OP-120 [Bee Health]
The effect of oxalic acid on honey bee (Apis mellifera) physiology

Eliška Pinďáková Beranová[1], Marek Petřivalský[1], Jaroslava Friedecká[2], Rene Lenobel[2], Per Kryger[3], Jiří Danihlík[1]
[1]Department of Biochemistry, Faculty of Science, Palacký University in Olomouc, Olomouc, Czech Republic
[2]Laboratory of Growth Regulators, Faculty of Science, Palacký University in Olomouc, Olomouc, Czech Republic
[3]Department of Agroecology, Aarhus University, Slagelse, Denmark

One of the main stressors to honey bee health worldwide is the Varroa destructor mite. The mites transmit viruses that can be lethal to honey bee colonies. Oxalic acid (OA) is a natural acaricide that has become widespread among beekeepers as an alternative to synthetic pesticides. However, oxalic acid with glycerol in strips also influences honey bee health. The strips activate the immune system and can be associated with high energy demands and stress, making bees more vulnerable to infections. Recent reports indicate that increased chalkbrood incidence may potentially be related to increased colony stress. Co-infection with two pathogens can increase each pathogen's virulence, increasing the risk of the host's defense being overcome.

Our research aimed to determine the effect of oxalic acid on honey bee brood with concomitant chalkbrood disease. Methods of molecular biology and proteomics were used to study protein components involved in the immune responses of honey bee brood. We used peptide sequencing and data-independent acquisition (DIA) of molecular structure determination.

Data from proteomic analysis of larvae show significant differences in proteome composition between the treated and untreated brood, identifying 1187 proteins. Due to the increase in OA concentration, there is an increase in oxalate and glyoxalate cycle proteins. Conversely, antioxidative enzymes are utilized and the detoxification system is affected. OA-G strips also affected the relative gene expression of AmPs defensin and hymenoptaecin, which was significantly increased both in clinically healthy larvae and those infected with A. apis. On the other hand, abaecin levels were decreased after OA treatment.

Our results indicate the exposure of bee larvae to the commonly used acaricide oxalic acid in concentrations used by some beekeepers can strongly affect the levels of peptides and proteins involved in immune responses. Beekeepers need to consider the possible negative implications on honey bee immunity when picking their preferred Varroa treatment and be aware of the total dosage and the timing of the treatment. This work was supported by NAZV, Czech Republic, project QL24010241.

The effect of oxalic acid on honey bee brood physiology

Eliška Beranová

Palacký University in Olomouc
Department of Biochemistry
eliska.beranova@upol.cz

Synthetic or natural acaricides?

Amitraz & flumethrin & fluvalinate & coumaphos

formic acid & oxalic acid & thymol

Oxalic acid

- strips with glycerol (Maggi et al., 2016) - slowly and continuously released;
  the brood does not have to be removed before the treatment, high concentration of OA

Chalkbrood

- Chalkbrood is caused by the fungus *Ascosphaera apis*.
- This disease is not devastating to the colony, but the lack of healthy brood results in a reduced bee population and lower production.
- Clinical signs are mummified pupae of bees, visible on the bottoms of hives and in combs.

Physical barrier

- Cuticle
- Midgut epithelium

Hummoral immunity

- Antimicrobial peptides
- Phenoloxidase cascade

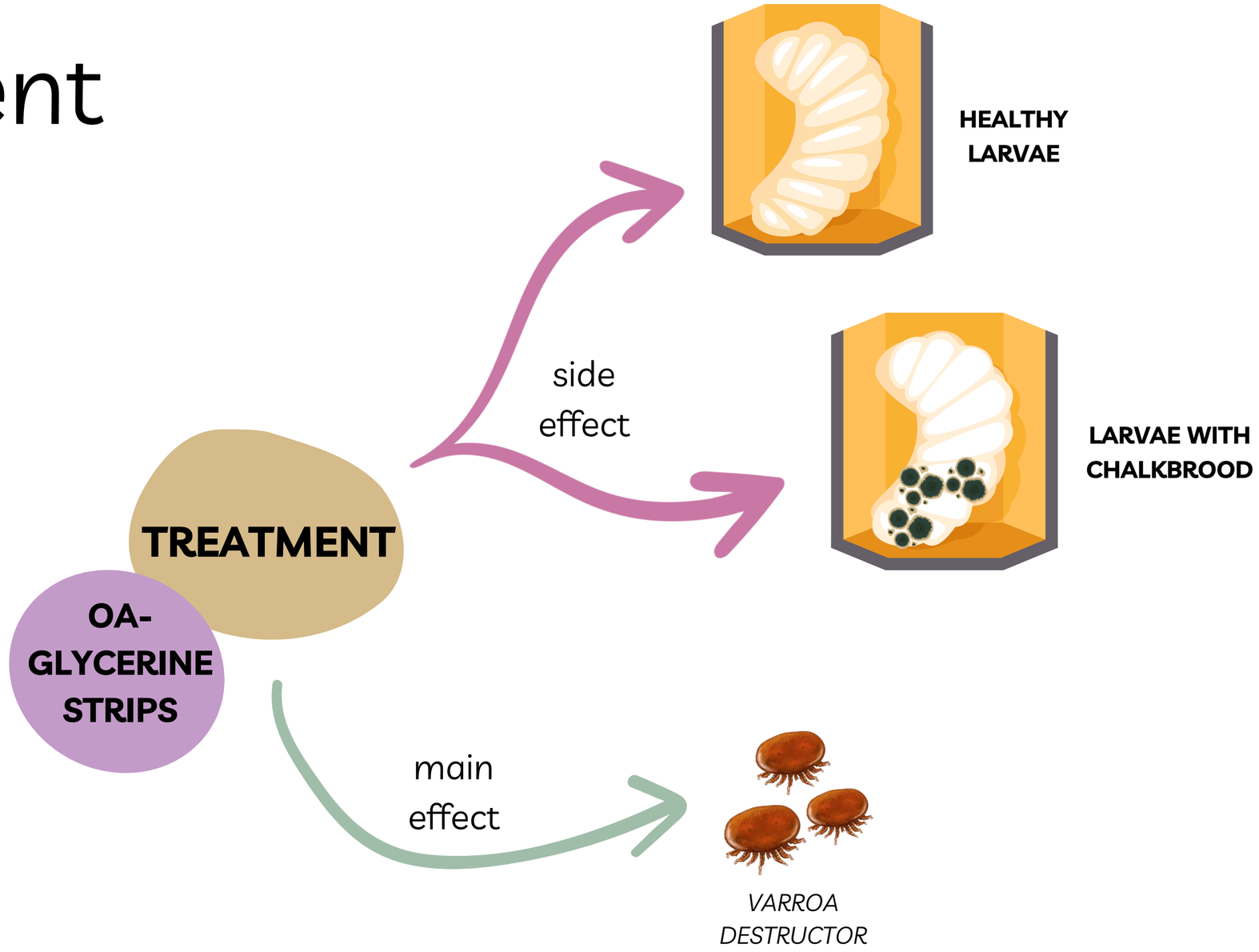
Cellular immunity

- Hemocytes
- Phagocytosis
- Encapsulation
- Nodulation

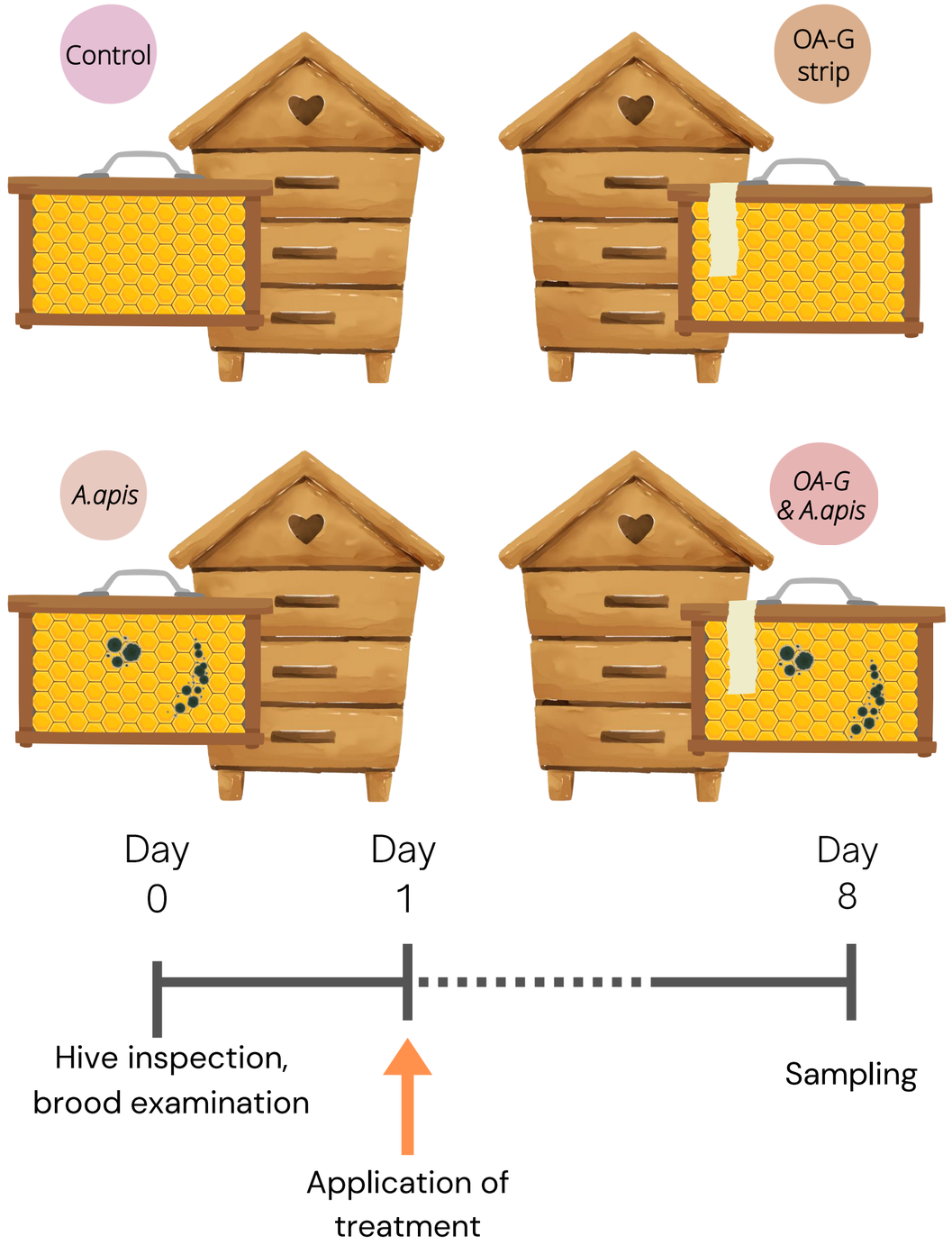
Social immunity

- Hygienic behavior
- Grooming

Experiment

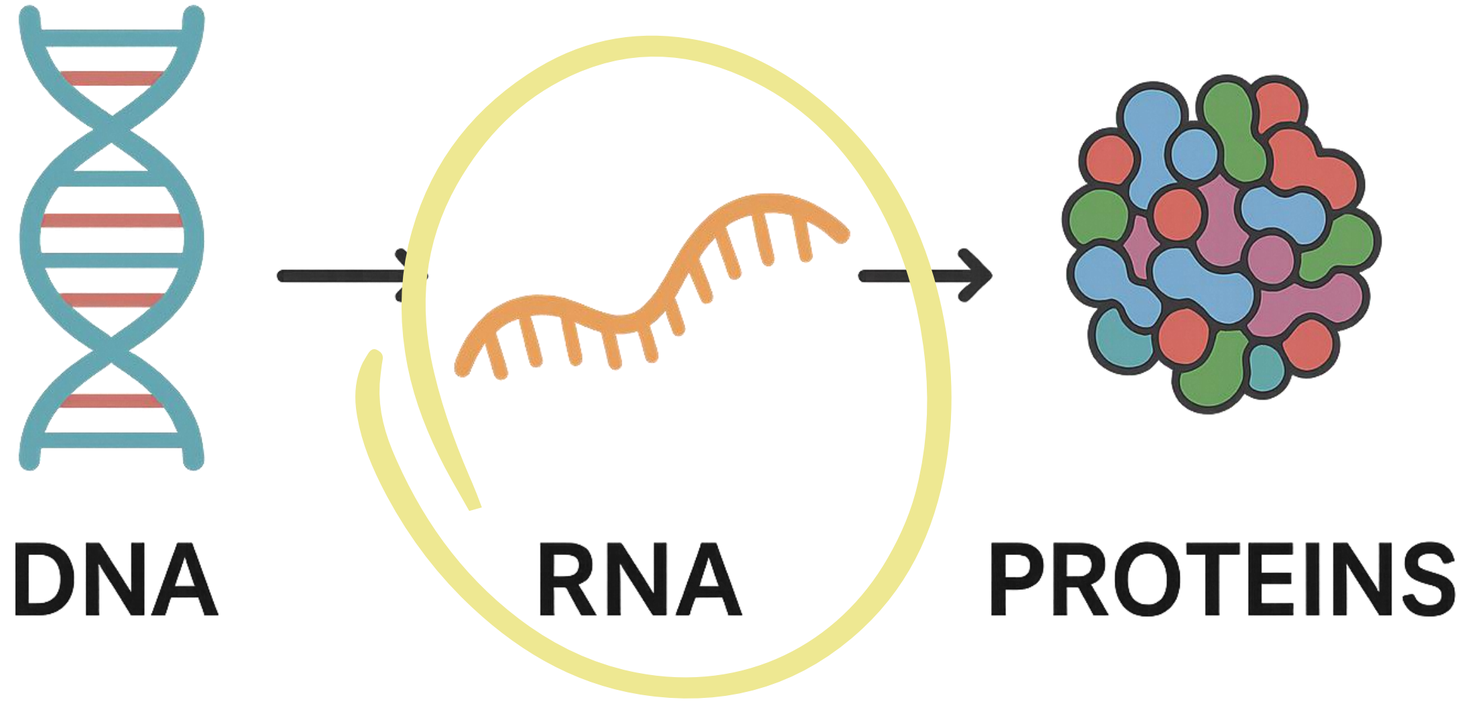

HEALTHY LARVAE
side effect
LARVAE WITH CHALKBROOD
TREATMENT
OA-GLYCERINE STRIPS
main effect
VARROA DESTRUCTOR

Experiment set-up



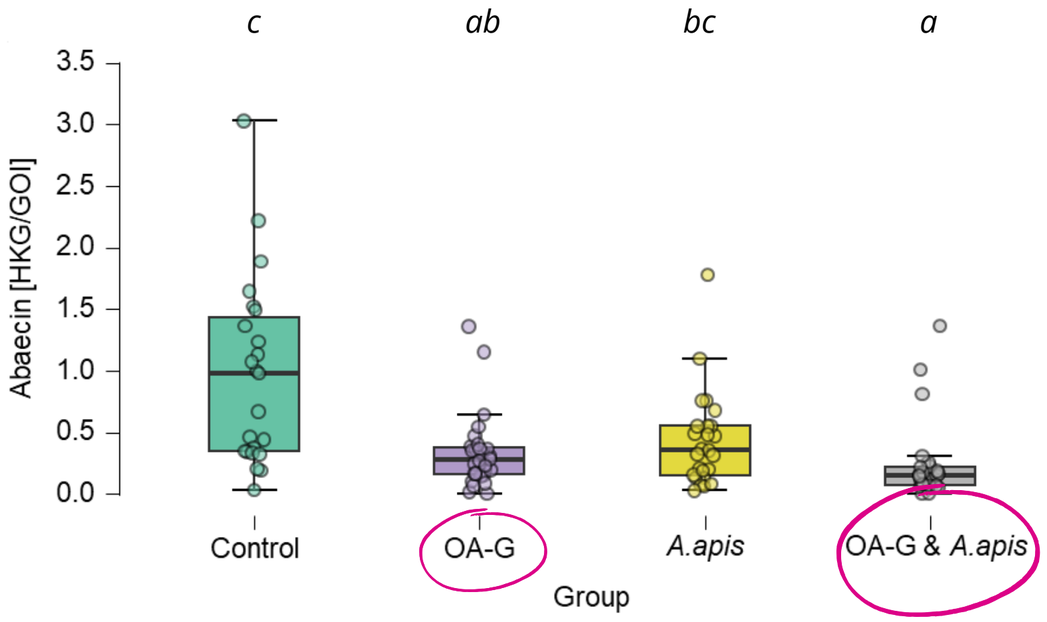
Gene expression

Gene expression means that a gene is “switched on” and the cell produces the protein it needs according to that gene.



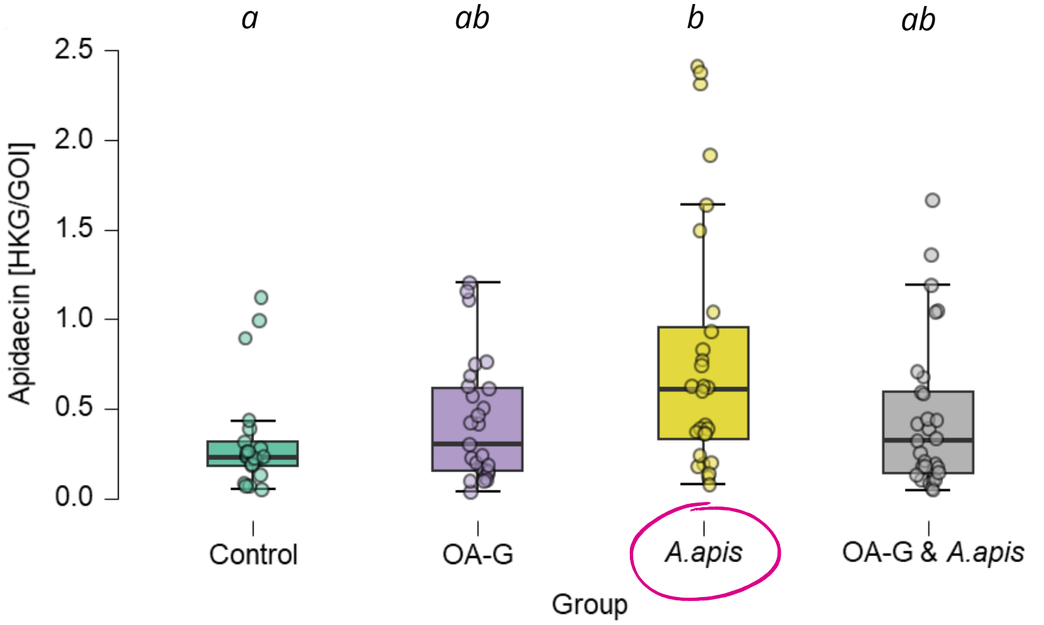
RESULTS

Relative gene expression of abaecin



- Relative gene expression of abaecin decreased after OA-G treatment in both bees from healthy colonies and bees from diseased colonies.
- The OA-G and OA-G & *A.apis* groups thus differed significantly from the control group ($p<0.001$).

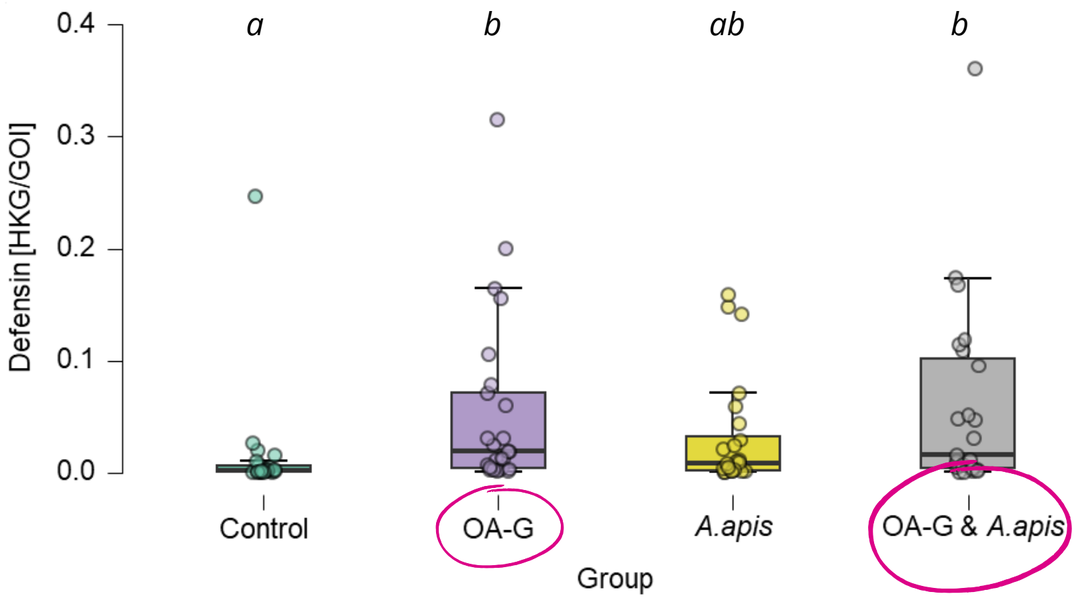
Relative gene expression of apidaecin



- In apidaecin, gene expression increased only in the *A. Apis* group.
- The *A. Apis* group thus differs significantly from the control group ($p<0.001$).

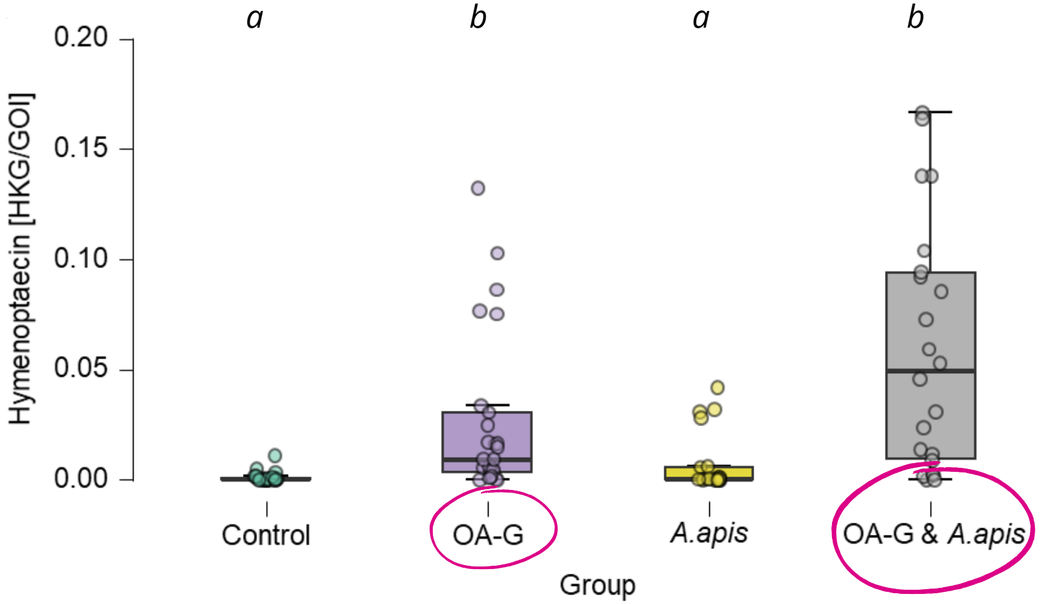
RESULTS

Relative gene expression of defensin



- Relative gene expression of AmP defensin increased after OA-G treatment, both in bees from healthy colonies and those from diseased colonies.
- Thus, the OA-G and OA-G & *A.apis* groups were significantly different from the control group ($p<0.001$).

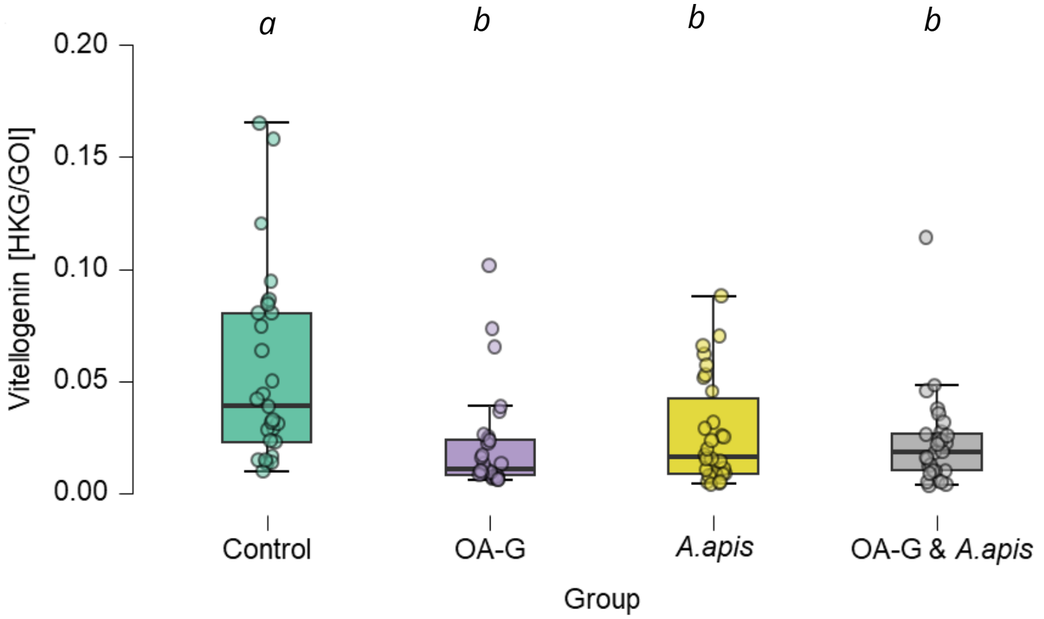
Relative gene expression of hymenoptaecin



- The same pattern can be observed for relative gene expression of hymenoptaecin.
- The OA-G and OA-G & *A.apis* groups are significantly different from the control and *A.apis* group ($p=0.003$, $p=0.004$, respectively).

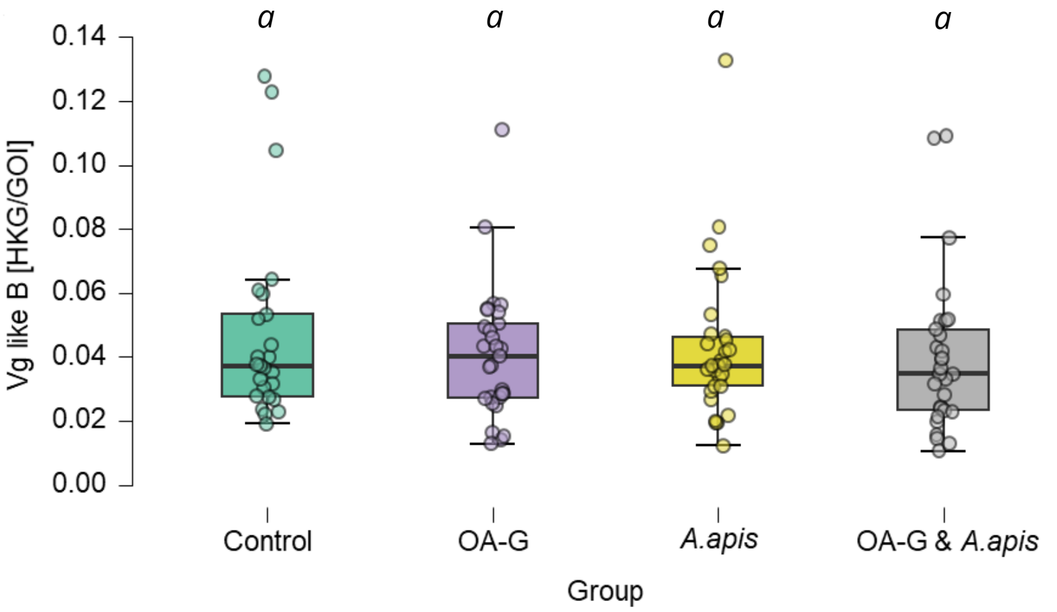
RESULTS

Relative gene expression of vitellogenin



- For vitellogenin, a decrease in expression was observed after OA-G treatment, both in healthy bees (OA-G group) and in diseased bees (OA-G & *A.apis* group).
- Fungal infection alone caused a decrease in relative vitellogenin gene expression in the *A.apis* group.

Relative gene expression of Vg-like B



- The gene expression of vg-like B was not affected by either disease or oxalic acid treatment
- Compared to the control, there was no significant change in gene expression in either group.

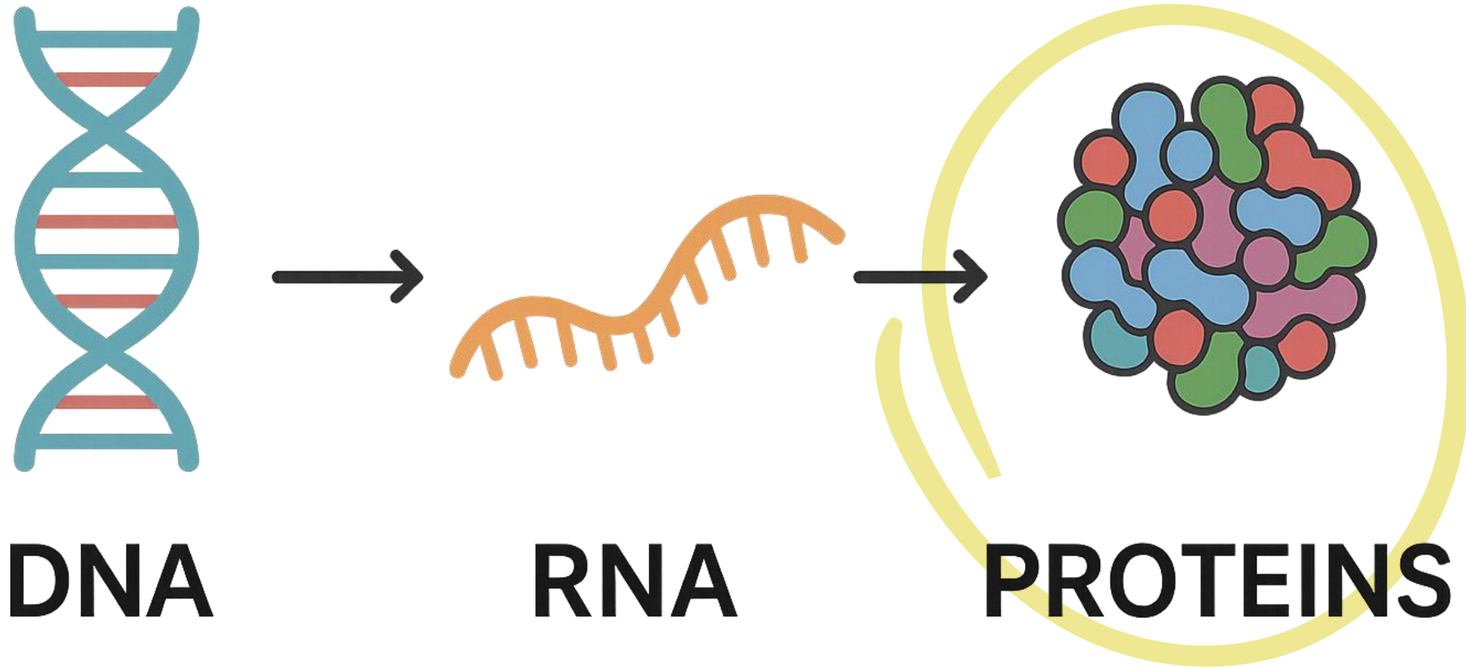
Summary

01. OA-G strips showed an effect on honey bee larvae where the relative gene expression of AmPs significantly changed in clinically healthy larvae and those with *A.apis*.

02. Expression of vitellogenin was consistently downregulated, whereas Vg-like B expression remained unchanged, indicating potential functional divergence and context-specific expression.

Proteomics

Proteomics reveals what is actually produced – the functional proteins that determine the state of the organism.

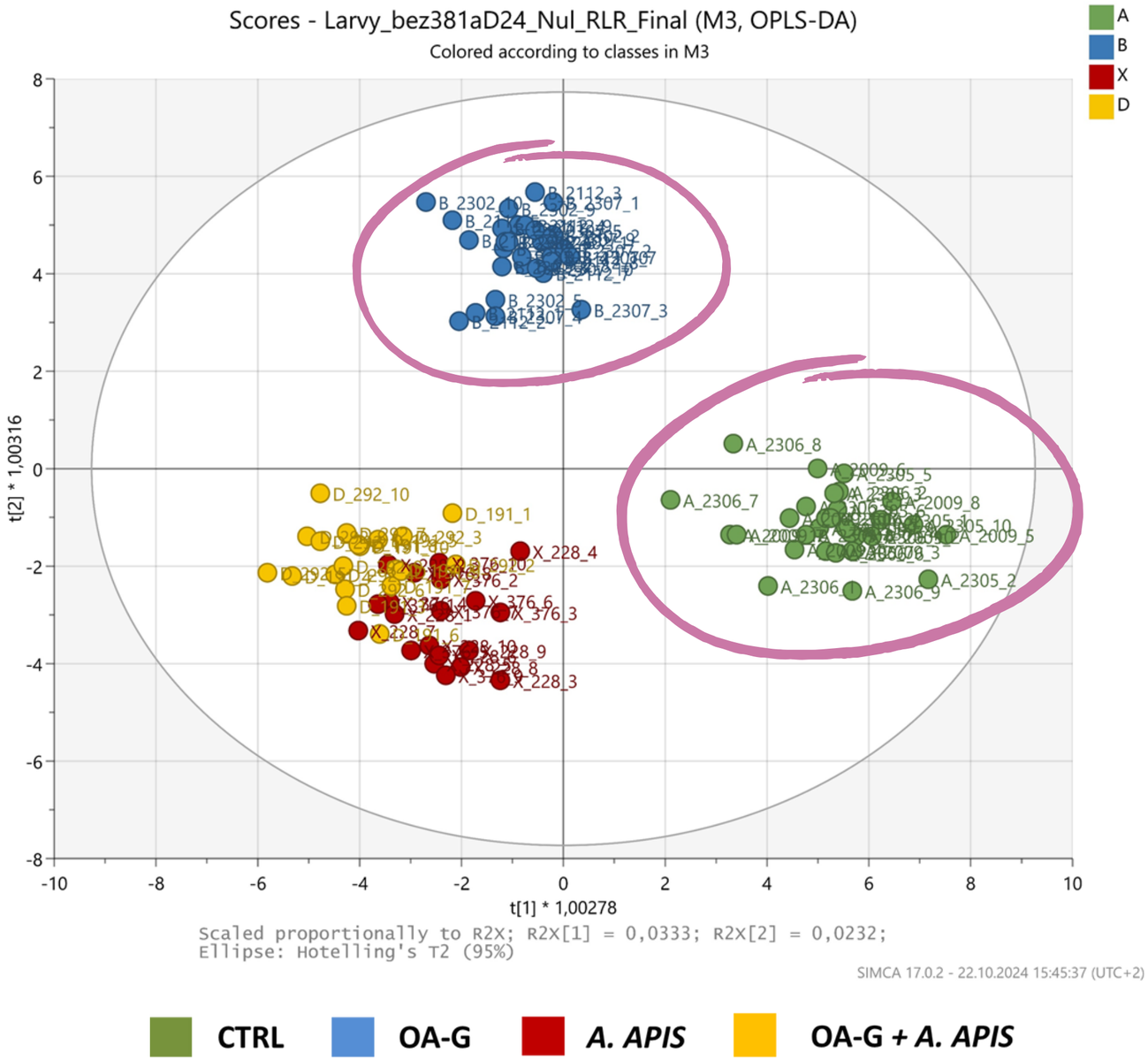


Results

OLPS-DA - supervised analysis

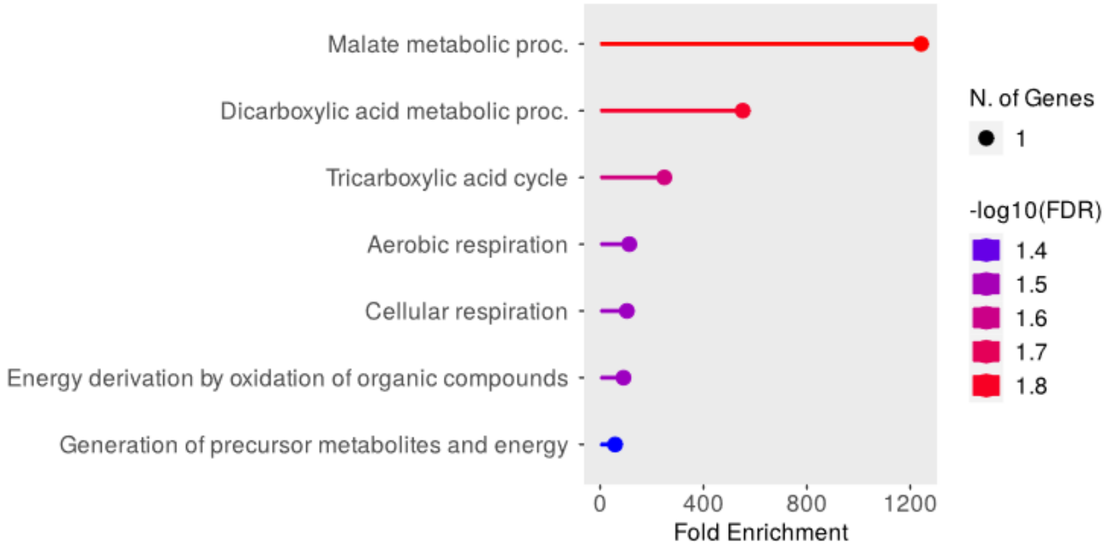
n = 30

- The distribution of proteins in the OA-G group differs from that in the control group.
- More than 1,187 proteins were identified.



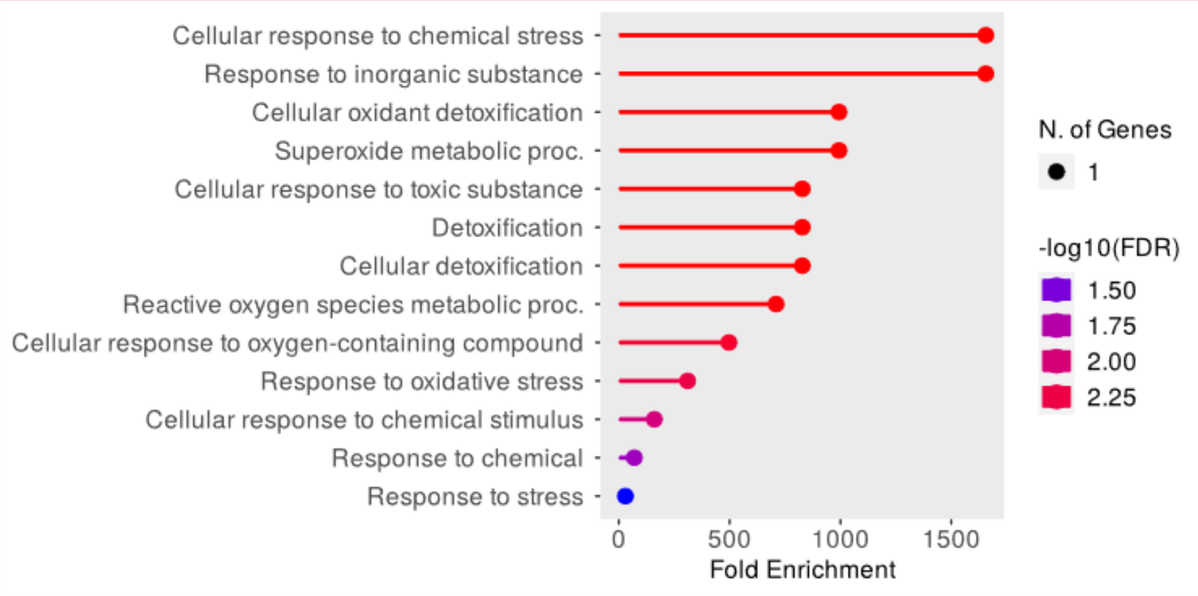
CTRL OA-G *A. APIS* OA-G + *A. APIS*

Higher abundance of proteins in the OA-G group compared to the control group



- As a result of increased OA concentration, there is an increase in the content of oxalate and glyoxalate cycle proteins.

Lower protein representation in the OA-G group compared to the control group



- On the other hand, antioxidant enzymes are utilized and the detoxification system is affected.

Key tasks for future research

- Which metabolic pathways are influenced by OA?

- Spread of OA across the colony?
  How does the brood come into contact with OA?

Take home message

Stress Oxalic acid (OA-G strips), though widely used, does not only target mites — it also affects bee larval immunity and metabolism.

Immunity OA exposure increases immune activation but also raises stress and energy demands, which may make colonies more vulnerable to diseases like chalkbrood.

Risk Proteomics revealed strong shifts in protein expression, including immune peptides and detoxification pathways.

Balance Beekeepers should carefully consider OA dosage and timing, as treatments can have unintended side effects on brood health.

Palacký University Olomouc

DEPARTMENT OF BIOCHEMISTRY

Endowment Fund of Palacký University

Eliška Beranová (Pinďáková)

Palacký University in Olomouc
Department of Biochemistry
eliska.beranova@upol.cz

Thank you for your attention.

AARHUS UNIVERSITY

Per Kryger, Ph.D.

René Lenobel, Ph.D.

Jaroslava Friedecká, Ph.D.

This work was supported by The Ministry of Agriculture of the Czech Republic (QK1910286 and QL24010241) and the Endowment Fund of Palacký University.