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Kmetijski inštitut Slovenije

6. ZNANSTVENO POSVETOVANJE O ČEBELAH IN ČEBELARSTVU Z MEDNARODNO UDELEŽBO

POKLUKARJEVI DNEVI 2026



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»Če sem videl dlje, je bilo to zato, ker sem stal na ramenih velikanov.«

“If I have seen further, it is by standing on the shoulders of giants.”

Isaac Newton

Pismo Robertu Hooku, 5. februarja 1676 / Letter to Robert Hooke, 5 February 1676





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Kje (kako) nadaljevati, ko je toliko nedokončanih poti?

Marjan Papež[1] *(marjan.papez@t-2.si)*

Docent dr. Janez Poklukar (1960–2004) je bil človek, ki je v slovenskem čebelarstvu pustil za sabo veliko nedokončanih poti. Tisti, ki smo bili njegovi sodobniki, vemo, da je Janez Poklukar suvereno deloval na številnih področjih: kot avtor znanstveno-strokovnih in poljudnih vsebin, kot tvorec sodobne čebelarske zakonodaje v samostojni Sloveniji, kot srčni prenašalec znanja na študente, kot spreten in prepričljiv prenašalec dosežkov znanosti v prakso ter kot človek, ki je kot vodja znal motivirati tako, da je iz ljudi prišlo tisto najboljše, kar so vedeli in znali o čebelarstvu.
Molekularna genetika, dedovanje in selekcija, bolezni čebel, prehrana in obnašanje čebel, (gozdno) medenje, tehnologija in ekonomika čebelarjenja, kakovost in trženje medu so bila njegova glavna znanstveno-strokovna področja, na katerih je s svojim delom pustil pomembno sled.
Kako so se te sledi nadaljevale? Kako so se te poti gradile po njegovi smrti 5. aprila 2004? Ker je Janez Poklukar pripravil dobro utrditev in premišljeno traso, se je kljub temu, da je takrat za njim zazevala izjemna praznina, slovensko čebelarstvo dobro gradilo naprej in se še vedno gradi.
Veliko je bilo storjenega in doseženega na podlagi tistega, kar je začrtal Janez Poklukar. Brez dvoma pa bi pod njegovo taktirko nastalo še bistveno več in bolje. Koliko, ne ve nihče. Ni vzporednega sveta. Je pa zavedanje, da takšne vsestranskosti, h kateri je Janez dodajal svoje izjemne človeške lastnosti, nenazadnje je bil tudi med ustanovitelji in prvi predsednik Slovenskega akademskega čebelarskega društva (SAČD), ni (bilo) mogoče nadomestiti.
Razširjeno je prepričanje, »da Bog rad pokliče k sebi take ljudi, kot je bil Janez Poklukar, ker jih želi imeti ob sebi«. Mi pa bi želeli drugače. Čeprav ga že več kot dve desetletji ni več med nami, njegov duh še živi. Življenje teče dalje, prihajajo nove generacije mladih, ki na znanstvenem in strokovnem področju nadaljujejo njegovo delo, ki je opredmeteno v izvlečkih v nadaljevanju.
In kot je zapisal evangelist Janez (4,38): »Jaz sem vas poslal, da boste želi, česar niste obdelovali. Drugi so se trudili, vi pa ste stopili v njihov trud.«

Ključne besede: Janez Poklukar, Slovensko akademsko čebelarsko društvo, genetika, selekcija, kakovost medu, čebelarska zakonodaja

Where (and how) do we carry on when so many paths remain unfinished?

Assistant Professor Janez Poklukar, PhD (1960–2004), was a man who left behind many unfinished paths in Slovenian beekeeping. Those of us who were his contemporaries know that he was a highly accomplished figure in numerous fields: as an author of scientific, professional and popular publications; as one of the architects of modern beekeeping legislation in independent Slovenia; as a passionate and dedicated teacher who shared his knowledge with students; as a skilled and persuasive communicator who successfully translated scientific advances into practical applications; and as a leader who knew how to inspire people to bring out the very best of their knowledge and expertise in beekeeping.
Molecular genetics, inheritance and selection, bee diseases, bee nutrition and behaviour, forest honey production, beekeeping technology and economics, as well as honey quality and marketing, were the main scientific

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and professional fields in which his work left a significant and lasting mark.
How have these contributions been carried forward? How have these paths continued to develop since his death on 5 April 2004? Thanks to the solid foundations he laid and the carefully considered course he charted, Slovenian beekeeping continued to develop successfully, despite the immense void his passing left behind. And it continues to do so today.
Much has been accomplished and achieved on the foundations laid by Janez Poklukar. There is no doubt, however, that under his guidance even more could have been achieved, and to an even higher standard. How much more, no one can know. There is no parallel world in which to find out. What we do know is that such extraordinary versatility, combined with Janez's exceptional personal qualities – not least that he was one of the founders and the first president of the Slovenian Academic Beekeeping Society (SAČD) - could not, and cannot, be replaced.
There is a widely held saying that God calls people like Janez Poklukar to Himself because He wants them by His side. We, however, would have wished things to be different. Although he has been gone for more than two decades, his spirit lives on. Life goes on, and new generations of young people are emerging, continuing his work in the scientific and professional fields. Their contributions are reflected in the abstracts presented in the following pages.
And, as the Evangelist John wrote (4:38): “I sent you to reap that for which you did not labour. Others have laboured, and you have entered into their labour.”

Key words: Janez Poklukar, Slovenian Academic Beekeeping Society, genetics, selection, honey quality, beekeeping legislation



Primjena Beesmoke peletiranog goriva za dimilicu

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Sažetak

Kao inovativni pristup održivom pčelarstvu u svakodnevni rad na pčelinjaku može se dodati komercijalno dostupna peletirana goriva za dimilicu BeeSmoke Forte i BeeSmoke. Istraživanjem učinkovitosti navedenih goriva bilo je obuhvaćeno određivanje jačine pčelinjih zajednica, ponašajne osobitosti pčelinjih zajednica, učinkovitost primjene na otpadanje nametničke grinje *V. destructor*, te analizu na kvantifikaciju rezidua insekticida/akaricida u medu. Prilikom primjene dima zapaljenih goriva (BeeSmoke Forte, BeeSmoke i drvenih peleta) iz dimilice u košnice, na pčelinjim zajednicama nisu uočene promjene uobičajenog ponašanja niti ugibanja pčela. Utvrđeno je učinkovito djelovanje BeeSmoke Forte i BeeSmoke peletiranaog goriva na otpadanje nametničke grinje *V. destructor*. Analiziran je zreli med prije i nakon primjene dimilice iz pčelinjih zajednica na prisutnost insekticida / akaricida, rezultati kojih su bili negativni, a što potvrđuje da je med sakupljen tijekom aktivne pčelarske sezone u kojoj su primijenjena istraživana peletirana goriva siguran kao hrana za pčele i ljude.

Ključne riječi: zajednice medonosne pčele, peletirana goriva za dimilicu, pčelarenje, *Varroa destructor*

Use of Beesmoke smoker pellets

Abstract

An innovative and sustainable approach to beekeeping involves using commercially available pellet fuels, such as BeeSmoke Forte or BeeSmoke, for daily work in the apiary. This study aimed to determine the effectiveness of these pellets, including their impacts on the strength and behaviour of honeybee colonies, their ability to reduce the number of parasitic mites *Varroa destructor*, and the presence of insecticide/acaricide residues in honey. During the study, the smoke produced by burning BeeSmoke Forte, BeeSmoke, and pure wood pellets in a smoker caused no changes to honeybee behaviour and resulted in no fatalities. BeeSmoke Forte and BeeSmoke pellets increased the number of fallen parasitic mites *V. destructor*. Furthermore, honey collected before and after the use of pellets and analysed for the presence of insecticide/acaricide residues gave negative results, indicating that the honey extracted during the active beekeeping season when these fuels were used is safe for bees and for human consumption.

Key words: honeybee colonies; smoker pellet fuels; good beekeeping practices; *Varroa destructor*

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Eksperimentalna vzreja diploidnih trotov *Apis mellifera* in možnosti njihove genomske ter epigenetske karakterizacije

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Izvleček

Pri medonosni čebeli (*Apis mellifera*) ima pri določanju spola osrednjo vlogo gen complementary sex determiner (csd). Iz neoplojenih haploidnih jajčec se razvijejo troti, iz oplojenih jajčec, heterozigotnih na lokusu csd, pa samice – delavke ali matice. Ob homozigotnosti na lokusu csd se iz oplojenega jajčeca razvije diploidni trot. Njihov pojav je povezan z zmanjšano genetsko pestrostjo in parjenjem osebkov s skupnimi aleli csd, kar lahko negativno vpliva na vitalnost in produktivnost čebeljih družin.

Delavke diploidne trotovske ličinke praviloma prepoznajo in odstranijo že v zgodnjem obdobju razvoja, zato je njihovo pridobivanje za raziskovalne namene zahtevno. V okviru doktorskega raziskovalnega dela vzpostavljamo eksperimentalni pristop, ki bo omogočal vzrejo diploidnih trotov ter pripravo vzorcev za nadaljnje molekularne analize. V poskus smo vključili matice, ki so bile umetno osemenjene s svojimi brati. Razvoj jajčec in izleganje ličink smo spremljali z rednimi pregledi pod nadzorovanimi pogoji. Ličinke smo hranili z različnimi tipi hrane, pripravljene po protokolu OECD. V različnih razvojnih stadijih smo vzorčili ličinke in bube ter jih shranili za nadaljnje molekularne analize. Vzpostavili smo tudi metodo za določanje ploidnosti čebel s pretočno citometrijo (CyFlow). Metodo smo prilagodili za analizo vzorcev glav ter jo preverili na osebkih z znano ploidnostjo, haploidnih trotih (1n) in diploidnih delavkah (2n), z namenom nadaljnjega določanja ploidnosti potencialnih diploidnih trotov (2n).

Pridobljene vzorce bomo uporabili za genotipizacijo, sekvenciranje celotnega genoma (WGS) in epigenetske analize. Z genotipizacijo bomo proučevali alelne kombinacije na lokusu csd, z WGS genomsko variabilnost in stopnjo heterozigotnosti, z epigenetskimi analizami pa razlike v epigenetskih vzorcih med haploidnimi in diploidnimi troti ter diploidnimi delavkami. S povezovanjem rezultatov različnih molekularnih pristopov želimo bolje razumeti genetsko in epigenetsko ozadje nastanka in razvoja diploidnih trotov.

Ključne besede: *Apis mellifera*, csd gen, diploidin troti, genotipizacija, pretočna citometrija

Experimental Rearing of Diploid Apis mellifera Drones and Opportunities for Their Genomic and Epigenetic Characterization

Abstract

In the honey bee (*Apis mellifera*), the complementary sex determiner (csd) gene plays a central role in sex determination. Unfertilized haploid eggs develop into drones, whereas fertilized eggs that are heterozygous at the csd locus develop into females—workers or queens. When a fertilized egg is homozygous at the csd locus, it develops into a diploid drone. The occurrence of diploid drones is associated with reduced genetic

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diversity and the mating of individuals carrying identical csd alleles, which may negatively affect colony vitality and productivity.

Worker bees generally recognize and remove diploid drone larvae during the early stages of development, making their production for research purposes challenging. As part of our doctoral research, we are establishing an experimental approach that will enable the rearing of diploid drones and the preparation of samples for subsequent molecular analyses. The experiment included queens that were artificially inseminated with semen from their brothers. Egg development and larval hatching were monitored through regular inspections under controlled conditions. The larvae were provided with different types of diet prepared according to an OECD protocol. Larvae and pupae were sampled at different developmental stages and stored for subsequent molecular analyses. We also established a method for determining bee ploidy using flow cytometry (CyFlow). The method was adapted for the analysis of head samples and validated using individuals of known ploidy—haploid drones (1n) and diploid workers (2n)—with the aim of subsequently determining the ploidy of potential diploid drones (2n).

The collected samples will be used for genotyping, whole-genome sequencing (WGS), and epigenetic analyses. Genotyping will be used to investigate allelic combinations at the csd locus, WGS to assess genomic variation and the degree of heterozygosity, and epigenetic analyses to examine differences in epigenetic patterns among haploid drones, diploid drones, and diploid workers. By integrating the results obtained through these molecular approaches, we aim to gain a better understanding of the genetic and epigenetic basis underlying the origin and development of diploid drones.

Keywords: *Apis mellifera*, csd gen, diploid drones, sex determination, genotyping, flow cytometry



Potencial bakterij rodu *Bacillus* za zatiranje povzročitelja poapnele zalege *Ascosphaera apis*

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Izvleček

Poapnela zalega, ki jo povzroča gliva *Ascosphaera apis*, je najpogostejša glivična bolezen čebelje zalege. Bolezen praviloma ne povzroči propada čebeljih družin, jih pa oslabi in povzroča ekonomsko škodo. Ker trenutno ni registriranih učinkovitih načinov zatiranja bolezni, predstavljajo bakterije rodu *Bacillus* obetavno možnost za razvoj bioloških pristopov zatiranja. Namen raziskave je bil ovrednotiti protiglivni potencial bakterij rodu *Bacillus* proti *A. apis* ter raziskati možne mehanizme njihovega delovanja.

V raziskavo smo vključili 1182 bakterijskih izolatov, izoliranih iz cvetnega prahu in ocenili njihovo inhibitorno delovanje proti *A. apis*. Za nadaljnje raziskave smo izbrali nekatere izolate z največjim inhibitornim učinkom. Njihovo vrsto smo določili s celogenomskim sekvenciranjem in analizo povprečne nukleotidne identitete (ANI). Prisotnost genskih gruč za sintezo sekundarnih presnovkov smo analizirali z orodjem antiSMASH. Izbrali smo po en sev vrst *Bacillus velezensis, Bacillus amyloliquefaciens* in *Bacillus subtilis* z najboljšim inhibitornim delovanjem. Ekstrahirali smo sekundarne presnovke ter jih analizirali s tekočinsko kromatografijo ultra visoke ločljivosti sklopljeno z visokoločljivostno masno spektrometrijo. Njihovo protiglivno delovanje smo ovrednotili na desetih različnih izolatih *A. apis*. Dodatno smo preverili tudi vpliv hlapnih organskih spojin na rast glive.

V poskusih dvojnih kultur so vsi trije reprezentativni sevi učinkovito zavirali rast *A. apis*, pri čemer je *B. velezensis* dosegel najvišjo povprečno inhibicijo rasti micelija (83,3 %) in statistično značilno višjo učinkovitost v primerjavi z *B. subtilis* ($p = 0,018$). Genomske in metabolomske analize so pokazale prisotnost več sekundarnih presnovkov s protiglivnim potencialom, pri čemer smo z referenčnimi standardi potrdili prisotnost surfaktinov. Analiza hlapnih organskih spojin ni pokazala pomembnega vpliva na rast glive, kar nakazuje, da je inhibitorno delovanje predvsem posledica difuznih sekundarnih presnovkov.

Rezultati raziskave potrjujejo potencial bakterij rodu *Bacillus* kot kandidatov za razvoj bioloških pripravkov za omejevanje poapnele zalege ter predstavljajo pomembno izhodišče za nadaljnje raziskave njihove uporabe v čebelarstvu.

Ključne besede: *Ascosphaera apis*, poapnela zalega, *Bacillus*, biološko zatiranje, sekundarni presnovki

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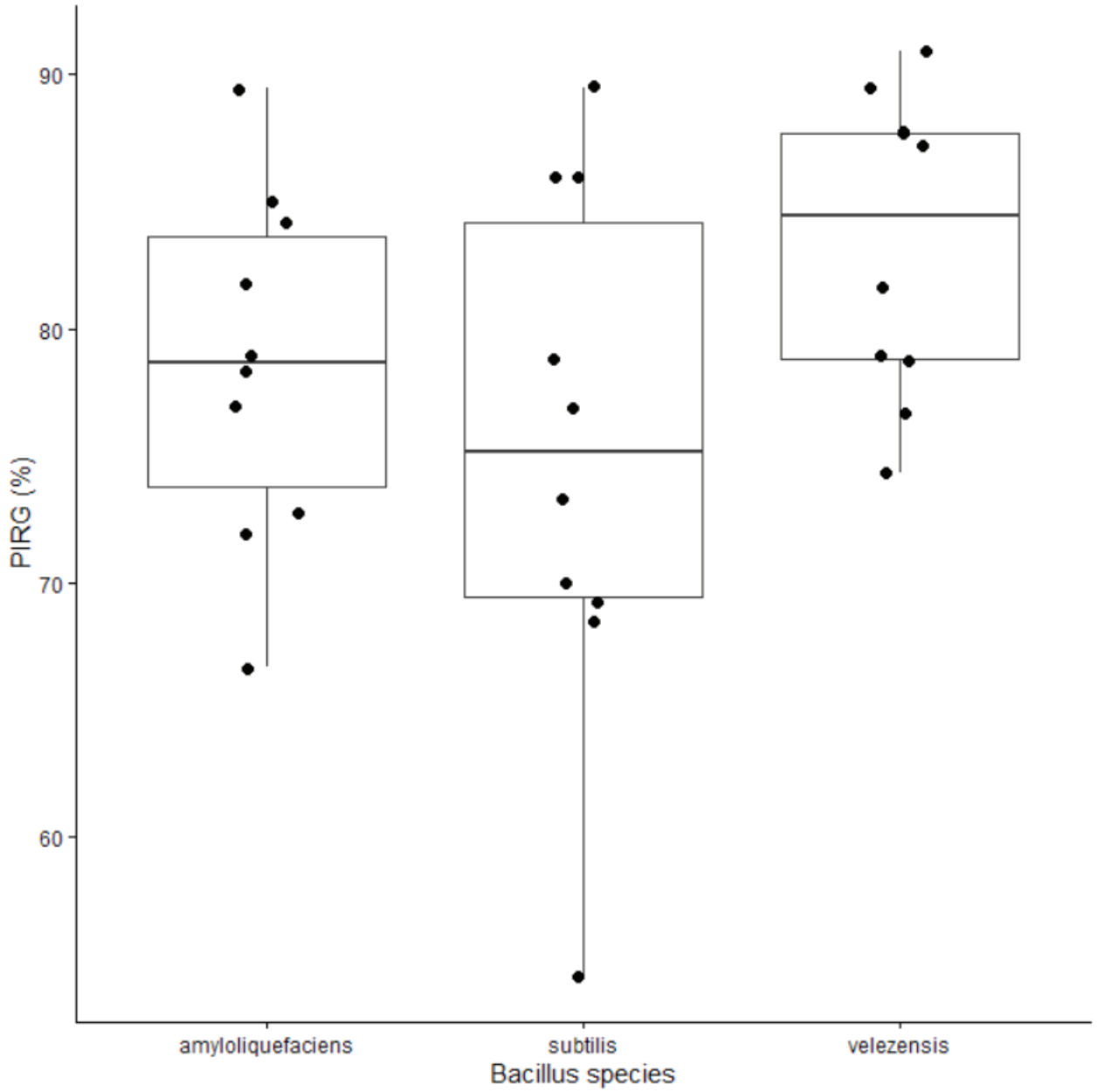
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Slika 1: Škatlasti diagram porazdelitev vrednosti inhibicije radialne rasti (%) pri treh različnih bakterijskih vrstah rodu *Bacillus*.
Figure 1: Box plot showing the distribution of radial growth inhibition (%) for three different *Bacillus* species.

Potential of *Bacillus* spp. for the control of the chalkbrood pathogen *Ascosphaera apis*

Abstract

Chalkbrood, caused by the fungus *Ascosphaera apis*, is the most common fungal disease of honey bee brood. Although the disease generally does not lead to colony loss, it can weaken colonies and cause economic losses. As no effective registered treatments are currently available for disease control, bacteria of the genus *Bacillus* represent a promising option for the development of biological control approaches. The aim of this study was to evaluate the antifungal potential of *Bacillus* spp. against *A. apis* and to investigate the possible mechanisms underlying their activity.

The study included 1182 bacterial isolates obtained from pollen, which were screened for inhibitory activity against *A. apis*. Isolates exhibiting the strongest inhibitory effects were selected for further investigation. Their species were determined by whole-genome sequencing and average nucleotide identity (ANI) analysis. The presence of biosynthetic gene clusters associated with the production of secondary metabolites was analysed using antiSMASH. One strain each of *Bacillus velezensis, Bacillus amyloliquefaciens*, and *Bacillus subtilis* showing strong inhibitory activity was selected for further analyses. Secondary metabolites were extracted and analysed by ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). Their antifungal activity was evaluated against ten different *A. apis* isolates. In addition, the effect of volatile organic compounds (VOCs) on fungal growth was investigated.

In dual-culture assays, all three representative strains effectively inhibited the growth of *A. apis*, with *B. velezensis* showing the highest mean inhibition of mycelial growth (83,3%) and significantly greater inhibition than *B. subtilis* ($p = 0{,}018$). Genomic and metabolomic analyses revealed several secondary metabolites with potential antifungal activity, and the presence of surfactins was confirmed using reference standards. Analysis of volatile organic compounds showed no substantial effect on fungal growth, suggesting that the observed inhibition may be primarily associated with diffusible secondary metabolites.

The results demonstrate the potential of *Bacillus spp*. as candidates for the development of biological control agents against chalkbrood and provide an important basis for further research into their application in apiculture.

Keywords: *Ascosphaera apis*, chalkbrood, *Bacillus*, biological control, secondary metabolites



Hipertermija čebelje zalege spremeni izražanje antimikrobnih peptidov pri odraslih čebelah

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Izvleček

Hipertermija čebelje zalege se vse pogosteje uporablja kot biotehnična metoda za zatiranje pršice *Varroa destructor*. Njena učinkovitost temelji na večji občutljivosti pršic na povišano temperaturo v primerjavi s čebeljo zalego. Izpostavitev pokrite zalege natančno nadzorovanemu temperaturnemu režimu zato omogoča zmanjšanje populacije pršic brez pomembnejših negativnih učinkov na razvoj in preživetje zalege. Zaradi naraščajoče odpornosti varoj proti sintetičnim akaricidom predstavlja hipertermija obetavno in trajnostno alternativo kemičnemu zatiranju. Kljub temu pa so njeni vplivi na fiziologijo medonosnih čebel, zlasti na delovanje imunskega sistema, še vedno slabo raziskani.

V raziskavi smo preučevali neposredne učinke hipertermije na bube ter dolgoročne posledice pri odraslih delavkah kranjske sivke (*Apis mellifera carnica*). Pokrito zalego smo za dve uri izpostavili temperaturi 42 °C, medtem ko je bila kontrolna skupina ves čas vzdrževana pri 34 °C. Štiriindvajset ur po izpostavitvi smo del bub imunostimulirali z vbodom sterilne igle, preostale pa pustili brez dodatnega posega. Vzorčenje je sledilo po nadaljnjih 24 urah. Preostalo zalego smo do izleganja vzdrževali v inkubatorju, nato pa novo izležene delavke namestili v poskusne kletke. Pri starosti enajst dni smo na enak način spodbudili imunski odziv tudi pri odraslih čebelah, ki smo jih vzorčili 24 ur pozneje.

Z metodo RT-qPCR smo analizirali izražanje genov za štiri antimikrobne peptide (defensin-1, abaecin, hymenoptaecin in apidaecin) ter izbrane proteine toplotnega stresa. Pri bubah je hipertermija povzročila le omejene spremembe v izražanju proučevanih genov. Nasprotno pa so odrasle čebele, ki so se razvile iz zalege, izpostavljene hipertermiji, kazale značilno nižje osnovno izražanje vseh štirih genov za antimikrobne peptide v primerjavi s kontrolnimi čebelami. Imunostimulacija je v obeh skupinah sprožila močan porast njihovega izražanja, pri čemer med skupinama nismo ugotovili razlik v intenzivnosti odziva. Rezultati kažejo, da lahko izpostavljenost povišani temperaturi med razvojem povzroči dolgotrajne spremembe v delovanju imunskega sistema odraslih čebel, ne da bi zmanjšala njihovo sposobnost učinkovitega odziva na imunski izziv.

Ključne besede: *Apis mellifera, Varroa destructor*, toplotno tretiranje čebelje zalege, antimikrobni peptidi, proteini toplotnega šoka

Brood hyperthermia for Varroa control reduces antimicrobial peptide expression in adult honey bees

Abstract

Brood hyperthermia is increasingly used as a biotechnical method to control *Varroa destructor*, exploiting the mite's greater sensitivity to heat compared with developing honey bee brood. Short-term exposure to elevated temperatures can effectively reduce mite populations without causing brood mortality. As resistance to synthetic acaricides continues to rise, such thermal treatments represent a promising sustainable alternative; however, their effects on honey bee physiology, particularly immune function, remain poorly understood.

In this study, we evaluated the immediate effects of heat treatment on pupae and the long-term effects on

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adult *Apis mellifera carnica* workers. Late-stage pupae were exposed to 42°C for 2 hours, while control brood was maintained at 34°C. Twenty-four hours after treatment, a subset of pupae was either left untreated or subjected to immune stimulation by cuticular puncture with a sterile needle. Pupae were sampled 24 hours later to assess their immune response. The remaining brood was allowed to emerge, and newly emerged workers were maintained in laboratory cages. At 11 days of age, adult bees were either left untreated or immune stimulated using the same procedure and sampled 24 hours later.

Gene expression of four antimicrobial peptide genes—*defensin-1, abaecin, hymenoptaecin*, and apidaecin—as well as selected heat shock protein genes, was quantified by qPCR. Adult bees originating from heat-treated pupae exhibited significantly lower basal expression of all four antimicrobial peptide genes compared with controls. However, mechanical injury induced a strong upregulation of these genes in both groups, with a comparable magnitude of response.

These results indicate that while brood hyperthermia reduces resting immune investment of adult workers, it does not impair the functional capacity to mount a robust humoral response. This suggests that early-life thermal stress alters the immune set-point without compromising the bee's ability to defend against acute challenges.

Key words: *Apis mellifera, Varroa destructor*, brood heat treatment, antimicrobial peptides, heat shock proteins



Kalorimetrija kao metoda procjene energetskog statusa pčelinjih zajednica i učinkovitosti prihrane

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Sažetak

Prihranjivanje zajednica medonosne pčele (*Apis mellifera L.*) temeljna je sastavnica dobre pčelarske prakse. Provodi se u skladu s prehrambenim potrebama zajednice tijekom određenih razdoblja u sezoni te ima važnu ulogu u razvoju pčelinje zajednice. Optimalna prihrana osobito je važna tijekom razdoblja bez paše i nakon vrcanja meda, kada prirodni izvori hrane nisu dovoljni za održavanje normalnog razvoja, vitalnosti i proizvodnosti zajednice. Istraživanja utjecaja različitih režima prihrane na energetski status medonosnih pčela još su uvijek nedostatna. U istraživanju su analizirane 34 komercijalne i jedna „kod kuće" pripremljena pogača te je proveden pokus na pčelinjaku u kolovozu 2024. godine na 36 pčelinjih zajednica raspoređenih u četiri skupine: šećerni sirup (I), komercijalna proteinska pogača (II), peludna pogača (III) te šećerna pogača s dodatkom 2 % spiruline (IV). Uzorci ličinaka i odraslih medonosnih pčela prikupljeni su 0., 22. i 43. dana pokusa. Ličinke su prikupljene izravno iz stanica saća. Nakon liofilizacije, uzorci ličinaka te osušeni uzorci pčela i pogača analizirani su metodom kalorimetrije (IKA C6000 global standard, Staufen, Njemačka) radi određivanja bruto energetske vrijednosti (BE), izražene u MJ/kg.

Kalorimetrijskom analizom utvrđeno je da pogače s dodatkom proteina nisu imale značajno veće vrijednosti BE u odnosu na šećerne pogače istog proizvođača bez dodatka proteina. Pogače iz skupina II (15,63 ± 0,14 MJ/kg), III (15,64 ± 0,02 MJ/kg) i IV (15,72 ± 0,14 MJ/kg) imale su slične energetske vrijednosti, dok je šećerni sirup imao najvišu vrijednost BE (16,45 ± 0,02 MJ/kg). Prosječna vrijednost BE-a odraslih pčela povećala se s 10,25 ± 0,29 na 10,75 ± 0,25 MJ/kg tijekom pokusnog razdoblja, pri čemu je najviša vrijednost zabilježena u skupini I (10,83 ± 0,19 MJ/kg). Energetski sadržaj ličinki prikupljenih 43. dana pokusa značajno se povećao u skupinama I, III i IV u usporedbi s uzorcima prikupljenima 0. dana. Najviše vrijednosti BE-a ličinki zabilježene su u skupinama I (22,31 ± 0,56 MJ/kg) i IV (22,20 ± 0,80 MJ/kg) (sve, $p < 0,05$). Rezultati pokazuju da prihranjivanje zajednica može značajno utjecati na energetske vrijednosti medonosnih pčela tijekom ontogeneze. Kalorimetrijska analiza predstavlja koristan pristup za procjenu energetskih učinaka prihrane te može pridonijeti boljem razumijevanju uloge prehrane u razvoju pčelinjih zajednica.

Ključne riječi: kalorimetrija, prihranjivanje pčela, pogače, energetski status

Calorimetry as a method for assessing the energy status of honey bee colonies and the efficiency of supplemental feeding

Abstract

Supplemental feeding is a fundamental aspect of good beekeeping practice for managing honey bee (*Apis mellifera L.*) colonies. It is implemented according to the colony's nutritional requirements during specific periods of the season and plays a crucial role in colony development. Providing adequate nutrition is

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particularly important during periods of nectar dearth and after honey harvest, when natural food resources are insufficient to sustain normal colony growth, health, and productivity. Current studies of how different feeding regimes influence the energy content of honey bees are still limited.
The study included the analysis of 34 commercially available patties and one homemade patty, as well as a field trial conducted in August 2024, with thirty-six honey bee colonies allocated to one of four feeding treatments: sugar syrup (I), a commercial protein patty (II), a pollen patty (III), or a sugar patty supplemented with 2% spirulina (IV). Samples of larvae and adult honey bees were collected during three sampling periods, on days 0, 22, and 43. Larvae were collected directly from wax combs. Following lyophilization, larval samples were analyzed, as well as dried bee samples and the experimental feed patties, using bomb calorimetry (IKA C6000 global standard, Staufen, Germany) to determine their gross energy (GE) content, expressed as MJ/kg. Calorimetric analysis showed that protein-enriched feed patties produced by the same manufacturers did not have significantly higher GE values than patties without added protein. Comparable energy densities were observed among patties from groups II (15.63 ± 0.14 MJ/kg), III (15.64 ± 0.02 MJ/kg), and IV (15.72 ± 0.14 MJ/kg), whereas sugar syrup exhibited the highest GE value (16.45 ± 0.02 MJ/kg). The mean GE content of adult bees increased from 10.25 ± 0.29 to 10.75 ± 0.25 MJ/kg over the experimental period, with the highest value recorded in group I (10.83 ± 0.19 MJ/kg). At the third sampling point (Day 43), larval GE increased significantly in groups I, III, and IV compared with the initial sampling. The highest larval GE values were observed in groups I (22.31 ± 0.56 MJ/kg) and IV (22.20 ± 0.80 MJ/kg) (all, $p < 0.05$).
These findings demonstrate that supplemental feeding can significantly influence the energy content of honey bees during ontogenesis. Calorimetric assessment provides a useful approach for evaluating the energetic effects of supplemental feeding and may improve understanding of nutritional contributions to honey bee colony development.

Keywords: calorimetry, honey bee nutrition, patties, energy status



Vpliv kronične izpostavljenosti broflanilidu na preživetje in fiziološke biomarkerje pri različnih starostnih skupinah kranjske čebele (*Apis mellifera carnica*)

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Izvleček

Broflanilid je razmeroma nov insekticid s širokim spektrom delovanja, ki v EU sicer še ni dovoljen, zunaj EU pa se v kmetijstvu vse pogosteje uporablja za zatiranje škodljivih žuželk na različnih kulturah. Deluje kot alosterični antagonist ionotropnega receptorja za γ-aminomasleno kislino (GABA), s čimer zavira pomembno inhibitorno signalizacijo v živčnem sistemu žuželk. Ker je broflanilid izrazito lipofilen, se lahko kopiči v vosku in tako prispeva k dolgotrajni izpostavljenosti čebel. Kljub visoki akutni toksičnosti za medonosne čebele, zaradi katere bi lahko predstavljal tveganje za neciljne opraševalce, sta njegova kronična toksičnost in fiziološki učinki pri medonosni čebeli še vedno slabo raziskani.

V raziskavi v okviru doktorskega dela smo preučevali vpliv kronične oralne izpostavljenosti broflanilidu na preživetje in izbrane fiziološke biomarkerje pri medonosni čebeli. Poskuse smo izvedli na mladih, tj. dva dni starih čebelah delavkah ter na panjskih in pašnih čebelah, s čimer smo želeli preveriti, ali se občutljivost na broflanilid razlikuje med čebelami različnih starosti. Poleg smrtnosti smo spremljali tudi aktivnost acetilholinesteraze, ki je povezana z delovanjem živčnega sistema, in glutation S-transferaze, ki sodeluje pri razstrupljanju ter odzivu na oksidativni stres. Pri mladih čebelah smo dodatno spremljali porabo kisika posameznega osebka in aktivnost citokrom c oksidaze v možganih kot pokazatelja presnovne aktivnosti.

Rezultati kažejo, da je kronična izpostavljenost broflanilidu negativno vplivala na preživetje čebel, pri čemer se je občutljivost razlikovala med starostnimi skupinami. Dva dni stare čebele so se na izpostavljenost odzvale z jasnim koncentracijsko odvisnim vzorcem, medtem ko so bili odzivi starejših, predvsem panjskih in pašnih čebel, bolj variabilni in odzivi niso vselej sledili naraščajočim koncentracijam. Pri pašnih čebelah je bila smrtnost relativno visoka v primerjavi z dva dni starimi čebelam tudi pri nižjih koncentracijah pesticida, vendar zaradi večje variabilnosti in povišane smrtnosti v kontrolnih skupinah odziva ni bilo mogoče enako jasno povezati s koncentracijo. Ti rezultati kažejo na starostno odvisne razlike v občutljivosti na broflanilid. Kljub učinkom na preživetje nismo zaznali značilnih razlik v aktivnostih acetilholinesteraze in glutation S-transferaze. Pri porabi kisika smo zaznali vpliv mase čebel, ki je bila odvisna od koncentracije pesticida. Aktivnost citokrom c oksidaze je bila po izpostavljenosti broflanilidu nekoliko povišana v primerjavi s kontrolo.

Ključne besede: kranjska čebela, broflanilid, starost, preživetje, toksikologija, živčni sistem

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Effects of Chronic Broflanilide Exposure on Survival and Physiological Biomarkers in Different Age Groups of the Carniolan Honey Bee (*Apis mellifera carnica*)

Abstract

Broflanilide is a relatively new broad-spectrum insecticide that is not currently authorised in the EU but is increasingly used in agriculture outside the EU to control insect pests on a variety of crops. It acts as an allosteric antagonist of the ionotropic γ-aminobutyric acid (GABA) receptor, thereby inhibiting an important inhibitory signalling pathway in the insect nervous system. Because broflanilide is highly lipophilic, it may accumulate in wax and thus contribute to prolonged exposure of bees. Despite its high acute toxicity to honey bees, which suggests that it may pose a risk to non-target pollinators, its chronic toxicity and physiological effects in honey bees remain poorly understood.

As part of this doctoral research, we investigated the effects of chronic oral exposure to broflanilide on survival and selected physiological biomarkers in honey bees. Experiments were conducted on young, two-day-old worker bees, as well as on in-hive workers and foragers, to determine whether sensitivity to broflanilide differs among age groups. In addition to mortality, we measured the activity of acetylcholinesterase, which is associated with nervous-system function, and glutathione S-transferase, which is involved in detoxification and the response to oxidative stress. In young bees, we also measured individual oxygen consumption and cytochrome c oxidase activity in the brain as indicators of metabolic activity.

The results indicate that chronic exposure to broflanilide adversely affected bee survival, with sensitivity differing among age groups. Two-day-old bees exhibited a clear concentration-dependent response, whereas responses in older bees, particularly hive bees and foragers, were more variable and did not consistently increase with concentration. Mortality in foragers was relatively high compared with two-day-old bees, even at lower pesticide concentrations. However, because of greater variability and elevated mortality in the control groups, the response could not be linked to concentration as clearly. These findings indicate age-dependent differences in sensitivity to broflanilide. Despite the effects on survival, we detected no significant differences in acetylcholinesterase or glutathione S-transferase activity. Oxygen consumption was influenced by bee body mass, which varied with pesticide concentration. Cytochrome c oxidase activity was slightly higher following broflanilide exposure than in the control group.

Keywords: Carniolan honey bee, broflanilide, age, survival, toxicology, nervous system



Praćenje pojavnosti stršljenova štetnika u pčelarstvu: *Vespa velutina* i *Vespa orientalis* u Istarskoj, Zagrebačkoj i Krapinsko-zagorskoj županiji

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Sažetak

Monitoring invazivnih vrsta stršljena predstavlja važan dio zaštite pčelarstva zbog njihove sposobnosti uzrokovanja značajnih gospodarskih gubitaka i narušavanja prirodnih ekosustava. Cilj ovog diplomskog rada bio je utvrditi prisutnost invazivnih vrsta *Vespa velutina* i *Vespa orientalis* te analizirati populacijsku dinamiku autohtonog europskog stršljena (*Vespa crabro*) na području Istarske, Zagrebačke i Krapinsko-zagorske županije. Istraživanje je provedeno od početka svibnja do sredine srpnja 2024. godine na ukupno dvadeset lokacija primjenom cilindričnih visećih klopki sa selektivnim tekućim šećernim mamcem i repelentom za pčele. Uzorkovanje je provedeno u deset uzastopnih termina, a svi prikupljeni primjerci morfološki su identificirani te opisno statistički obrađeni. Tijekom istraživanja nije potvrđena prisutnost invazivnih vrsta *V. velutina* niti *V. orientalis*. Svi ulovljeni primjerci pripadali su autohtonoj vrsti V. crabro. Uočena je izražena sezonska dinamika s niskom aktivnošću u proljetnom razdoblju te naglim porastom brojnosti krajem lipnja i tijekom prve polovice srpnja, što odgovara razvoju kolonija. Najveći broj jedinki zabilježen je u Istarskoj županiji, sa 476 stršljenova. Krapinsko-zagorska županija pokazala je stabilnu i kontinuiranu aktivnost vespida kroz cijelu sezonu sa 400 stršljenova dok je Zagrebačka županija pokazala najniži populacijski pritisak sa 245 stršljenova. Dobiveni rezultati potvrđuju izostanak invazivnih vrsta na istraživanom području tijekom razdoblja istraživanja te naglašavaju važnost kontinuiranog monitoringa radi njihova pravodobnog otkrivanja i provedbe mjera ranog suzbijanja.

Ključne riječi: *Vespa crabro, Vespa velutina, Vespa orientalis*, pčelarstvo, monitoring, invazivne vrste.

Monitoring the Occurrence of Hornet Pests in Beekeeping: *Vespa velutina* and *Vespa orientalis* in Istria, Zagreb, and Krapina-Zagorje Counties

Abstract

Monitoring invasive hornet species is an essential component of beekeeping protection due to their potential to cause significant economic losses and disrupt native ecosystems. This diploma thesis aimed to determine the presence of the invasive species *Vespa velutina* and *Vespa orientalis* and to analyse the population dynamics of the native European hornet (*Vespa crabro*) in the counties of Istria, Zagreb, and Krapina-Zagorje. The study was conducted from early May to mid-July 2024 at twenty sampling sites using commercially available cylindrical hanging traps baited with a selective liquid sugar attractant containing a honeybee repellent.

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Sampling was performed on ten consecutive occasions, and all collected specimens were morphologically identified and analysed using descriptive statistics. No specimens of the invasive species V. velutina or V. orientalis were detected during the study. All captured hornets were identified as the native European hornet (V. crabro). A clear seasonal pattern was observed, with low activity during spring followed by a marked increase in abundance in late June and the first half of July, corresponding to colony development. The highest number of hornets was recorded in Istria County, with 476 hornets. Krapina-Zagorje County exhibited stable and continuous vespid activity throughout the entire season, with 400 hornets, whereas Zagreb County exhibited the lowest population pressure, with 245 hornets. These findings confirm the absence of invasive hornet species within the surveyed area during the study period and highlight the importance of continuous monitoring for the early detection and timely control of potential biological invasions.

Keywords: *Vespa crabro, Vespa velutina, Vespa orientalis*, **beekeeping, monitoring, invasive alien species.**



Biomonitoring teških metala i toksičnih elemenata u okolišu i pčelinjim zajednicama (*Apis mellifera* L.)

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Sažetak

Na području Republike Hrvatske tijekom 2023. i 2024. godine provedeno je znanstveno istraživanje koje je obuhvatilo terenska uzorkovanja i laboratorijske analize specifičnih pokazatelja onečišćenja. U pet različitih matriksa uzetih iz neživih i živih sastavnica pčelama i ljudima zajedničkog okoliša, određivani su teški i toksični metali i metaloidi. Utvrđivana je njihova koncentracija u vodi i tlu te u biljnom materijalu i pčelinjim zajednicama *Apis mellifera* L., odnosno u odraslim pčelama i medu. Medonosne pčele se uslijed svoje građe i načina života već desetljećima pokazuju kao organizmi vrlo osjetljivi na promjene u okolišu, a samim time i kao dobri bioindikatori, odnosno biomonitori prisutnosti određenih onečišćivala. Uzorci navedenih materijala uzimani su s 20 lokacija u tri regije RH, u kontinentalnom nizinskom dijelu, u gorskom te primorskom dijelu. Lokacije su bile grupirane kao one vezane uz intenzivnu poljoprivrednu proizvodnju, zatim uz urbana i prometna područja, industrijska područja te ona šumska s jednom otočnom lokacijom. Određivani su sljedeći teški metali i toksični elementi: As, Cd, Co, Cr, Cu, Hg, Fe, Mn, Ni, Pb, Se i Zn. Navedeni elementi analizirani su atomskom apsorpcijskom spektrometrijom (AAS) i induktivno spregnutom plazmom s masenom spektrometrijom (ICP-MS). Dobiveni rezultati uspoređeni su s važećim propisima o kvaliteti vode, tla i meda. Antropogeni pritisak na okoliš i medonosne pčele kvantificiran je kroz faktore bioakumulacije, indekse kvalitete i ukupni pokazatelj antropogenog utjecaja. Rezultati su pružili jedinstveni uvid u stanje okoliša na istraživanim lokacijama, povezujući različite dijelove okoliša putem konkretnih onečišćivala opasnih za zdravlje biljaka, pčela i ljudi ukazujući na štetne učinke antropogenih aktivnosti posebice vezanih za intenzivnu poljoprivredu i industriju.

Ključne riječi: *Apis mellifera* L., biomonitoring okoliša, teški metali i toksični elementi, voda, tlo, medonosne biljke, med

Biomonitoring of heavy metals and toxic elements in the environment and honey bee colonies (Apis mellifera L.*)*

Abstract

A scientific study was conducted in the Republic of Croatia during 2023 and 2024, which included field sampling and laboratory analysis of specific contamination indicators. Heavy and toxic metals and metalloids were determined in five different matrices from non-living and living components of the environment shared by bees and humans. Their concentrations were determined in water and soil, as well as in plant material and bee colonies of *Apis mellifera* L., i.e., in adult bees and honey. Due to their anatomy and way of life, honey bees have been proven for decades to be very sensitive to changes in the environment, and therefore good

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bioindicators, or biomonitors, of certain contaminants. Samples of the mentioned materials were taken from 20 locations in three regions of the Republic of Croatia, in the continental lowland part, in the mountain and coastal part. The locations were grouped as those related to intensive agricultural production, urban and traffic areas, industrial areas and forest areas with one island location. The following heavy metals and toxic elements were determined: As, Cd, Co, Cr, Cu, Hg, Fe, Mn, Ni, Pb, Se and Zn. The mentioned elements were analyzed by atomic absorption spectroscopy (AAS) and inductively coupled plasma with mass spectrometry (ICP-MS). The obtained results were compared with the current regulations on the quality of water, soil and honey. Anthropogenic pressure on the environment and honey bees was quantified through bioaccumulation factors, quality indices and a total indicator of anthropogenic impact. The results provided a unique insight into the state of the environment at the researched locations, linking different parts of the environment through specific contaminants hazardous to the health of plants, bees and humans, indicating the harmful effects of anthropogenic activities, especially related to intensive agriculture and industry.

Keywords: *Apis mellifera* L., environmental biomonitoring, heavy metals and toxic elements, water, soil, melliferous plants, honey



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