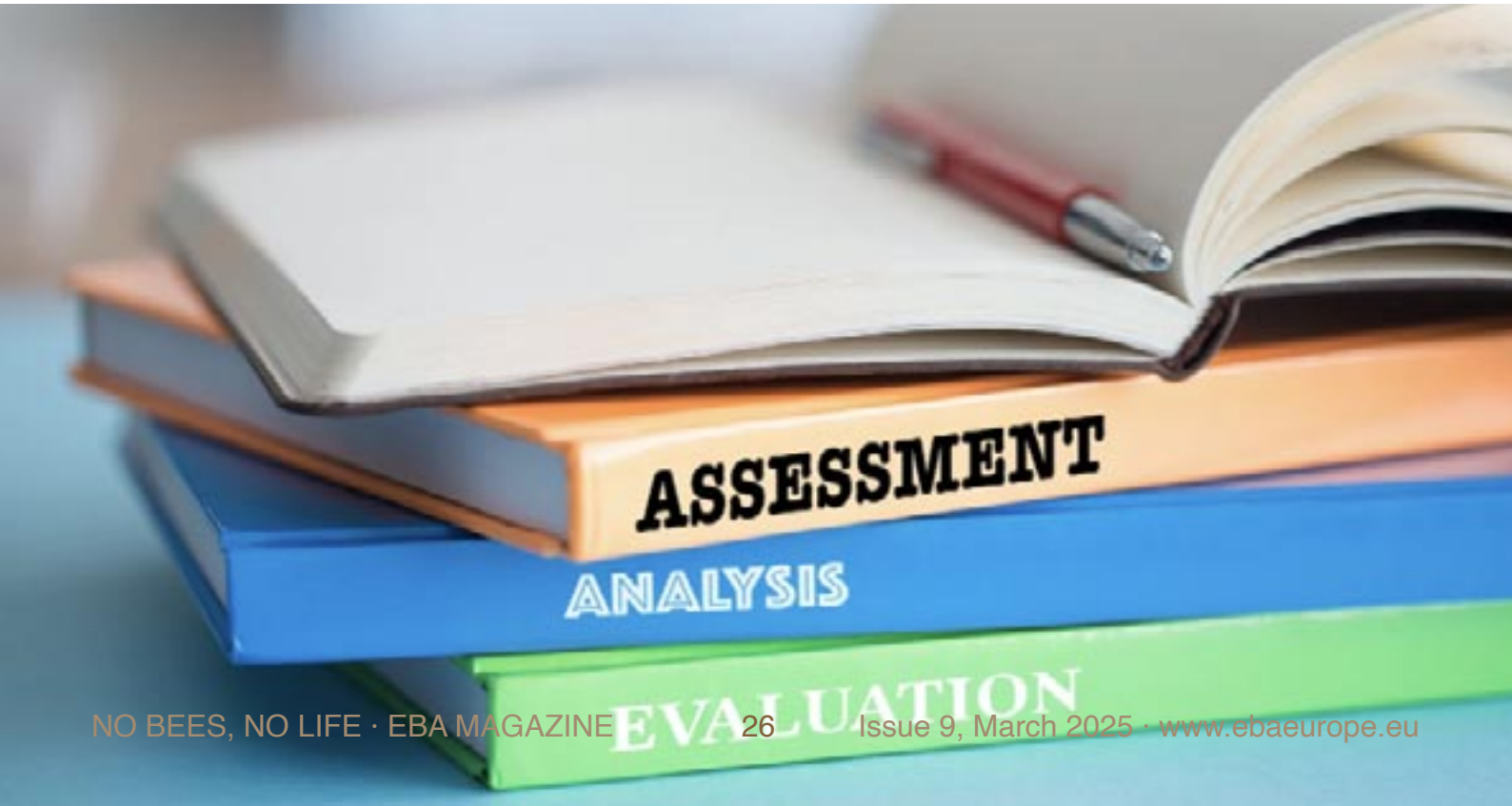
1. INTRODUCTION

European honey bee (Apis mellifera L.) colonies have experienced widespread losses in the past decades in the US and Europe, which is a particular concern due to the importance that honey bees play in agricultural pollination services critical to both the economy and human health (National Research Council 57; Pettis and Delaplane 59). While honey bees are facing numerous challenges, from pesticides to land use changes, parasites have emerged as a significant factor in these losses (Potts et al. 60). In the first half of the 20th century, the obligate ectoparasitic mite Varroa destructor (Acari: Mesostigmata: Varroidae) made a sustained host switch from the Asian honey bee (Apis cerana) to the European honey bee (Rosenkranz et al. 65). Since that time, V. destructor has spread around the world and become the largest biotic threat, termed “varroosis”, currently facing the beekeeping industry (Sammataro et al. 66; Rosenkranz et al. 65). In addition, V. destructor is a vector for a range of economically important viruses, and the interaction between these viruses and V. destructor is considered the single most important factor in honey bee colony losses worldwide (Boecking and Genersch 9; Wegener et al. 71).

In the honey bee\ system, the dynamics by which V. destructor mites interact with honey bee colonies can vary drastically. Feral honey bee colonies, those colonies that are unmanaged by humans, typically occur at a density of around one per square kilometer in the USA (Seeley 67). In these isolated settings, bees and mites are not likely to interact with individuals from other honey bee colonies on a regular basis. In contrast, industrial beekeeping operations manage thousands of colonies in a much smaller area. Virulence-transmission trade-off theory (Boots

and Sasaki 11; Boots et al. 12; Alizon et al. 2; Lion and Boots 50; Webb et al. 70) suggests that the higher colony densities and high rates of between-colony mixing foun d i n m a n ag ed op erations fav o r V. destructor mites with increased reproduction and virulence. According to trade-off theory, natural selection favors virulent parasites that cause reductions in host fitness by selecting for between-host parasite transmission (Levin and Pimentel 49; Anderson and May 5; Ewald 31; Bremermann and Pickering 14; Antia et al. 6; Bull 16; Levin 48; Boots and Mealor 10). This theory is based on the assumption that both between-host transmission and virulence (usually defined as parasite-induced host mortality) increase with increasing within-host parasite reproduction, an assumption that has found empirical support in a wide range of systems (Messenger et al. 54; Mackinnon and Read 51, 52; Jensen et al. 40; De Roode et al. 25; Hawley et al. 37). As a result, parasites are generally expected to evolve an intermediate level of within-host growth and consequent virulence: parasites with low growth rates are selected against because of low between-host transmission, while parasites with high growth rates are selected against by killing the host before transmission can occur (Levin and Pimentel 49; Lenski and May 46). The expected level of optimal virulence, however, depends strongly on the density of susceptible host individuals, as well as the spatial structure of the population (Kamo and Boots 42; Boots and Mealor 10). In well-mixed high-density host populations, transmission opportunities are ample and the cost of high virulence in terms of killing hosts before transmitting is low. This type of environment is common in agricultural settings and according to theory can favor the evolution of higher virulence (Kennedy et al. 43). In contrast, in highly structured low-density host populations, transmission opportunities are rare and costs of virulence are high. As a result, evolutionary theory predicts selection for greater virulence in highly dense and well-mixed populations than in low density populations with high spatial structure. Evidence for such increased virulence evolution due to greater host density remains lacking outside of laboratory settings (Kerr et al. 44; Boots and Mealor 10), but it is now clear that practices imposed by agriculture can select for more deadly parasites, as has been demonstrated, for example, in the increased virulence of the virus causing Marek's disease due to vaccination of chickens with a vaccine that provides tolerance, but not resistance, to the target virus (Atkins et al. 7; Read et al. 62).

The contrasting transmission conditions driven by density and population mixing are crucial to honey bees, where industrial beekeeping practices have shifted the host-parasite interaction from low densities with high spatial structure in feral bees to highly dense and well-mixed



populations in industrially managed bees. Thus, based on virulence-transmission trade-off theory, we would expect greater selection for parasite growth and virulence in managed honey bee colonies than in feral colonies (Brosi et al. 15). By promoting increased transmission opportunities, management practices such as moving frames of brood to boost struggling colonies (a common beekeeping practice) and the high rates of mixing of managed bees due to migratory beekeeping could contribute to Varroa destructor virulence evolution and be responsible for maintaining virulent Varroa destructor genotypes in managed honey bee colonies (Fries and Camazine 32; Calderón et al. 17; Guzmán-Novoa et al. 36; Brosi et al. 15).

Our current understanding of these relationships in the honey bee system is limited, but there is a small amount of research that is consistent with the virulence-transmission trade-off hypothesis. Based on a comparison of bee colonies infected with mites from different backgrounds, Seeley (67) proposed that avirulent mite strains may explain feral colonies surviving V. destructor better than feral bee resistance to the mites. Migratory beekeepers have reported more colony mortality than small-scale beekeepers (Dahle 22). More V. destructor transmission has been observed in higher-density (compared to lower-density) honey bee colonies (Nolan and Delaplane 58; Dynes et al. 30). Furthermore, studies indicate a genetic basis for variation in mite virulence, confirming that virulence could be acted upon by natural selection (De Jong and Soares 23; Anderson 4; Corrêa-Marques et al. 20, 21).

To understand if mites from different management regimes have evolved contrasting virulence, we completed a large and replicated study at the apiary level to examine varroosis using a highly standardized approach which to our knowledge has not been previously attempted. Specifically, we compared how mites evolved from different honey bee management histories (feral, lightly managed, or heavily managed) reproduced and affected bee colonies from a common, lightly managed background. We hypothesized that V. destructor mites that evolved under more intensive honey bee management regimes had greater population growth rates and increased virulence compared with lower honey bee management intensity. We measured both mite burdens and effects on colony strength over more than 2 years. The strength of our approach lies in our colony and queen standardization, mite clearance, standardized inoculations, and replication at the apiary level.

2. MATERIALS AND METHODS

2.1. Overview

We performed a virulence assay on V. destructor mites collected from different honey bee management backgrounds on bees obtained from a lightly managed background such as one would find with backyard beekeepers. Our purpose was to determine whether management conditions have selected for mites with differential growth rate and/or virulence and whether colony response differs among these backgrounds. We established eight apiaries, each consisting of 11 colonies, for a total of 88 colonies, in June 2015 around Athens, GA, USA, maintained by the University of Georgia Honey Bee Lab. Colonies were initially cleared of mites and subsequently inoculated with mites (N = 100 in multiple doses over the course of 2 months). We used 7–9 mite donor colonies for each management background type (feral, lightly managed, and heavily managed). In order to ensure a sufficient quantity of mite inoculations for each experimental colony, mites were pooled from between 1 and 3 of the 7–9 possible donor colonies (Table I). Colonies in two apiaries each were inoculated with mites from feral, lightly managed, or heavily managed backgrounds, while two apiaries were established as negative controls and were not inoculated with mites.

2.2. Mite and honey bee backgrounds

2.2.1. Mite sources

We collected live mites from different source backgrounds by dusting colonies with powdered

sugar and gathered mites that were dislodged and fell onto a piece of cardboard placed on the bottom of the hive. Mites from feral backgrounds were obtained from honey bee colonies that originated from swarm traps placed in remote forest settings (to reduce likelihood of swarms from recently managed colonies) in Georgia (Oconee National Forest or the Okefenokee National Wildlife Refuge), while mites from lightly managed backgrounds originated from colonies from typical backyard beekeeper management systems. For the heavily managed mites, we acquired mites from a migratory beekeeper that manages thousands of colonies. Colonies were housed in standard five-frame Langstroth nucleus hive boxes and we attempted to minimize drift by arranging colonies in a circular layout with all entrances facing outwards from the center of the circle, with 1 m between the colonies. We further attempted to minimize drift by maximizing bees' ability to visually distinguish between colonies (Dynes et al. 30). The colonies were painted different colors, placed at different heights above the ground (5, 20, or 40 cm), with different symbols painted at the hive entrance.

USA, and added 1.1 kg (2.5 lb) adult bees from a common genetic background to each package. To clear mites from the standardized packages, we placed them in a dark room overnight at 16.6 °C (62 °F) and sprayed with sugar water 1 h prior to the application of 30 mL of a 2.8% oxalic acid solution (Milani 55). Each package was installed 3 days later into a nucleus colony in a randomly assigned apiary at least 5 km from any known colonies (Figure S1, map). Mites were collected from source colonies outside of the experiment by sifting powdered sugar over the colony and collecting dislodged mites at the bottom of the colony. We used small natural fibered paintbrushes to place mites on damp coffee filters. We kept mites in an incubator set at 35 °C (95 °F) until all mites were collected for each dose. We then transferred all mites (N = 100 mites per colony) evenly to an uncapped brood frame and waited to ensure that mites were crawling before returning the frame to the colony.

To maintain our focus on these original colonies (and their queens), we enacted swarm control on colonies likely to swarm by splitting those colonies. We standardized swarm control in this

Table I. Mite inoculation sources within each apiary

Apiary	Mite background	Number of colonies receiving mites (mite donor source)
1	Negative control	NA
2	Heavily managed	5 (HM7), 2 (HM1/6), 1 (HM8/13), 1 (HM10/12), 1 (HM6/10/12)
3	Lightly managed	3 (LM1/8), 2 (LM2), 2 (LM3), 2 (LM6/29), 1 (LM5)
4	Feral	4 (F7/13), 2 (F1), 2 (F3/10), 1 (F6), 1 (F2/14), 1 (F6/13)
5	Lightly managed	3 (LM5), 2 (LM2), 2 (LM3), 2 (LM6/Farm9), 1 (LM1/8), 1 (LM1/2/8)
6	Heavily managed	5 (HM7), 2 (HM1/6), 2 (HM10/12), 1 (HM2/27), 1 (HM8/13)
7	Negative control	NA
8	Feral	5 (F7/13), 3 (F6), 1 (F1/2), 1 (F2/14), 1 (F3/F10)

2.2.2. Colony standardization, mite clearance, and mite inoculation

We started with highly standardized colonies to minimize variation. We obtained mated queens from a single queen breeder in southern Georgia,

manner to ensure that small colonies were not jeopardized by the procedure. A total of 33 out of the 72 colonies that remained alive were split in March and April of 2016. We employed a Fisher's exact test to determine that there was not a statistically significant difference (X 2(3) = 6.44, P = 0.092) in amount of splitting between our treatment groups. During the experiment, we did not conduct any control measures against V. destruc-

tor . We continued the experiment from June 2015 through December 2017, at which point only 12 of the original 88 colonies were surviving.

2.3. Data collection

2.3.1. Measuring Varroa destructor infestation

We measured V. destructor infestation levels using three different methods. First, we used an alcohol wash method described by Fries et al. (33). This method involves destructively sampling approximately 300 bees from a colony in alcohol and counting bees and mites (which detach from the bees allowing easier counting) to get a relative mite level on the adult bee population. We took eight alcohol wash samples throughout the experiment (roughly once a month during summer and fall and once every 3 months at other times of the year). Second, we used sticky boards (Branco et al. 13), a standard method to evaluate V. destructor levels in a colony by collecting mites that fall and become entrapped on a board placed at the bottom of a colony. We measured mite levels with sticky boards six times throughout the experiment including one measurement immediately following package installation to confirm that colonies were V. destructor free (roughly every 3 months during the first year and at the end of the experiment). Third, we measured the mite population in brood cells by opening 100 covered brood cells in each colony and counting the number of mites. We measured mite levels in brood cells five times throughout the experiment (roughly every 4 months).

2.3.2. Colony strength assessments

We took periodic strength assessments throughout the experiment in order to evaluate the effect of mite background on colony strength. We followed the assessment guidelines outlined in Delaplane et al. (27) to measure colony strength in terms of (1) adult bee population, (2) amount of brood, and (3) amount of honey stored for each colony. We performed these colony assessments five times over the 2 years of the experiment (roughly every 4 months). We also recorded the date each colony was found to be dead and last known date it was alive for survival analyses.

2.4. Statistical analysis

2.4.1. Overview

We explored how our treatment levels (mites from feral, lightly managed, and heavily managed backgrounds) affected the mite burdens and health response outcomes at the colony level. We also assessed the effects of mites from our different mite donor colonies within each treatment level to determine whether variation exists within the treatment levels. We conducted analyses based on three classes of response variables: (1) colony-level mite infestation levels, (2) colony strength parameters, and (3) colony-level survival.

2.4.2. Mite infestation levels and colony strength

Our experiment used longitudinal repeated measures and nested random effects which can result in temporal and within-subject autocorrelation and violates the assumption of independence for parametric and linear regression methods. Therefore, we used generalized estimation equations (GEE) to account for repeated measures including temporal autocorrelation. GEE models are similar to the more common generalized linear mixed models (GLMM), but

handle within-group correlation as a marginal model rather than as a conditional model found in GLMMs (Hubbard et al. 39). We used the 'geeglm' function in the 'geepack' package v1.2-1 (Højsgaard et al. 38) in R v.3.4.2 (R Core Team 19) to specify and evaluate the GEE models in particular because it allows for longitudinal data with missing observations. We blocked the data by apiary and c olony a nd utilized an autoregressive (AR1) autocorrelation structure to compare treatment levels with negative control colonies. We used the 'lsmeans' package v. 2.27 in R to conduct post hoc pairwise comparisons of response variables of mites from different donor colonies using Tukey's method for multiple comparisons (Lenth 47). We used the 'missMDA' package v.1.12 in R (Josse and Husson 41) to impute missing values (N = 917 out of a total of 1869 values) for mite measurements that did not occur in the same months and then created a composite index combining the three methods of mite measure using a unity-based normalization index (Dodge et al. 28). This index takes each method of mite measurement and scales the measurement to a value between 0 and 1 by comparing the measurement to the minimum and maximum value for that method. The normalized value for each method of measurement is then added to the other methods for that particular sample for a composite index value. We employed a GEE model to evaluate this composite index in addition to each of the individual mite measures. We similarly assessed colony strength measures (adult bee population, brood production, and honey stores) using GEE models to compare treatment levels to negative control colonies.

2.4.3. Survival analysis

We performed survival analyses to determine whether there was a difference in colony survival based on mite background.

Colonies were inspected periodically throughout the ex- periment and exact timing of colony death could not be determined.

Therefore, we used an interval of date of observed colony death and date of last known colony viability. Given this data structure, we analyzed survival with mixed-effects survival (frailty) Cox proportional hazard models, with interval censoring via the 'frailtypack' package (Rondeau et al. 64) in R.

3. RESULTS

3.1. Overview

We collected data on mite levels and colony strength parameters for each colony. The colony strength assessments resulted in 231 measurements from each colony on the adult bee population, brood coverage, and honey storage. In order to evaluate V. destructor levels throughout the experiment, we collected 413 sticky boards, 353 alcohol washes (each containing approximately 300 worker bees), and 189 counts of mites in the brood (each including 100 brood cells).

3.2. Mite infestation levels

The GEE model for mite levels as assessed by sticky boards showed that colonies inoculated with mites from heavily managed backgrounds had significantly (Wald = 4.06, P = 0.044) higher mite levels over the course of the experiment than the negative control colonies (Figure 1a). The model for the alcohol wash data showed that colonies inoculated with mites from lightly managed backgrounds had significantly (Wald = 3.94, P = 0.047) higher mite levels (Figure 1b). The mites in brood measurement did not show any treatment level significantly different from negative controls (Figure 1c).

However, the trend in this measurement is consistent with the other two measures with colonies inoculated with feral mites tending to have the lowest mite levels and the treatment groups from managed backgrounds having the most mites.

The GEE for the composite index, which combines the three measurements of mite level, indicated that colonies inoculated with mites from both lightly and heavily managed backgrounds had significantly (Wald = 5.99, P = 0.014 and Wald = 4.55, P = 0.033, respectively) higher mite levels than the negative controls (Figure 1d). We did not find significant differences in mite levels within mite donor colony treatment groups.

3.3. Colony strength and survival analysis

The GEE model for the amount of brood showed that colonies inoculated with mites from feral backgrounds had significantly (Wald = 8.27, P = 0.0040) lower levels of brood production (Figure 2). The models for adult bee population and honey stores did not show any significant differences between the treatment groups and the negative control colonies. The feral and heavily managed treatments showed pairwise with treatment differences for adult bees based on mite donor colonies. The feral treatments had three significantly different pairwise comparisons (Wald = 19.67, P = 9.2 × 1 –6 to Wald = 4.13, P = 0.042). The heavily managed treatments had five significantly ifferent pairwise comparisons (Wald = 14.38, P = 0.00015 to Wald = 3.91, P = 0.048). Eighty-six percent (76 of 88) of the colonies died over the 2-year experiment. The Cox survival analysis did not show a significant difference in survival between the different treatment groups (Figure 3).

4. DISCUSSION

4.1. Overview

The conditions for V. destructor are substantially different in managed bee colonies versus feral bee colonies (Seeley 67). The colony densities found in managed colonies far exceed those found in feral populations and may facilitate disease transmission (Seeley and Smith 68). According to theory, increased transmission between honey bee colonies may alter selection pressure to favor increased replication and virulence (Brosi et al. 15). We performed a large replicated study assessing how mites from different management backgrounds interacted with honey bees from a single background. We were able to replicate varroosis by standardizing bee background, clearing mites, and inoculating with controlled doses of mites in a large replicated study, which has not been documented before. Our work provides evidence consistent with theory that densities in managed colonies have favored Varroa destructor strains with increased growth rates. Specifically, we found increased levels of mites in colonies inoculated with mites taken from managed honey bee populations. However, we did not find the negative consequences we expected for colony strength and survival based on increased mite levels. In fact, for one response variable (brood production), we found that colonies inoculated with mites from feral backgrounds had a negative colony strength outcome relative to bees inoculat- ed with mites from managed backgrounds.

4.2. Mite infestation

Our finding of increased levels of V. destructor mites in colonies inoculated with mites from managed backgrounds (Figure 1) suggests that honey bee management conditions have favored increased mite reproductive rates. While these levels were not always significantly different from negative controls for each mite measure (Figure 1a–c), the trend was always consistent with our predictions, with colonies inoculated with mites from feral backgrounds exhibiting the lowest mite levels and mites from managed backgrounds showing increased mite burdens. The composite index of all three mite measures (Figure 1d) reduced within-group variation and showed that colonies inoculated with mites from managed backgrounds had increased levels of infestation. This is consistent with the idea that mites from feral vs managed backgrounds are under different selection pressures with potential differences in mite growth and/or virulence (Corrêa-Marques et al. 20, 21).

4.3. Colony strength and survival analysis

We found significant within-treatment differences based on mite donor colony for adult bee population in apiaries inoculated with mites from feral or heavily managed bees. This indicates genetic variation in mites among feral and heavily managed bee populations, as has been found in other studies (Dynes et al. 29). While we did not find significant differences in adult bee population or honey stores across treatment groups, we found that bees inoculated with feral-background

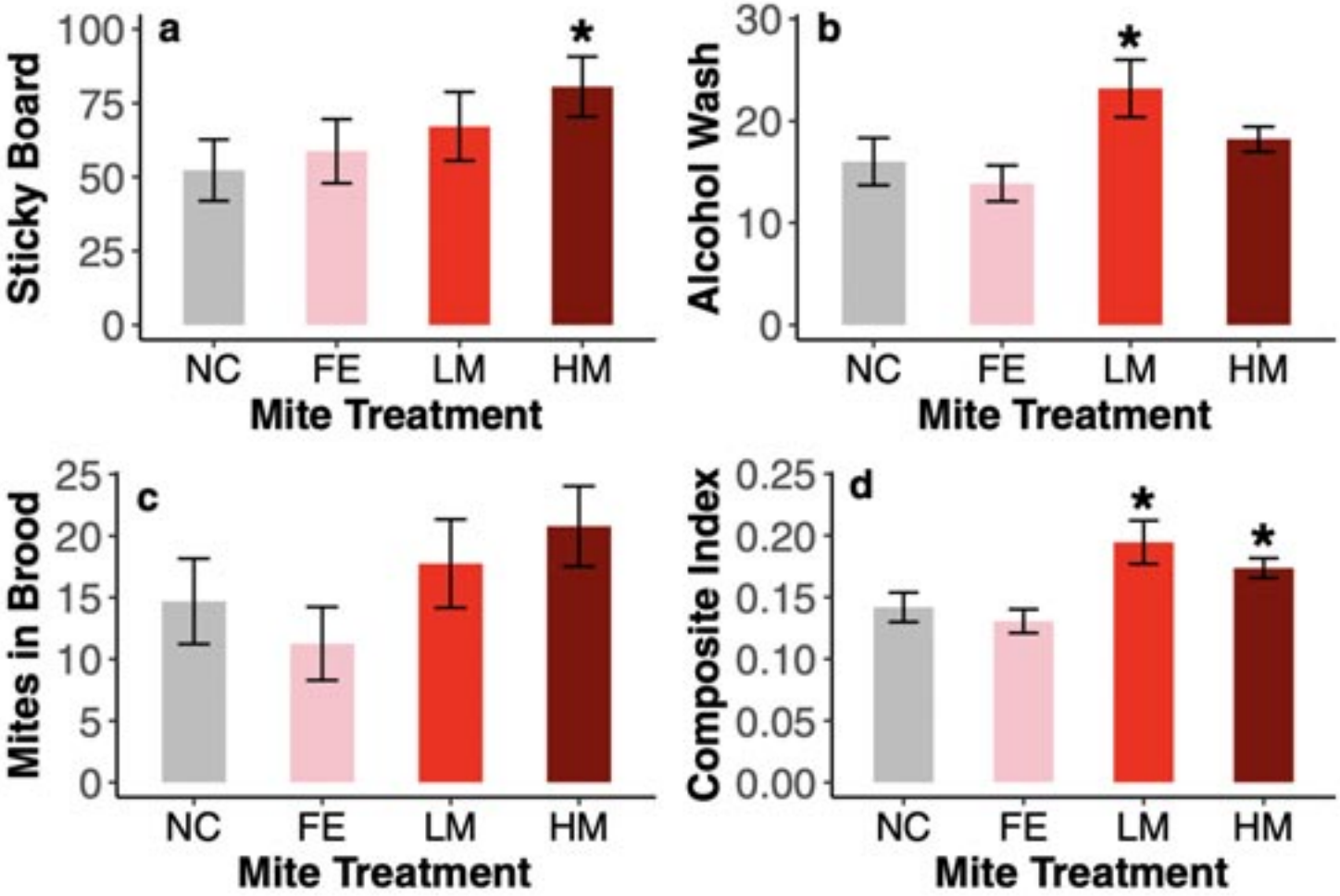


Figure 1. Measures of mite abundance by treatment over the course of the experiment (NC = Negative Control, FE = Feral, LM = Lightly Managed, HM = Heavily Managed). a Sticky board, b alcohol wash, c mites in brood, and d composite index of all three measurements. GEE models were employed for data in each panel to determine significant differences from the negative controls. More mites were found in colonies with mites from heavily managed backgrounds (a Wald = 4.06, P = 0.044) and lightly managed backgrounds (b Wald = 3.94, P = 0.047). Note that while significance was not always found in each mite measurement (a –c), the trend in each is consistent with our hypothesis. A unity-based normalization index was used in panel d to combine all three mite measure- ments. This reduced the measurement variation and showed a significant difference between mites from the lightly managed (Wald = 5.99, P = 0.014) and heavily managed (Wald = 4.55, P = 0.033) backgrounds from the negative controls which is consistent with our hypothesis. Error bars represent SEM

mites produced less brood than bees inoculated with mites from managed backgrounds (Figure 2). This was surprising because we expected the opposite: that higher levels of mites would lead to negative colony strength outcomes. There are five potential explanations for this pattern that we consider here.

First, the bees we used could be adapted to the mite strain that they coevolved with. Predicting the outcome of host-parasite interactions, such as in the honey bee—V. destructor system—can be complicated by interactions between host and par asite genotype. Genotype-by-genotype (G × G) interactions mean that some parasite strains are more successful against some hosts and some hosts less susceptible to certain parasite s t rains (Lambrechts et al. 45). When G × G interactions occur, no single parasite strain optimally infects all hosts, while no single host strain is optimally de-

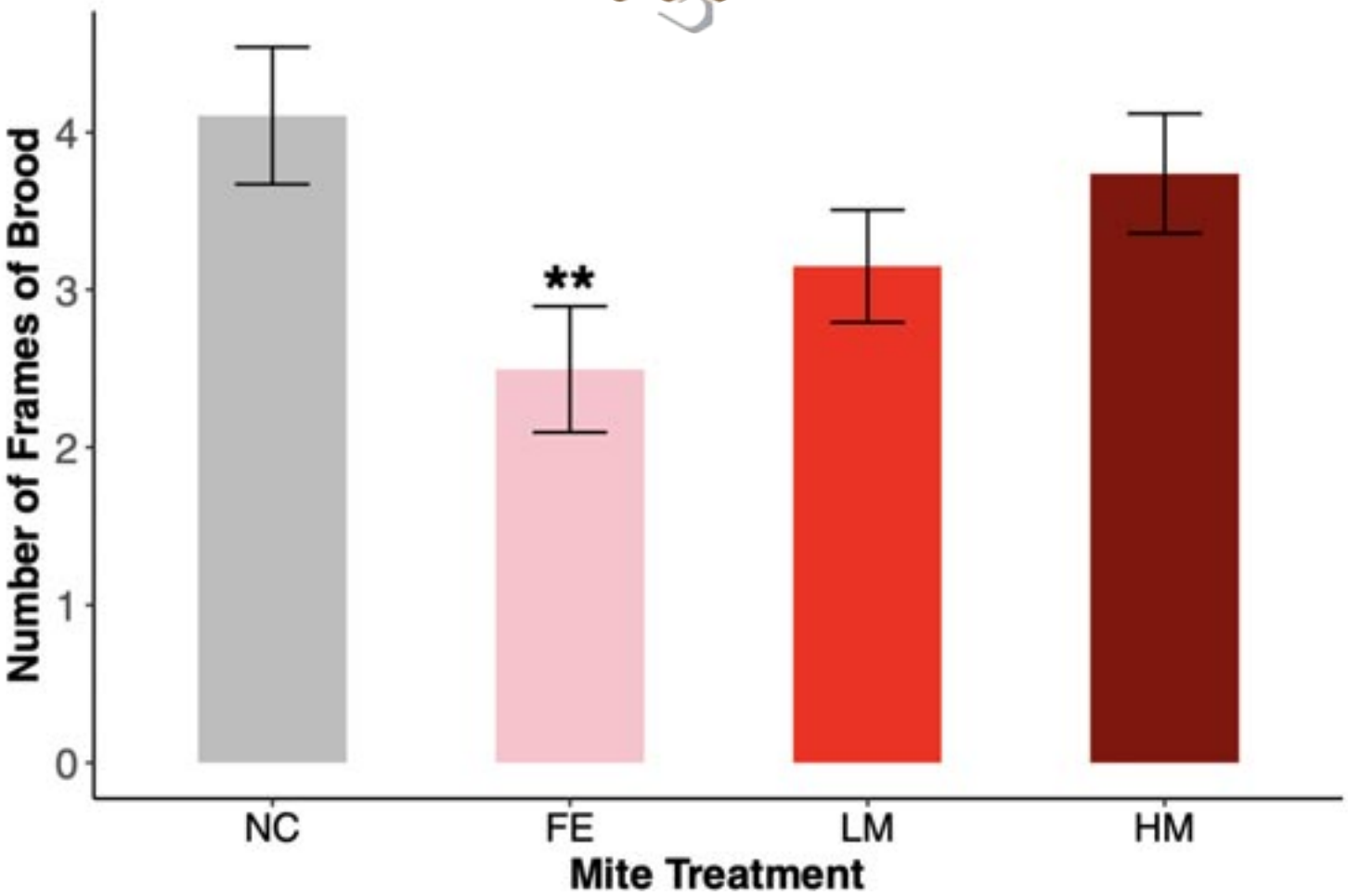


Figure 2. Number of frames of brood by treatment over the course of the experiment (NC = Negative Control, FE = Feral, LM = Lightly Managed, HM = Heavily Managed). A GEE model found significantly (Wald = 8.27, P = 0.0040) fewer frames of brood in the colonies inoculated with mites from a feral background. Note that the trend in the experimental treatment groups is opposite to what we predicted. Error bars represent SEM

fended against all parasite strains (Carius et al. 18; Lambrechts et al. 45; de Roode and Altizer 24). Both theory and empirical studies indicate that coevolution can lead to increased host tolerance; as a consequence, a novel parasite strain from another evolutionary background can lead to more virulence than a coevolved parasite (Greischar and Koskella 35; Miller et al. 56; Read et al. 61; Hawley et al. 37; Gibson et al. 34). If this is the case, the observed patterns of mite growth and colony strength may be due to a genetic mismatch between lightly managed bees and mites from feral colonies, with lightly managed bees resisting, but not tolerating, mites from feral colonies. This means that the bees are able to keep parasite population levels in check (resistance) but are unable to cope with the damage caused by these lower levels of parasites (tolerance) (Restif and Koella 63; Best et al. 8). Thus, while we would predict that the higher transmission opportunities in managed honey bees select for greater mite virulence, we may also predict greater selection for host resistance and tolerance, and the existence of mismatches in coevolved mite and honey bee strains may make virulence outcomes more difficult to predict. A full cross-infection experiment using bees from different backgrounds (in addition to mites of different backgrounds, as we assessed here) is needed to follow up and explore this hypothesis.

Second, honey bee queens may adjust their egg laying frequency based on mite-induced bee mortality. This pattern of increased brood production as a potential means of compensation for higher brood parasitism in V. destructor -infested colonies was noted by Delaplane and Hood (26). Third, our negative controls, which were initially cleared of mites and not inoculated, had greater mite levels than we expected. This suggests that horizontal transmission of mites from outside the

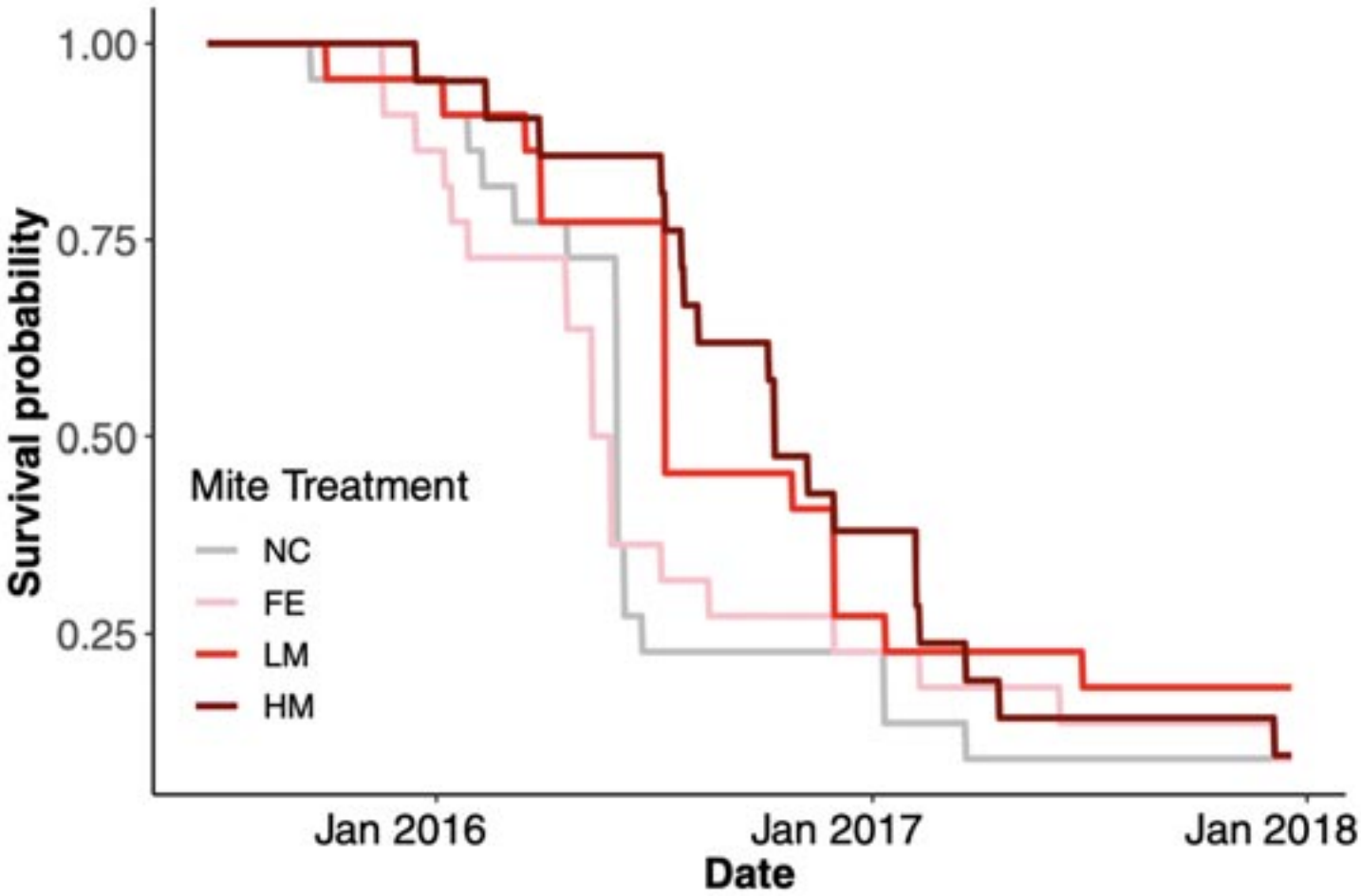


Figure 3. Survival curves by mite treatment (NC = Negative Control, FE = Feral, LM = Lightly Managed, HM = Heavily Managed). A Cox proportional hazard model with interval censoring did not find a significant difference between the groups

experiment could have occurred (Nolan and Delaplane 58). We isolated our experimental apiaries from all known colonies by at least 5 km to minimize this potential, but we cannot discount this as a possibility. Fourth, our mite clearance protocol may not have been as successful as we anticipated, and residual mite populations could have overtaken the inoculated population. However, our first sticky board samples taken after clearance and before inoculation showed most colonies having zero mites and an overall low average of 2.29 mites detected in the 72-h sample per colony. Thus, our inoculation of 100 mites should have overwhelmed any residual mite population. Finally, it is well known that the negative consequences of Varroa destructor infestation are both due to the mites themselves and the viruses they transmit, and differences in viral virulence are well established (Anderson 4; Vojvodic et al. 69; McMahon et al. 53). As such, it is possible that feral mites harbor different populations of viruses than those circulating in managed colonies and these feral viruses could have differential virulence or G × G interactions, leading to distinct health outcomes relative to mite infestation on their own in the absence of viruses.

Colony level mortality was a key measurement in our assessment of virulence of Varroa destructor on the honey bee colonies. The level of colony mortality (86%) across 2 years by the simple addition of mites indicates just how virulent V. destructor mites are for honey bee colonies. These findings are in line with another study that determined V. destructor was responsible for > 85% of the colony mortalities (Guzmán-Novoa et al. 36). However, we did not find an effect of mite background on colony survival (Figure 3). We had expected that the higher mite levels in colonies inoculated with mites from managed backgrounds would translate into worse health outcomes and reduced colony survival in these

colonies. That we did not see these results suggests that there are other factors such as queen health (Amiri et al. 3) or viral infections that play a more important role than mite infestation. Additionally, the finding that our negative controls had similar survival outcomes as our treatment groups demonstrates that a single treatment for Varroa destructor infestations is ineffective, even when that treatment clears all or nearly all mites from a colony. One study found that while a single treatment of oxalic acid caused 97.6% mortality in V. destructor mites, an additional treatment resulted in 99.6% mortality leaving the possibility that a small population of mites could reestablish after a single treatment (Al Toufailia et al. 1).

4.4. Future research

While our study provides insights into how mites from different backgrounds interact with bee colonies of a similar background, our results also indicate that a cross-infection study with bees from different backgrounds would help us further understand the trade-offs that occur in this system.

Specifically, we suggest that future studies explore how human management contributes to virulence-transmission trade-offs by measuring transmission and virulence of mites introduced into mite-free apiaries such as Hawley et al. performed with a bird disease (2013). Additionally, we need to determine the conditions under which mite levels are dissociated from colony harm. Future work needs to focus on the role viruses play in the Varroa destructor - honey bee system. This three-way system could interact in potentially unexpected ways including mechanisms that confound our present understanding.

5. CONCLUSION

Host population densities in managed honey bee apiaries are vastly different than what Varroa destructor experiences in feral honey bee populations. We provide evidence consistent with the idea that selection pressures on mites in these managed conditions favor increased reproductive rates. This could act to increase the transmission rate in these managed environments. However, we did not find negative strength and survival outcomes that we expected with these higher mite burdens.

Mites from feral backgrounds may have caused negative health outcomes due to a mismatch in coevolved bee and mite strains. Future research needs to determine the conditions under which mite levels are dissociated from virulence and whether human management of bee colonies is driving selection for more damaging mites.

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Travis L. Dynes
Department of Environmental Sciences, Emory University, Atlanta, GA 30322, USA
travis.dynes@gmail.com

Jennifer A. Berry
Keith S. Delaplane
Department of Entomology, University of Georgia, Athens, GA, USA

Jacobus C. de Roode
Department of Biology, Emory University, Atlanta, GA, USA

Berry J. Brosi
Department of Environmental Sciences, Emory University, Atlanta, GA 30322, USA

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CONTROLLED INFESTATION OF HONEYBEE COLONIES WITH VARROA DESTRUCTOR FEMALES

Abstract

The development of female Varroa destructor mites in the bee colonies was examined in the apiculture season through a developed research system in which bee colonies were experimentally infested with fifty V. destructor females. Differences in infestation rates were observed between the control group (C) and the infested group (E). The average number of female mites per colony was determined at 513 in group E and 261.6 in group C. Natural daily mortality reached 0.16 mites in group E and 0.09 mites in group C. In group E, the number of V. destructors increased 7.96 to 13.32-fold, subject to colony. The size of V. destructor populations increased at a higher rate in group E than in group C (F= 12.39, P= 0.047). At the end of the experiment, the percentage of infested honey bee workers was determined at 0.97% in group E and 0.46% in group C. The results of this study confirmed that V. destructor mites continue to proliferate rapidly in honey bee colonies, and that the population growth rate in bee colonies and apiaries has to

be closely monitored due to growing levels of resistance to acaricides.

INTRODUCTION

V. destructor is regarded as a leading cause of colony collapse disorder (CCD) (Guzmán-Novoa et al., 2010; Le Conte, Ellis, & Ritter, 2010). In 2007-2008, CCD caused a 35.8% loss in bee colonies in the USA (van Engelsdorp et al., 2008), and in 2003 and 2006 similar losses were noted in Southern and Central Europe (Hendrikx et al., 2009). Untreated bee colonies die in the third or fourth year of V. destructor invasion (Korpela et al., 1992; Romaniuk, Sokół, & Witkiewicz, 1992). Mite infestations are controlled in bee colonies with acaricide which exhibit different mechanisms of action. Various control methods are used, including biological methods which rely on pathogenic fungi and biotechnological

methods where the drone brood is removed from bee colonies. Integrated management strategies are also applied in late autumn. Al- ternative control methods include the use of organic acids and thymol formulations. Despite these efforts, V. destructor continues to infest bee colonies, which is attributed to its ability to proliferate in wild bee colonies (Madras-Majew- ska et al., 2016).

The development of V. destructor mites is linked with the life cycle of bees. Varroa females are more likely to reproduce at the beginning of the spring-summer season when drone cells are not present in the hive, which prevents a detailed assessment of infestation severity (Fuchs, 1990). Females born in spring live for two or three months (De Ruijter, 1987), and their prolif- eration rate can be influenced by such factors as permanent infertility, laying of unfertilized eggs, and damage to eggs and young mites caused by moving bees inside cells (Donzé & Guerin, 1994; Martin, 1998). During the breeding season, one V. destructor female can produce 2.5 fertile females (Martin, 1998), of which 1.3-1.4 survive into adulthood per one worker cell and 2.2-2.6 per one drone cell (Schulz, 1984; Fuchs & Langenbach, 1989). One female mite can undergo up to seven reproductive cycles during one beekeeping season (De Ruijter, 1987), and twelve generations of bees can be produced in a bee colony within one year (Ben, 1997). Hy- pothetically, a single Varroa female present in a bee colony can contribute to producing up to 1500 offspring by the fourth year of infestation (Martin, 1998).

In Europe, attempts to eliminate V. destructor have been made for nearly forty years in various beekeeping systems, but the problem has not been resolved yet. In the fight against such a parasite, its biology and behavior must be monitored to determine whether these actions based

on selection did not change the biology and behavior of the parasite. This is important due to the possibility of modifying methods of controlling the development of the parasite population. The aim of this study was to monitor the size and growth rate of V. destructor popu- lations during the beekeeping season in bee colonies experimentally infested with female mites.

MATERIAL AND METHODS

In early May 2015, ten bee colonies with a similar number of workers (weight-based measurement - 1.5 kg of bees per colony, average body weight of workers - 112 mg in experimental group - group E, and 110 mg in control group – group C). The average body weight was determined based on one-hundred randomly CO2 euthanized workers. The number of bees was determined based on the body weights of individual workers. Bees were established in twelve-frame Dadant beehives on four frames with wax foundation sheets from the same batch, complying with PN-R-78894: 1997 standards. Naturally in- seminated Carnolian honey-bee queens were introduced to the beehives, and in order to standardize the results, only one-year queens were used. The colonies were established far from other apiaries (r=3 km) near Mierki, Poland (Degree-Minute-Second- DMS: 53°35'35.085" N 20°20'34.696" E) and fed 1 L of sugar syrup three times every two days, before Varroa mites were introduced. The bee colonies in a group were separated from one another by a distance of 10 m, and group E hives were separated by a distance of around 100 m from group C hives. V. destructor mites were controlled with an Apiwarol tablet (active ingredient - amitraz, 12.5 g/tablet), which was placed on the bottom board in the hive at 6 p.m. once every 24h. During Apiwarol administration, the hive entrance was blocked for around thirty minutes. Mites were combated with the presence of a bee queen. The treatment was continued daily for four to five days until dead female mites were no longer found on the mesh screen at the bottom of the hive. Before the experiment, twenty bees from each hive had been tested with standard diagnostic methods for the presence of infectious diseases, laboratory tests found no pathogens present, and the observation of bee behavior did not indicate any ongoing diseases. A colony was regarded as non-infested when female mites were not detected on the mesh screen after two consecutive treatments. After seven days, fifty live V. destructor females with evenly colored exoskeletal plates obtained directly from a hatching brood were introduced to five bee colonies (group E) directly onto built combs with open brood cells. The group C comprised five bee colonies without mites. The mites were obtained by uncapping the worker's brood and the drone with a fork to uncork the honeycombs from another apiary. Bees foraged on winter rapeseed, raspberry, linden, weeds and mixed forest vegetation. Colony growth was not stimulated after the introduction of V. destructor to observe the natural development (abundance) of mite populations.

The experiment was conducted between 25 May and 21 September throughout one beekeeping season. Mite drops were monitored every fourteen days, and dead mites were removed from the mesh screen and their number recorded. The colonies in each group were pro-

Table 1.

Number of bee workers and *V. destructor* females, and infestation rates in bee colonies in experimental group (E) and control group (C)

Group	Colony	Weight		Calculated number of workers	Weight		Final number of workers	Number of *V. destructor* mites on workers	Infes- tation rate (%)
		bees in hive (kg)	worker bee (mg)		final weight of bees in hive (kg)	worker bee (mg)			
E	1	1.5	112 (± 2.094)	13393	3.8	116 (± 2.54)	32759	271	0.83
	2				2.9		25000	334	1.34
	3				3.4		29310	362	1.24
	4				3.1		26724	402	1.50
	5				3.9		33621	296	0.88
C	1	1.5	110 (± 2.10)	13636	3.6	114 (± 2.28)	31579	167	0.53
	2				3.5		30702	144	0.47
	3				3.0		26316	120	0.46
	4				3.5		30702	163	0.53
	5				3.3		28947	184	0.64

Key: ± denote to standard deviation

vided with frames and wax foundation sheets. On 21 September, bee queens were caged. Mites were removed from all open and capped worker- and drone-brood cells that had been cut out from combs.

No combs with drone cells had been placed in the hives, because the bees were building them spontaneously.

The mites in every cell was counted, and the bees from the colonies were euthanized and weighed at the end of the experiment. The bee to mite ratio (infestation rate) was calculated using the formula: number of V. destructor mites on workers/final number of workers × 100%. Infes- tation rate was calculated based on the number of mites on workers.

The research results were tested with the use of the T-Student test for independent samples, and standard deviation in the evaluated population was determined.

The results were in- terpreted through repeated measures one-way ANOVA to determine whether the experimental infestation influenced the population dynamics of V. destructor. The results were analyzed sta- tistically in the Statistica 12.5 program with a medical application.

RESULTS

Bee colonies developed normally, as every colony built eight combs on the wax foundation sheets and raised a similar numbers of drones. The measurement was performed three times and the average surface area of drone cells in the season was 2.4 dm2/colony. On average eleven kilograms of honey were harvested from every colony. No workers or drones were found with developmental anomalies and other bee diseases.

During the experiment, 513 V. destructor females, including fifty experimentally introduced females, were detected per bee colony in group E on average. The workers in this group were infested with 333 mites on average (271-402), and brood cells were infested with 180 mites on average (122-260). In group C, workers were infested with 155.6 mites on average (120-184), and brood cells were infested with 106 mites on average (86-124). The mite population in this group, increased from 7.96 to 13.32-fold, subject to colony (SD=2.31, +95% CI=1.38, -95% CI=6.63). Every group C colony was infested with 261.6 mites on average. The average mite drop was

Table 2.

Number of *V. destructor* females in experimental group (E) and control group (C)

Group	Number of *V. destructor* females				
	Introduced to colonies	Mite drops	At the end of the experiment		Total (average)
			In brood cells	On workers	
E (n=5)	50	3.8 (± 0.98)	180* (± 58.38)	333* (± 46.51)	513* (± 92.94)
C (n=5)	-	2.2 (± 0.75)	106* (± 14.12)	155.6* (± 21.88)	261.6* (± 30.89)

Key: ± denote to standard deviation in population σ;
* - statistical differences between groups (P value < 0.05).

determined at 3.8 mites per colony in group E and 2.2 mites per colony in group C. The first mite drops were observed on 29 June in group E and on 24 August in group C. Daily mite mortality reached 0.16 mites in group E and 0.09 mites in group C.

During the experiment, the number of workers increased by 220% in group E and 217% in group C, and the observed increase was not statisti- cally significant. The infestation rate increased from 0.33% at the beginning to 0.97% at the end of the experiment in group E, and from 0% to 0.46% in group C; these differences were in- significant. The differences in mite drops in the analyzed groups were significant ($t = 4.26$, $df = 8$, $p = 0.0054$. The V. destructor populations grew at a faster rate in group E than in group C ($F = 12.39$, $df = 8$, $p = 0.047$) (Tab. 1 and 2).

DISCUSSION

The increase in the development dynamics of V. destructor population among bees, as indicated by Gliński (1994), Hubert et al. (2014) and González-Cabrera et al. (2016) may be affected by its period of presence and control methods. This study documents the develop- ment dynamics of the V. destructor population after for

the first time since they were detected in Poland forty years ago. Population develop- ment was assessed through the composing of a monitoring system, based on published scientific observations, to monitor the behavior of V. destructor in bee colonies. The reason for this experiment was mainly the progres- sive V. destructor resistance phenomenon on acaricides, confirmed by many researchers (Hubert et al., 2014; González-Cabrera et al., 2016). In the study, the mites were eliminated with an effective short-acting preparation (active ingredient - amitraz) with low accumulation in wax, and the observations were carried out on new generations of workers and drones. The study was deliberately conducted in one beekeeping season because such an approach allowed an objective assessment of the V. destructor population threatening overwin- tering bees. When comparing several seasons, the number of factors that may affect the behavior of the mite population is too high.

The conditions for monitoring the V. destructor population in each season should be performed in the same research system, i.e. the type of beehive, number of workers, the starting number of combs, young queens of the same species and study site, which allows the same swarm structure to be obtained (Seeley & Smith, 2015).

The size of the V. destructor population in a colony is influenced by the species of bees. Carnolian honey bees (Apis mellifera carnica), described in many experiments on the building of the largest cells (5.27 mm), are particularly susceptible to the rapid development of mite populations (Piccirillo & De Jong, 2003). Piccirillo & De Jong (2003) found that V. destructor invaded a greater number of brood cells by in freshly Carnolian honey bee built combs than in that of other species. We did not compare the development of mite population in different bee species but instead in order to eliminate the impact of these factors on the size of the studied population deliberately created new colonies with the Carnolian queens, the most common species in Poland, which were building new honeycombs. In the future, such a monitoring system will allow the V. destructor population to be assessedly, and its proper func- tioning will be partially confirmed with very low natural daily mite drop. Akyol et al. (2007) proved that the colony mite infestation increased with the queen's age, so we used young queens at the same age in the study. In addition, new honeycombs eliminated both the possibility of V. destructor development being inhibited by acaricides, found in the wax of colonies treated for varroosis, as well as the possibility of its development being stimulated by pheromones, found in the wax of heavily infected colonies (Thrasyvoulou & Pappas, 1988; Slabezki, Gal, & Lensky, 1991; Piccirillo & De Jong, 2004).

The conductedexperimentclearlyconfirmedthat V. destructor still has a great ability to multiply in bee colonies. According to Gliński (1994), a 10% infestation rate in summer leads to a 30% infestation rate in autumn. In our studies, we showed an increased rate of V. destructor population development, as indicated in Tab. 1 and Tab. 2. In group E, the number of introduced fifty females increased over ten-fold. Ritter (1988) and Seeley & Smith (2015) suggested that in field conditions a mite population in bee colonies can be strengthened by parasites from other apiaries, and reinvasions may occur during the robberies from other hives and during bees' wandering. It is also favoured by a lack of mite control in apiaries or poorly conducted antivarroa therapy (Komeili, 1988). The above facts cannot be excluded in the conducted study. We

believe, however, that it is difficult to estimate the number of parasites that entered the tested colonies from the environment and the number of mites that left on the foraging bees flying out for food. The conducted experiment minimized these phenomena through the appro- priate setting of beehives in the apiary (material and methods). This arrangement also allowed the eliminate of V. destructor drift between colonies resulting from the crowding of colonies in the apiary (Seeley & Smith, 2015; Nolan & Delaplane, 2016).

In conclusion, the size of the female V. destructor population is still important for the function- ing of the bee colonies. Our research indicates the increased dynamics of population devel- opment of this mite than previously reported. With reference to the Polish conditions (Central Europe), despite the use of various prepara- tions and methods of control, V. destructor still threatens the development of bee colonies, and our studies shows that it can increase its numbers by nearly 1000% even in colonies previously free of this mite.

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Rajmund Sokół
rajmund.sokol@uwm.edu.pl

Remigiusz Gałęcki
Maria Michalczyk

University of Warmia and Mazury in Olsztyn
Faculty of Veterinary Medicine
Department of Parasitology and
Invasive Diseases. Olsztyn, Poland

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DIETARY SUPPLEMENTS IN HEALTH IMPROVEMENT OF BEES INFECTED WITH NOSEMA CERANAE

Abstract

Nosemosis, caused by microsporidium Nosema ceranae, is a widespread disease affecting honey bee (Apis mellifera) colonies, leading to reduced longevity, weakened immune responses, and increased colony losses. Various treatment approaches have been explored, including natural compounds, probiotics, chemically synthesized substances, and dietary supplements. Essential oils, plant extracts, and mushroom-derived bioactive compounds have shown potential in reducing N. ceranae infection levels and improving bee survival. Natural-based substances, including kaempferol, sulforaphane, and caffeine, exhibited varying anti-nosemosis effects. Additionally, dietary supplements, such as "B+" and "BEEWELL AminoPlus", enhanced colony strength, improved immune responses, and mitigated oxidative stress. Field studies confirmed their potential to reduce pathogen loads, increase hygienic behavior, and strengthen bee resistance to stressors. While these alternative treatments offer promising avenues for nosemosis control, further research is needed to optimize their application and understand their long-term effects on honey bee health and colony sustainability.

1. Introduction

The honey bee (Apis mellifera) is one of the most important pollinators worldwide. Efficient pollination improves the quality and increases the yield of fruits, nuts, vegetables, seed crops, oilseeds, and fiber plants (Giannini et al., 2015; Khalifa et al., 2021). On the other hand, bee nutrition depends on nectar and pollen, which are transformed in the hive into honey and bee bread, providing essential carbohydrates, proteins, lipids, vitamins, and minerals. Food availability and nutritional balance play a key role in

regulating physiological processes, including resistance to oxidative stress, an adequate immune response, interactions with pathogens, brood development, and colony survival during winter (Stanimirovic et al., 2019).

Bees are exposed to various abiotic and biotic stressors, some of which act synergistically, negatively impacting colony health and reducing their survival rates. Among the main factors contributing to bee colony losses are infectious and parasitic diseases, in-hive chemicals, agrochemicals, beekeeping management practices, climate change, and land-use changes. The lack of plant diversity in agroecosystems can limit the availability of pollen and nectar, negatively affecting pollinators (Stanimirovic et al., 2019).

Nutritional stress directly impacts bee behavior and physiology, shortens their lifespan, reduces immunocompetence, and weakens resistance to pathogens (Di Pasquale et al., 2016). In the long term, this type of stress can severely threaten colony health, reducing productivity and reproductive capacity (Branchiccela et al., 2019; Ricigliano et al., 2019; Jovanovic et al., 2021). Additionally, poor nutrition can weaken bees' resistance to other stressors and increase mortality rates (Dolezal and Toth, 2018; Dolezal et al., 2019). Infection with the parasitic microsporidium Nosema ceranae is one of the most common biotic stressors affecting bees worldwide, including Serbia (Stevanovic et al., 2011, 2013, 2016). It has been shown that N. ceranae suppresses the bee immune system, induces oxidative (Glavinić et al., 2017, 2021a, 2021b, 2022, 2024; Jovanovic et al., 2023) and energetic stress (Martín-Hernández et al., 2011; Papežíkova et al., 2020), and reduces colony strength and hygienic behavior (Stanimirovic et al., 2022; Jovanovic et al., 2025).

Many studies indicate that N. ceranae virulence increases in combination with other stressors, including chemical substances and other pathogens or parasites. Such interactions may partially explain the massive bee colony losses recorded worldwide. Poor-quality forage and/or nutritional deficits promote the proliferation of N. ceranae (Stanimirovic et al., 2019). Good beekeeping practices, particularly in terms of hygiene, are crucial for preventing the spread of this endoparasite in hives (Formato et al., 2022). Additionally, supplementing bee diets with natural-origin products, including essential oils, plant extracts, mushroom extracts, organic acids, beneficial bacteria and their metabolites, and dietary supplements, represents a significant strategy for preventing and treating nosemosis.

2. Natural compounds

2.1. Essential oils

Natural compound-based therapies can help control nosemosis in bees. Essential oils represent a potential alternative to synthetic compounds. They are used in beekeeping due to their antiseptic, antimicrobial, and other beneficial properties (Raut and Karuppayil 2014).

One such preparation, Supresor1, contains essential oils of peppermint (Mentha pepper L.), lemon balm (Melissa officinalis L.), coriander (Coriandrum sativum L.), and summer savory (Satureja hortensis L.). The most effective dose Supresor1 (5 mL/L of sugar syrup) reduced the number of Nosema spores by 80% in laboratory tests without adverse effects on bees (Dumitru et al., 2017).

Essential oils from Chilean oak (Cryptocarya alba Looser) have also effectively controlled nosemosis. A dose of 4 µg per bee was non-toxic and effective, with the crude extract showing greater efficacy than individual isolated compounds (β-phellandrene, eucalyptol, and α-terpineol). Similarly, methanol extracts (2–16%) of Chilean plants (Aristotelia chilensis and Ugni molinae), rich in rutin and myricetin, as well as propolis, reduced Nosema ceranae spore loads and extended lifespan of honeybees (Bravo et al., 2017)

A herbal essential oil extract mixture was tested against Nosema apis, Nosema ceranae, and mixed infections. This formulation contains extracts from Rumex acetosella L., Achillea millefolium L., Plantago lanceolata L., Salvia officinalis L., Thymus vulgaris L., Rosmarinus officinalis L., and Laurus nobilis L. Laboratory and apiary experiments showed that the most effective dose was 500 µL in sugar syrup in laboratory experiment, and 2 mL per hive frame. The greatest reduction in spore count was observed on days 9

and 12 of the experiment, although the difference between these days was not statistically significant. The recommended application is every three days for at least 15 days. Due to its natural composition, a herbal essential oil extract mixture may serve as an alternative to synthetic products for nosemosis control (Özkırım and Küçüközmen 2021).

However, natural substances do not always guarantee a positive effect. Vetiver oil (Vetiveria zizanoides L.) from the Poaceae family did not exhibit anti-nosemosis properties. On the contrary, it increased infection between days 19 and 25 of the experiment. Although essential oils represent a natural alternative to fumagillin in nosemosis therapy, they do not surpass its efficacy (Maistrello et al., 2008).

2.2. Plant extracts and plant-based compounds

Plant extracts have been used in treating Nosema ceranae infections, similar to essential oils.

Particularly promising extracts tested both in the laboratory and in the apiary were those extracted from the root of Siberian Ginseng (Eleutherococcus senticosus). These extracts reduced nosemosis levels, extended bee lifespan, and can be used for preventive purposes (Ptaszynska et al., 2020).

The extract of Laurus nobilis L. (bay laurel), applied at a 1% concentration, reduced the spore load of N. ceranae (Damiani et al., 2014). After 17 days of treatment, an extract of Artemisia absinthium L. inhibited the development of N. apis in artificially infected worker bees in the laboratory. However, this treatment also increased bee mortality (Pohoreck, 2004). Among plants from the Compositae family, only Artemisia dubia (Wall.) and Aster scaber (Thunb.) exhibited anti-nosemosis effects (Kim et al., 2016), while other tested extracts showed no impact on nosemosis.

A decoction of the Chinese plant Andrographis paniculata (Burm.f.) Nees, applied at a 1% concentration, supported regeneration of bee gut epithelium during N. ceranae infection and reduced the number of spores. However, other plants used in this experiment, such as Cyrtomium fortunei J. Sm., Cinnamomum cassia (L.) J.Presl, and Eucalyptus citriodora (Hook.) K.D. Hill & L.A.S. Johnson, were ineffective, as they increased bee mortality in the laboratory compared to the control group (Chen et al., 2019).

The extracts of Origanum vulgare L. and Rosmarinus officinalis L. applied at a 0.7% concentration, as well as their essential oils, demonstrated anti-nosemosis effects by reducing the number of spores under laboratory conditions (Radoi et al., 2019).

On the other hand, ineffective extracts originated from the following plants:

Amaranthus mango-stanys L., Mentha ar-

vensis L., Allium senescens L. var. senescens, Astilboides tabularis (Hemsl.) Engl., Veratrum oxysepalum Turcz., Achyranthes japonica (Miq.) Nakai, Lythrum salicaria L., Symphytum officinale L., Schisandra chinensis (Turcz.) Baill., Perilla frutescens var. acuta Kudo, Physalis alkekengi var. francheti (Mast.) Hort., Rheum undulatum L., Aster scaber Thunb., Cirsium nipponicum (Maxim.) Makino, Achillea alpina (Ledeb), Disporum uniflorum Baker, Astragalus membranaceus Bunge var. membranaceus, Aster tataricus L.f., and Artemisia dubia (Wall.).

Another plant with anti-nosemosis activity is Lespedeza cuneata, an invasive species threatening native flora in many regions. Song et al. (2019) tested its extract in concentrations ranging from 12.5 μg/mL to 800 μg/mL against N. ceranae, using an alternative cell line, Trichoplusia ni BTI-TN5B1-4. Infected cells were deformed compared to healthy cells, which maintained their proper shape. After treatment with L. cuneata extract, the cells resembled healthy ones, with most spores located outside the cells. The lowest concentration that inhibited nosemosis development was 50 μg/mL, while the highest tested concentration (200 μg/mL) had no negative effects (Song et al., 2019).

In laboratory experiments conducted by Braglia et al. (2021) the inhibitory effect of Opuntia ficus-indica extract on nosemosis development was investigated. The extract, applied at a 0.005 μL/mL concentration in sugar syrup (1:1 w: v), did not show a positive effect. On the contrary, it stimulated nosemosis development and was toxic to bees, resulting in complete bee mortality by day 9 of the experiment (Braglia et al., 2021).

Another compound tested in the laboratory was thymol, which has shown potential in reducing N. ceranae infections in some studies. Bees fed with candy containing thymol (0.12 mg/g) and resveratrol (0.001 mg/g) exhibited lower N. ceranae infection levels (Borges et al., 2020). Additionally, bees that consumed thymol or resveratrol syrup lived longer than those in the control group. However, pure resveratrol did not reduce N. ceranae spore loads (Costa et al., 2010; Borges et al., 2020). In apiary experiments, Vargas-Valero et al. (2021) examined honey bee colonies infected with Nosema ceranae in the tropical conditions of Yucatán, Mexico. Colonies were treated with thymol solution (66 mg of thymol crystals per 1 L of sugar syrup) or fumagillin (25.2 mg per 1 L of sugar syrup). The thymol solution showed 31.1% efficacy, whereas fumagillin was significantly more effective (95.2%). In the control group, nosemosis levels decreased after four weeks, possibly due to seasonal variations in infection intensity (Stevanovic et al., 2013).

In a study by Glavinić et al. (2022), the effect of thymol on bees infected with N. ceranae was investigated. The study included six experimental groups of bees: a non-infected control group, an infected control group, a group treated only with thymol, and three groups infected with N. ceranae and treated with thymol from the first, third, and sixth days post-infection. The researchers monitored bee survival, N. ceranae spore loads, expression of immune-related genes, and oxidative stress parameters. The results showed that thymol application in infected bees led to increased survival, reduced N. ceranae spore loads (Figure 1), enhanced expression of immune-related genes, and improved antioxidant protection. However, in non-infected bees, thymol treatment caused certain health disturbances, including reduced survival, diminished antioxidant capacity, and decreased expression of some immune-related genes. Therefore, caution is advised in the preventive and uncontrolled use of thymol in healthy bees.

In addition to natural substances, chemically synthesized compounds have also been used for the prevention of nosemosis. For example, Bernklau et al. (2018) tested phytochemicals such as caffeic acid, gallic acid, p-coumaric acid, and kaempferol. Caffeic acid at a concentration of 25 ppm, gallic acid at 250 ppm, and kaempferol at 2500 ppm were the most effective in extending bee lifespan. However, caffeic acid at its highest concentration (2500 ppm) had the opposite effect, shortening bee lifespan. A reduction in Nosema ceranae spore count was observed when caffeic and p-coumaric acids were applied together, as well as kaempferol at a 25 ppm concentration. Unlike the other compounds, kaempferol reduced spore counts at all tested concentrations.

Nicotine was not effective against nosemosis. Foraging bees avoided it at a 1 ppm dose,

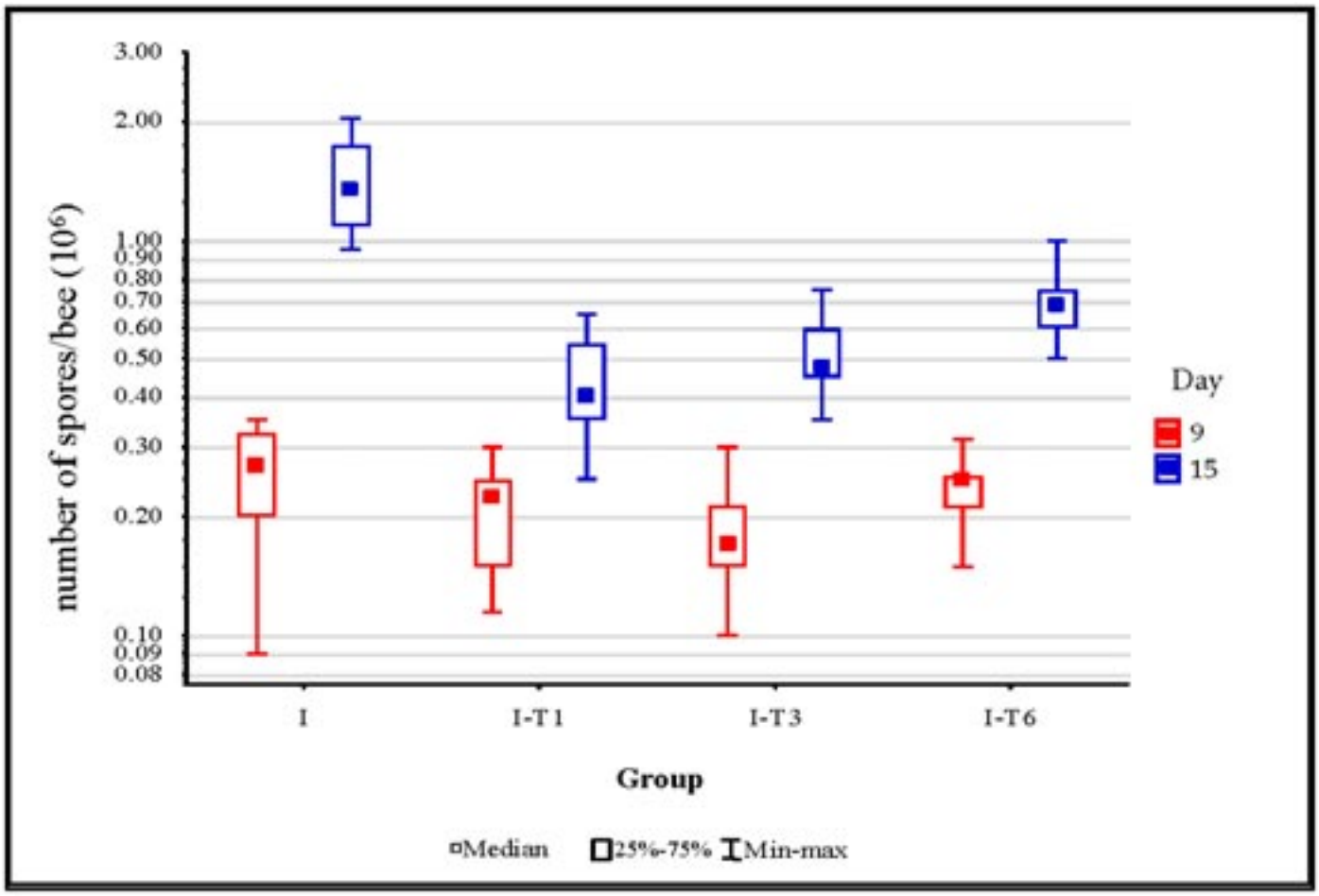


Figure 1.
N. ceranae spore loads on day 9 and day 15 in bees from the control group and from groups infected and treated with thymol. Group infected with N. ceranae (I) and groups infected with N. ceranae and treated with thymol from day 1 (I-T1), day 3 (I-T3), and day 6 (I-T6) (Glavinić et al., 2022)

while higher concentrations up to 104 ppm also had no therapeutic effect.

At high doses, nicotine increased bee mortality. In vitro studies showed that Nosema spores treated with nicotine remained infectious to bees (Hendriksma et al., 2020).

Caffeine, one of the purine alkaloids, is naturally present in plant species from the genera Camellia L., Coffea L., Theobroma L., Paullinia Kunth, Ilex L., and Cola H.W. Schott et Endlicher (Strachecka et al., 2014).

A caffeine solution at 5 µg/mL had a protective effect against Nosema infection and extended the lifespan of treated bees compared to the control group fed only with sugar syrup. Similar effects were observed in bees treated with curcumin (Strachecka et al., 2015) and piperine (Schulz et al., 2019).

Sulforaphane, a compound derived from plants in the Brassicaceae family, was also tested as a potential treatment against Nosema. At concentrations of 0.1250 mg/mL and 0.1667 mg/mL, it significantly reduced N. ceranae spore loads, while at the highest tested concentration (1.2500 mg/mL), it completely eliminated the infection but also increased bee mortality (Borges et al., 2020). Carvacrol, derived from oregano oil, reduced Nosema spore counts when applied at 0.1000 mg/mL in sugar syrup. On the other hand, naringenin, a flavonoid from citrus fruits, had a moderate effect on spore reduction but significantly extended bee lifespan (Borges et al., 2020). The majority of studies were conducted only in laboratory, so the results of those studies should be taken with caution, till the field investigations of their efficacy.

2.3. Mushroom extracts

Mushroom extracts from the genus Agaricus are used for nosemosis control due to the presence of biologically active compounds such as glucans, mannans, and lentinan, which exhibit immunostimulatory effects. In study Stevanovic et al., (2018), extract of Agaricus brasiliensis was tested on the strength of bee colonies. The results showed that the treatments significantly improved colony strength parameters, including an increase in brood production and adult bee population. Although increases in honey production and pollen reserves were observed less frequently, positive effects were mostly noted in April. The study concluded that A. brasiliensis extract is safe for bees and helps maintain strong colonies, particularly in the spring. These findings suggest that supplementation with this extract could be a beneficial practice in beekeeping to improve the health and productivity of bee colonies.

In laboratory experiments conducted by Glavinić et al. (2021a, 2021b), the effects of aqueous extracts from A. bisporus and A. blazei were examined on bee survival, nosemosis infection levels, and the expression of immune-related genes (abaecin, defensin, hymenoptecin, apidaecin, and vitellogenin). The application of aqueous extracts from both mushrooms reduced the number of Nosema ceranae spores and extended the lifespan of infected bees (Figure 2). Jelisic et al., (2024) investigated the potential of A. bisporus mushroom extract in mitigating oxidative stress in honey bees (Apis mellifera) caused by the pesticide deltamethrin and infection with the parasite Nosema ceranae. The study was conducted on bees exposed to deltamethrin, piperonyl butoxide (a synergistic component that enhances the effect of deltamethrin), and/or infected with N. ceranae, with or without the addition of A. bisporus extract in their diet. The results showed that supplementation with A. bisporus extract led to reduced bee mortality and a lower number of N. ceranae spores compared to control groups. Additionally, the extract demonstrated antioxidant properties by reducing the activity of enzymes associated with oxidative stress, including catalase (CAT), superoxide dismutase (SOD), and glutathione S-transferase (GST), as well as the concentration of malondialdehyde (MDA), an indicator of lipid peroxidation. Deltamethrin and its combination with piperonyl butoxide induced oxidative stress in bees, while the addition of A. bisporus extract alleviated these effects, particularly in bees infected with N. ceranae. The study concludes that natural extracts such as A. bispo-

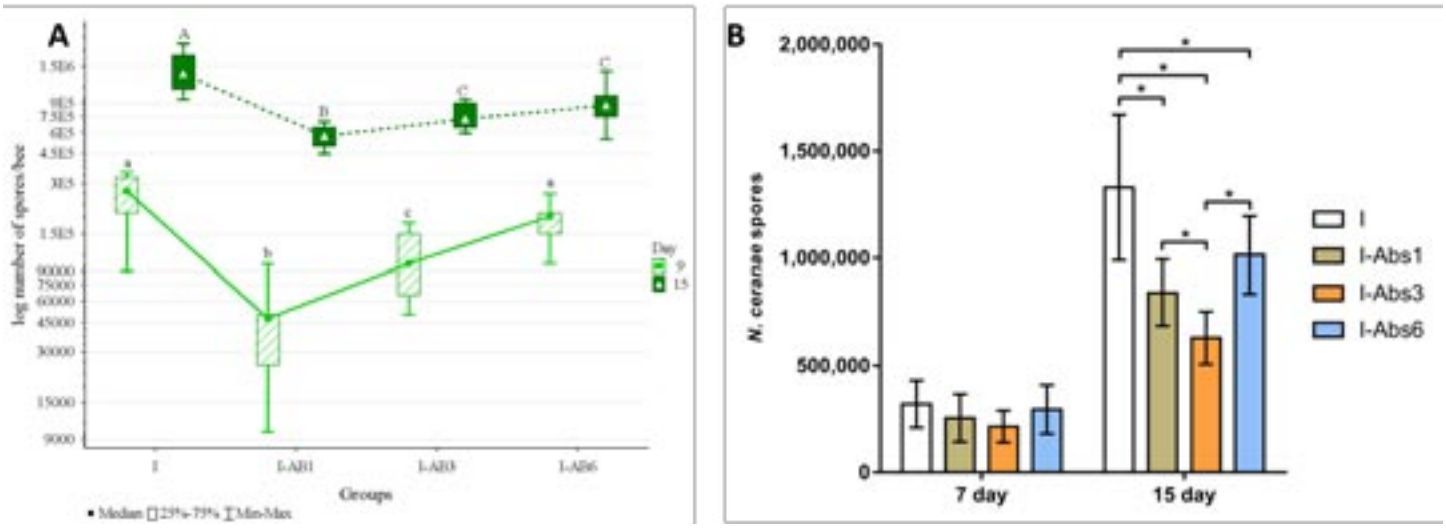


Figure 2.
A) N. ceranae spore loads on day 9 and day 15 in bees from the control group and from groups infected and treated with Agaricus blazei. Group infected with N. ceranae (I) and groups infected with N. ceranae and treated with A. blazei from day 1 (I-AB1), day 3 (I-AB3), and day 6 (I-AB6) (Glavinić et al., 2021a); B) Number of N. ceranae spores in infected group (I) and groups of bees infected with N. ceranae and treated with A. bisporus extract from day 1 (I-Abs1), day 3 (I-Abs3), and day 6 (I-Abs6) (Glavinić et al., 2021b)

rus can improve bee health by reducing the negative effects of pesticides and pathogens. These findings have significant ecological importance, as the use of natural supplements in bee nutrition could contribute to biodiversity conservation and the sustainability of bee colonies.

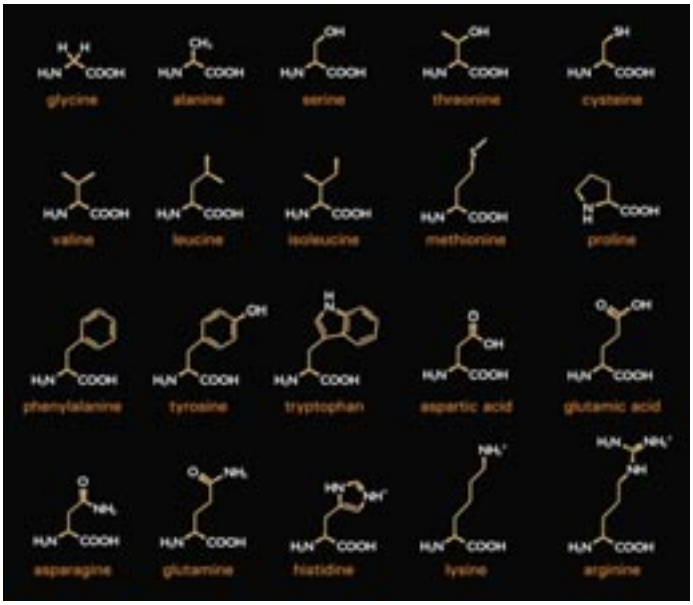
3. Probiotics

The first laboratory experiments with gut bacteria isolated from healthy bees, including bifidobacteria and lactobacilli, were conducted by Baffoni et al. (2016). The analyses showed that bees, whether healthy or infected, that were fed with bacteria had significantly lower levels of nosemosis compared to the control group fed only with sugar syrup. Later studies confirmed that the oral administration of bacterial metabolites produced by Lactobacillus johnsonii and L. kunkeei strains had no toxic effects on bees but reduced N. ceranae infection levels (Fiorella et al., 2017; Arredondo et al., 2018). Similar results were recorded in a study by Audisio et al. (2015), in which bees were fed Lactobacillus johnsonii CRL1647 at a concentration of 1×10^5 cfu/mL at intervals of either 15 days or once a month. The study showed that both administration methods reduced nosemosis levels and increased honey production. Additionally, monthly administration of lactobacilli to bees infected with Varroa reduced disease progression. Furthermore, organic acids produced by Lactobacillus johnsonii CRL1647 positively affected bee colonies by reducing the development of nosemosis. In vitro, administration of cell-free supernatant from L. johnsonii, rich in organic acids such as lactic, phenylacetic, and acetic acid, did not cause bee mortality even at high doses of 60 μL per bee (Maggi et al., 2013). Additionally, bacteriocins and surfactins derived from Bacillus strains inhibited nosemosis development (Porrini et al., 2010).

However, supplementing bee diets with inadequately selected bacteria can have negative consequences. Bees fed with Lactobacillus rhamnosus exhibited higher levels of nosemosis and a shorter lifespan than the control group fed only sugar syrup (Ptaszynska et al., 2016a). Therefore, it is important to emphasize that bacteria not adapted to bees not only fail to prevent nosemosis development but also may worsen the infection, weaken the immune system, and increase bee mortality (Ptaszynska et al., 2016a, 2016b). A metagenomic analysis of bee colonies from the United Kingdom, Spain, Poland, Greece, and Thailand revealed that nosemosis causes an increase in fungi and certain bacterial groups, such as Firmicutes (Lactobacillus), γ-proteobacteria, and Neisseriaceae. In contrast, healthy bees had a higher number of bacteria from the Orbales, Gilliamella, Snodgrassella, and Enterobacteriaceae groups (Zhang et al., 2019; Ptaszynska et al., 2021; Gancarz et al., 2021). Nosemosis infection also increased the presence of Bifidobacterium spp. in infected bees (Zhang et al., 2019).

4. Diet supplements

The effects of the plant-based supplement "B+" were investigated in both, field and laboratory experiment (Jovanović et al., 2021, 2023, 2025). Bee survival, Nosema ceranae infection levels, oxidative stress parameters, and the expression of genes for antioxidant enzymes and vitellogenin were monitored in the laboratory study (Jovanović et al., 2023). The results showed that groups receiving the supplement had lower mortality rates. In the N. ceranae-infected and treated group, the spore count was lower compared to the infected group. Additionally, oxidative stress parameters and the expression of antioxidant enzyme genes were lower in the treated groups, while vitellogenin gene expression was increased. The study concluded that the "B+" supplement could benefit bee survival, increasing the weight of fat body, reducing N. ceranae infection levels (Figure 3) and oxidative stress in infected bees (Jovanović et al., 2023). In a field experiment, the same supple-



ment was tested for its effects on colony strength parameters and pathogen presence (Jovanović et al., 2021), as well as oxidative stress and hygienic and grooming behavior (Jovanović et al., 2025). The study was conducted during late summer and early spring, evaluating the area of open and sealed brood, honey and pollen reserves, the number of adult bees, and the presence of N. ceranae spores (Figure 3), viruses, and Varroa destructor mite infestation levels. The results showed that colonies receiving the "B+" supplement had significantly higher strength parameters than the control group fed only sugar syrup. Additionally, these colonies had a significantly lower N. ceranae spore count and lower levels of acute bee paralysis virus, deformed wing virus, and sacbrood virus (Jovanović et al., 2021). The treated colonies also showed reduced oxidative stress. Furthermore, the supplement positively influenced hygienic and grooming behavior (Jovanović et al., 2025). The study concluded that "B+" supplementation could provide

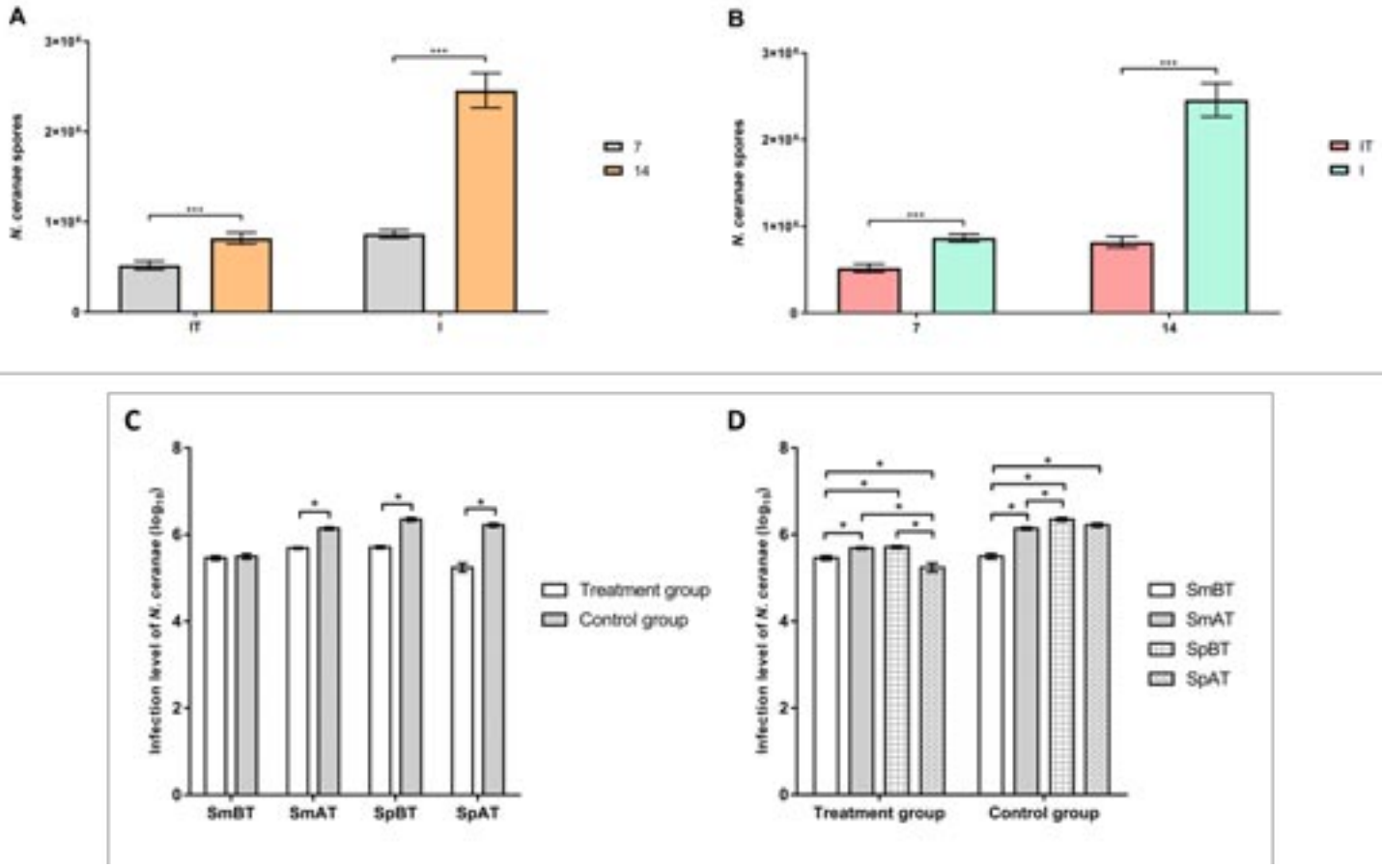


Figure 3. (A, B) Number of N. ceranae spores in infected group (I) and infected and treated group (IT). Comparison of N. ceranae spore loads within groups between samples collected on days 7 and 14 and comparison of N. ceranae spore loads between groups on samples collected on days 7 and 14 (Jovanović et al., 2023) (C, D) Comparison of average Nosema spore counts in bee colonies between treatment and control groups throughout the experiment and within each group at different sampling time points; SmBT - summer before treatment, SmAT - summer after treatment, SpBT -spring before treatment and SpAT - spring after treatment (Jovanović et al., 2021)

essential nutrients, strengthen colonies, prevent nutritional and oxidative stress, and enhance bee resilience to pathogens.

In a study by Shumkova et al. (2021), the effects of two plant-based supplements (NOZEMAT HERB® and NOZEMAT HERB PLUS®) on bee colonies infected with N. ceranae were investigated. The results suggest that these supplements could be an alternative therapy for controlling nosemosis and improving colony health and performance, but further research is needed to clarify the mechanisms of action of these supplements.

Glavinić et al. (2017) investigated the effects of an artificial supplement containing amino acids and vitamins (BEEWELL AminoPlus) on the immune response of bees infected with N. ceranae. In a laboratory experiment, bees were infected with N. ceranae and treated with the supplement on the first, third, sixth, and ninth days after emergence. The expression of immune-related peptide genes (abaecin, apidaecin, hymenoptaecin, defensin, and vitellogenin) was analyzed across different groups. The results showed that bees receiving the supplement had a significantly lower N. ceranae spore count than the control group, particularly on the twelfth-day post-infection (Figure 4). The supplemented bees maintained higher expression levels of these genes, while the expression of other immune peptides was reduced in the control group. These findings suggest that N. ceranae negatively affects bee immunity, while BEEWELL AminoPlus may modulate immune-related gene expression, enhance disease resistance, and reduce bee mortality. The supplement showed the highest efficacy when applied simultaneously with infection, which could help determine the optimal timing for its application in hives. The same supplement was also tested in field conditions for its effects on hygienic behavior and its role in combating infections caused by N. ceranae and viruses (Stanimirović et al., 2022). The study lasted one year and involved 40 bee colonies divided into five groups: one group received the supplement and was infected with N. ceranae and four viruses (deformed wing virus, acute bee paralysis virus, chronic bee paralysis virus, and sacbrood virus); three groups were infected without supplementation and one group served as a negative control. The results showed that supplementation with "BEEWELL AminoPlus" led to a significant and consistent increase in hygienic behavior despite the negative effects of infections. The supplement exhibited anti-nosemosis effects, as N. ceranae infection levels significantly decreased only in the supplemented group. Besides, only the supplemented group remained

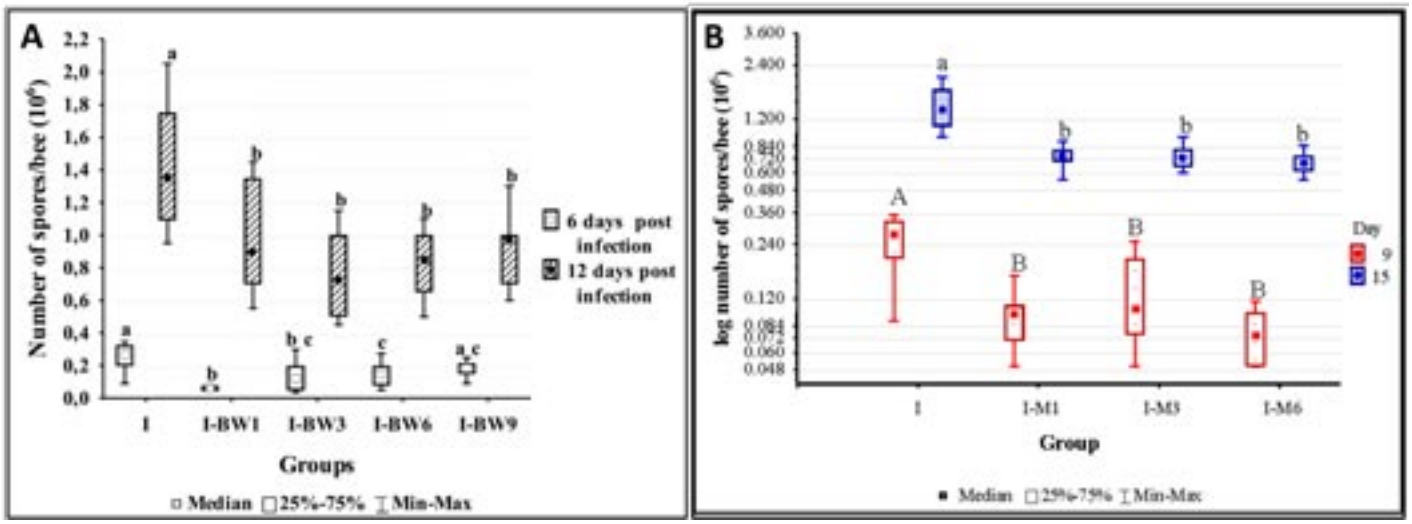


Figure 4. A) Nosema spore loads in control and groups treated with amino acid and vitamin complex BEEWELL AminoPlus on days 6 and 12 after the infection with N. ceranae. Groups were infected with N. ceranae spores on 3rd day after emerging and treated with ªBEEWELL AminoPlusº from 1st (I-BW1), 3rd (I-BW3), 6th (I-BW6) and 9th (I-BW9) day after emerging, while the control (I) was infected but not treated (Glavinić et al., 2017); B) Number of N. ceranae spores in infected group (I) and groups of bees infected with N. ceranae and treated with Medenko from day 1 (I-M1), day 3 (I-M3), and day 6 (I-M6) (Glavinić et al., 2024)

free of Lotmaria passim parasites throughout the study. The study concluded that dietary supplementation improves bee colony hygienic behavior and enhances their ability to fight common infections of parasitic and viral origin.

The effect of the "Medenko forte" supplement, which contains wormwood (Artemisia absinthium) and oak bark (Quercus robur) extracts, was tested for bee survival, N. ceranae infection levels, oxidative stress, and immune-related gene expression (Glavinić et al., 2024). The study was conducted under laboratory conditions, where bees were infected with N. ceranae and treated with the supplement at different time intervals after emergence (first, third, and sixth day). The results showed that the application of "Medenko forte" reduced N. ceranae spore counts (Figure 4) and oxidative stress in infected bees. Additionally, improved bee survival was observed regardless of the timing of administration. Analysis of immune-related gene expression (abaecin, defensin, hymenoptaecin, apidaecin, and vitellogenin) did not indicate any adverse effects of supplementation on the bee immune system. The study concluded that "Medenko forte" has the potential to control N. ceranae infection and improve honey bee health.

6. Conclusion

The control of Nosema ceranae infection in honey bees requires a multifaceted approach, integrating natural compounds, chemically synthesized substances, and dietary supplements. Studies demonstrate that essential oils, plant extracts, and mushroom-derived compounds can reduce spore loads and improve bee survival. Dietary supplements enhance immune responses and mitigate oxidative stress, contributing to colony resilience. However, improper probiotic supplementation and high doses of certain chemicals may have adverse effects. While these alternative treatments show promise, further research is needed to refine application methods, assess long-term impacts, and develop sustainable strategies for improving honey bee health and colony stability. Excessive application of any preparation, however, can have harmful effects and disrupt the conditioning and health of bee colonies.

Nemanja M. Jovanović
Teaching Assistant
University of Belgrade - Faculty of Veterinary Medicine, Department of Parasitology

Uroš Glavinić
PhD Assistant professor
Prof. dr Jevrosima Stevanović
PhD Full Professor
Tamara Markanović
Junior research assistant
Aleksa Mijatović
Junior research assistant
Jovana Stamenović
Junior research assistant
Zoran Stanimirović
PhD Full Professor
University of Belgrade - Faculty of Veterinary Medicine, Department of Biology

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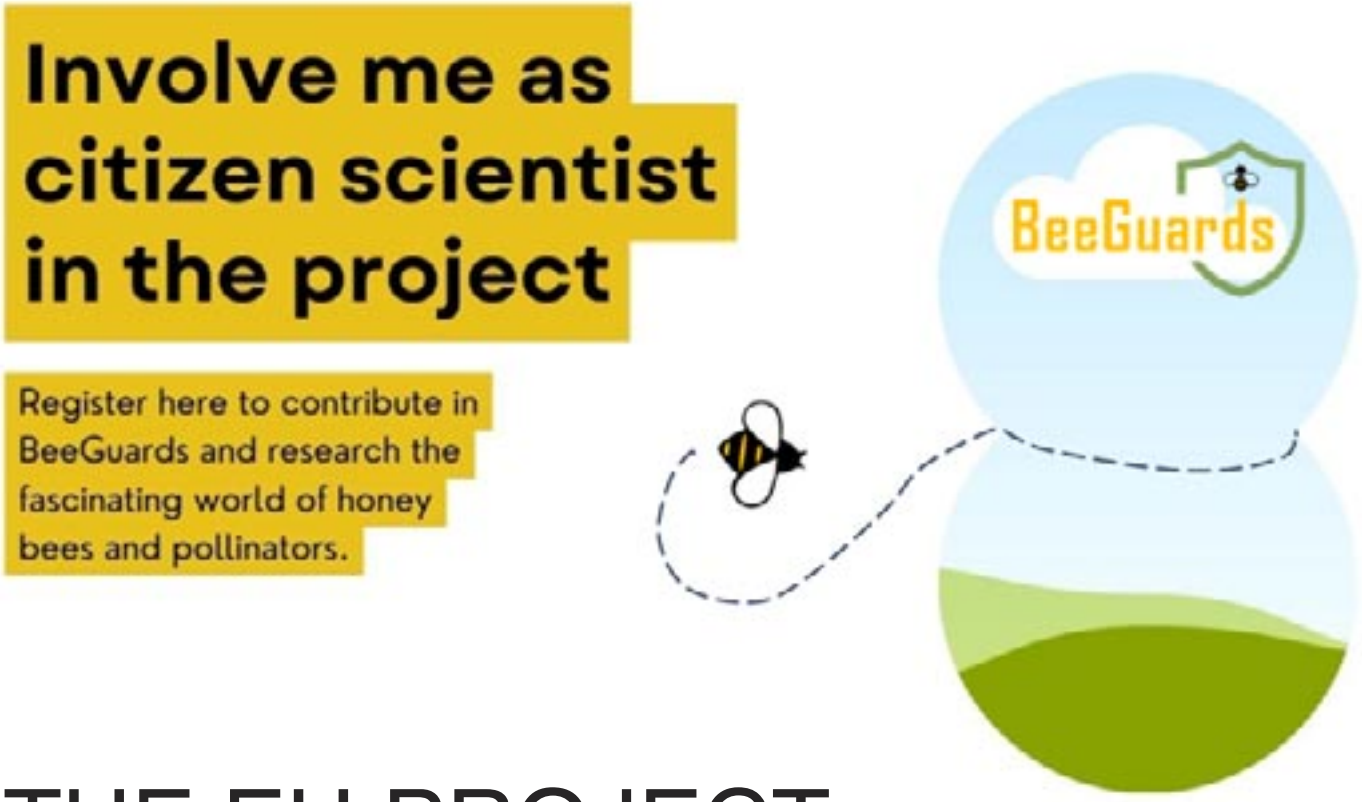

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Dr. Robert Brodschneider
Department of Biology
Honey bee, Environment and Society
University of Graz

A BRIGHT STAR ILLUMINATES THE WORLD OF BEES IN AFRICA

In this article, we will highlight an inspiring personality who is positive and influential, Engineer Ramadan Rafaa, an expert and consultant in beekeeping, Chairman of the Global Natural Food Company from North Africa, specifically the country of Libya.

For more than 40 years, he has been known and famous for his love of bees and pollinators, passionate about his profession, he does not see beekeeping as just a business, but rather a reflection of commitment to nature, and that quality is not just compliance, but rather leadership of high performance, innovation and continuous improvement to make the world safer and more sustainable.

Engineer Rafaa works hard to protect bees and pollinators, preserve biodiversity and enhance global conservation efforts.

Rafeh owns a picturesque nature reserve that takes you on a captivating journey into the depths of nature located east of Benghazi. His research has focused on bee health and his interest in rural development, pollination and bee plants is prominent. He always looks forward with renewed hope for the future of beekeeping and promoting sustainability. He takes awareness measures about bees and pol-

linators and their essential role in our ecosystem and urges the need to confirm the commitment to empowering the global beekeeping community and ensuring the well-being of pollinators. Mr. Rafaa has won many international awards from France, England, Turkey, Saudi Arabia, Egypt, Tunisia, etc. He was granted the title of "International Judge" in the field of beekeeping and conservation. These awards come in appreciation and honor of his efforts, which made Mr. Rafaa a role model worldwide.

Dr Sumaya Ramadan
Libya

APITHERAPY DAY

Invitation to the International Symposium on Apitherapy

saturday
29. march 2025

Location:

Faculty of Agriculture and Life Sciences

Pivola 10, Hoče, Slovenia

Programme

	8.00 - 8.45	Reception of participants
	8.45 - 9.00	Opening speeches
●	9.00 - 10.00	**Current Developments in Apitherapy in the World** dr. János Körmendy-Rácz
●	10.00 - 10.15	**Presentation of the Work of the Apitherapy Commission at the EBA** dr. Jana Irsáková, European Beekeeping Association (EBA)
●	10.15 - 10.45	**Products from Primary Bee Products *** Kristina Dolinar Paulič
		coffee break
●	11.15 - 12.15	**Application of Propolis-Herbal Ethanol Extract and Ointment in the Treatment of Slow-Healing Wounds. Case Reports. Basic Apitherapy Principles in Wound Treatment** dr. Plamen Entchev
●	12.15 - 12.45	**Integrative Homeopathy and Homeopathic Apis mellifica *** dr. Maruša Hribar
		coffee break
●	13.15 - 14.15	**Carpal Tunnel Syndrome - an Apitherapy Approach** dr. Jana Irsáková
●	14.15 - 14.45	**Reactions to Bee Venom *** dr. Aleš Rozman, University Clinic Golnik
●	14.45 - 15.00	**Results of Using Bee Venom in Skin Rejuvenation *** Urban Ropotar, Lamorix

* The presentation will be in Slovenian language.

INTERNATIONAL CONFERENCE

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The conference will feature inspiring keynote speeches, interactive workshops, and valuable networking opportunities. It's a unique chance to bridge science, education, and nature—bringing fresh perspectives into your teaching practice and beyond.

Save the Date April 22, 2025, and Register Now!

Don't miss out on this exceptional opportunity to explore how bees can transform education and contribute to a sustainable future.

Apipedagogy provides beneficial support for the health and immunity of children in kindergartens and schools, along with numerous developmental benefits: it aids in the development of concentration and perseverance in activities, fosters imagination, promotes intellectual development, encourages motor and mental development, supports prosocial growth and empathy, aids in speech development, fulfills emotional needs, and more. Through Apipedagogy, children feel comfortable, better cope with the challenges of adjusting to kindergarten, enjoy the activities, experience learning as a pleasant activity, and build a positive self-image.

For educators and teachers, Apipedagogy offers opportunities for career development, workplace health, and the freshness of new activities. The knowledge they gain also benefits their personal lives.

We extend an invitation to all apipedagogues, general educators, young students engaged in Apipedagogy, and other stakeholders to attend this conference, share their insights, and collaborate with us.

Website of the conference:

https://apisretis.wixsite.com/apipedagogy-2025

Organizers of the conference: Institute for the Development of Empathy and Creativity Eneja and Croatian Apitherapy Society.

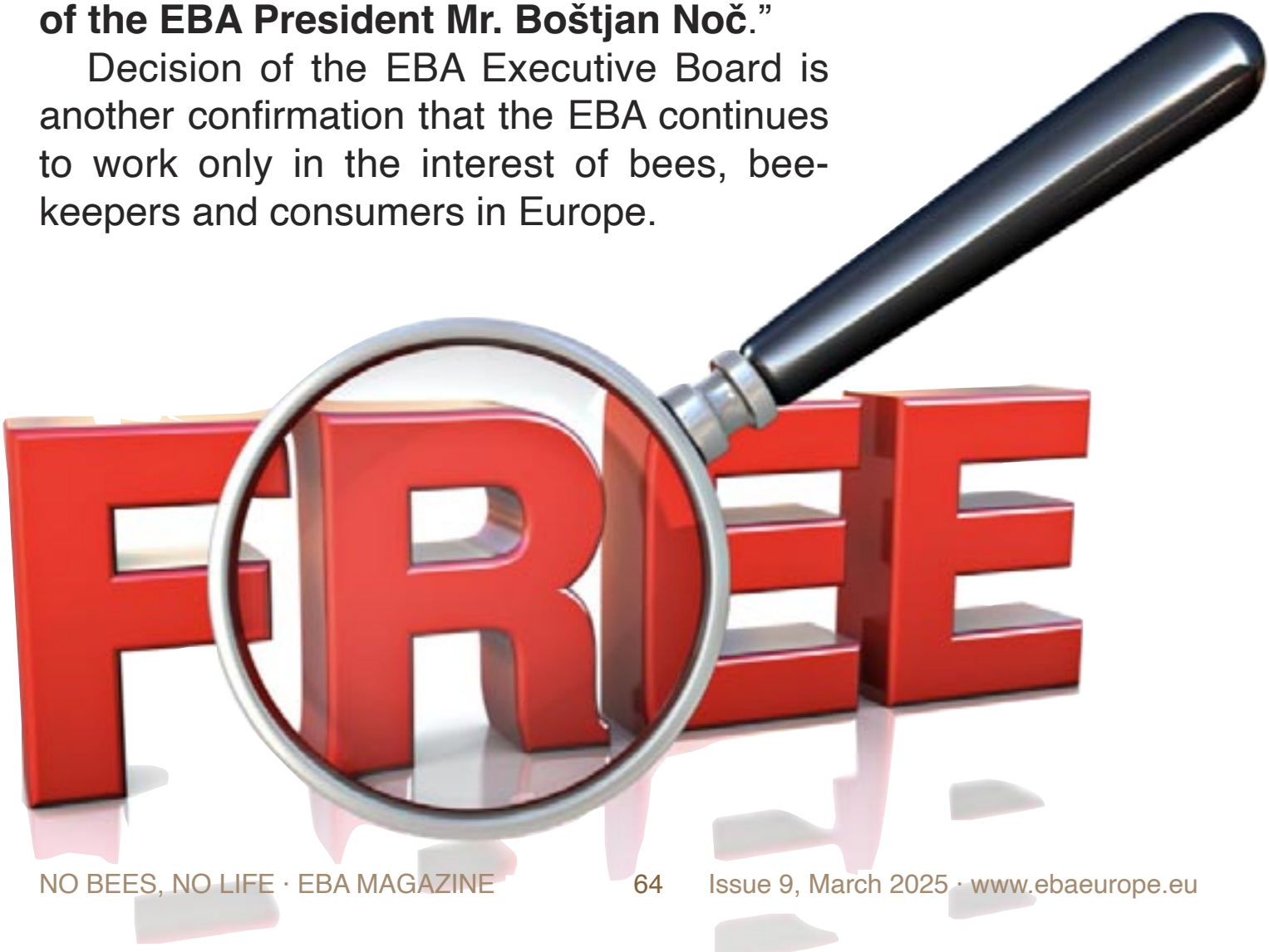
We look forward to welcoming you!

Nina Ilič
President of the International Conference
Apipedagogy 2025

TO THE EBA WITHOUT MEMBERSHIP FEE

At the meeting of the EBA Executive Board, on the proposal of the EBA President Mr. Boštjan Noč, an important decision was made regarding membership in the EBA in the upcoming period: "**Membership in the EBA is free for the duration of the mandate of the EBA President Mr. Boštjan Noč**."

Decision of the EBA Executive Board is another confirmation that the EBA continues to work only in the interest of bees, bee-keepers and consumers in Europe.



SPONSORSHIP REQUEST

AND METHOD OF ADVERTISING IN THE MAGAZINE

On behalf of the European Beekeeping Association (EBA),I am writing to seek your support in the form of sponsorship to help ensure the smooth and effective operation of our Association.

The EBA is dedicated to promoting and supporting beekeeping across Europe. The Association was founded out of necessity, as bees and beekeepers are essential for our ecosystem and society. Without beekeepers there are no bees, and whithout bees there is no pollination, leading to a lack of food on planet Earth.

EBA works for bees, beekeepers and consumers.

Our mission is to:

1. Fight against counterfeit honey that flooded the European market;
2. Introduction of incentives per beehive as agro-ecological programme;
3. Fight against the improper use of chemicals that are harmful to bees;

In return for your generous support, we offer various sponsorship benefits. We believe that this partnership would be mutually beneficial and would significantly contribute to the advancement of the european beekeeping sector.

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1. Through sponsorship packages;
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These are basic packages, but we are open to different forms of cooperation, which we agree on individually. We would be delighted to discuss this opportunity further and explore how we can align our goals with your organization's values.

Thank you for considering our request. We look forward to the possibility of working together.

Yours sincerely,

Boštjan Noč
President of the European Beekeeping Association

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eba@ebaeurope.eu
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Editor in chief of the electronic edition of the magazine:
MD Rodoljub Živadinović, Epidemiology Specialist, Apitherapist
apikult@gmail.com, +381 60 444 01 01 (Viber, WhatsApp, Telegram, Signal, WeChat, Daze)