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1.13 Using respiratory physiology techniques in assessments of pesticide effects on bees

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Abstract

The determination of sub-lethal effects of pesticides on beneficial insects is challenging topic because the vast number of different possible endpoints. Traditionally measured endpoints reflect the basic outcome but do not give any information about the mode of actions or the real non-harming dosages of the studied toxicants. Physiological changes, however, reflect even small deviations from normal state. The gas exchange patterns are sensitive cues to determine the sub-lethal toxicosis in insects. Methods of respiratory physiology have been used to detect sub-lethal toxic effects of many chemicals, but information for biological preparations is also needed, especially when bees are used in entomovectoring task.

The aims of this study were i) to clarify which are the effects of three microbiological preparations on two bee species, honey bees *Apis mellifera* L. and bumble bees *Bombus terrestris* L. and ii) could we compare the effects of the same preparations on different bee species. We saw that honey bees and bumble bees react similarly on microbiological preparations, however the reaction strength differed. We found that kaolin affects the survival of bumble bees and honey bees as much as did entomopathogenic preparations, whereas pure spores of a non-hazardous fungus and wheat flour did not. Bumble bees seem to be more tolerant to microbiological preparations than honey bees.

Keywords: measuring sub-lethal effect, honey bee, bumble bee, microbiological preparation

Introduction

Pesticide residues in environment are told to be among the reasons contributing to decreasing pollinator populations.[1] Establishment of lethal dosages or concentrations to both target and non-target organisms is demanded by legislation process of pesticides, but sublethal effects have gained much less attention. However, the sub-lethal effects of pesticides may affect insects

severely through chronic stress[2] or fostering the effects of other stress factors, ultimately leading to decreasing fitness of populations.[3]

Determination of such sub-lethal changes, which cannot be captured by a human eye, might give us knowledge to explain factors leading to bee declines for both domesticated and wild bees. We know much about the concentrations of residues in soils, plant tissues, nectar and pollen,[4] however we do not know how insects cope with the residues they are constantly in contact. Talking about non-harming dosages needs clarification of real versatile dosages of an active ingredient or a preparation. The behavioural changes might not reflect the effects[5] nor the border between real harming/non-harming level of toxicants due to the buffering capacity of the organisms or the bee colonies. Molecular and cellular methods typically require killing of the study-organism. Still, some physiological mechanisms allow working with living and intact insect. Among the latter, methods of respiratory physiology determine the rates of metabolic and water loss levels, muscle activity, heart pulsation and respiratory patterns, which easily react on any changes of stress factors.[6]

Respiratory measurements are highly sensitive and reflect any minor changes in environmental or organism functioning level. Metabolic rate that is calculated based on oxygen consumption or carbon dioxide release is most commonly measured parameter. Combining it with water loss rate and respiratory patterns gives understanding that is more detailed. Already in 1991, Kestler[7] has demonstrated the changes in respiratory patterns following to sub-lethal or lethal contact of an insecticide, which targets insect nervous system. He was first who described the respiratory pattern transitions due to poisoning and also determined the pattern, which indicates irreversible toxicosis.

Beside synthetic pesticides, also different biocontrol agents are used in plant production. These preparations also need detailed information about the modes of actions, lethal or sub-lethal dosages or harmful side-effects. More-over, when microbiological preparations are to be applied to crops using bees as vectors for preparations,[8-10] the safety of bees must be guaranteed. Both honey bees and bumblebees are used in bee-vectoring task, however the sublethal effects of preparations is not clear. The aims of this study were i) to clarify which are the effects of three microbiological preparations on two bee species, honey bees *Apis mellifera* L. and bumble bees *Bombus terrestris* L. and ii) could we compare the effects of the same preparations on different bee species.

Material and Methods

Bumble bees (2 hives) were purchased from Koppert Biological systems (Berkel en Rodenrijs, the Netherlands). Honey bees (one colony) were purchased from a local beekeeper. The exact age of the bees was unknown; however, we aimed to study only forager bees, bumble bees were captured from hive entrances and honey bees were caught with in insect net after when they were flying out for forage.

We used one biofungicide Prestop-Mix, which contains spores of *Gliocladium catenulatum* J1446 strain from Verdera (Espoo, Finland), and two bioinsecticides BotaniGard containing *Beauveria bassiana* GHA strain and Met52 *Metarhizium brunneum* Strain F52 (both from Borregaard BioPlant ApS, Aarhus, Denmark) in our experiments. In addition we tested the effects of pure *G. catenulatum* spores and some inert materials used as carrier compounds in preparations: kaolin ($[Al_2Si_2O_5(OH)_4]$, particle size: 3 microns, Bang to Bonsomer Estonia (Tallinn, Estonia) and wheat flower (Tartu Mill (Tartu, Estonia) since different corn flowers are also used as carrier materials.

Bees were treated individually with any of the powders with an amount that covered the bee with a thin powder layer by shaking them tenderly in a vial containing 20 mg for honey bees and 50 mg for bumble bees. Control bees were also treated similarly in an empty vial. All bees were kept individually in plastic vials (perforated walls to allow hearing and smelling of each-other) at a temperature of 28 ˚C and RH=60% in 12:12 light:darkness regime (SANYO - Versatile

Environmental Test Chamber, MLR-351, Japan). Each bee was provided 30% sugar solution as food.

The bee survival was monitored daily until all bees were dead. Metabolic rate (MR VCO_2, ml h^{-1}) and water loss rate (WLR VH_2O, µl h^{-1}) was measured by means of LI-7000 differential CO_2/H_2O analyser (LiCor, Lincoln, NE).[11] Each individual was measured 3 hours before and 3 hours after the treatment.

For statistical analyses of data Kruskal-Wallis ANOVA (survival data) and one-way or factorial ANOVA (MR and WLR data) (α=0.05) was used. In comparison of MR and WLR change in time (control groups only) paired t-test was performed.

Results

Bumble bees lived significantly longer than honey bees in such kind of experiment (KW-H(1;80)=44.9; p<0.001). In both groups the treatment affected the longevity of bees (bumble bees: KW-H(4;97)=16.2; p<0.01, honey bees: KW-H(6;480)=152.9; p<0.001). Control and wheat flour did not affect bee survival. Surprisingly, the biofungicide Prestop-Mix affected bee survival significantly in both bee species (see also Karise et al., 2016[11]), although pure *G. catenulatum* which was tested only on honey bees did not affect it. The kaolin caused as low survival as did bioinsecticides (Figure 1).

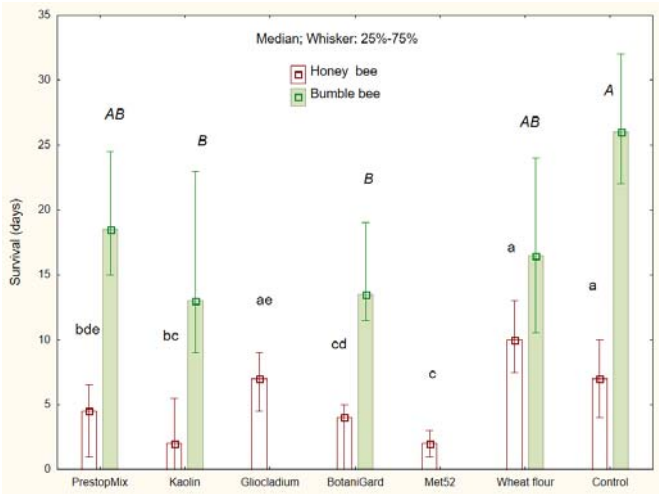

Figure 1 Mean survival of honey bees and bumble bees exposed to different biopesticides and inert materials. Letters indicate statistically significant (p<0.05) differences between treatments

Both metabolic rate and water loss rate in forced immobility are significantly lower in bumble bees compared to honey bees (MR: F(1;64)=3.9; p=0.05; WLR: F(1;64)=24.7; p<0.001). The MR of honey bees did not decrease in time (t=-0.37 df=3 p=0.74) as well did not change the WLR (t=0.68 df=3 p=0.55). In bumble bees, however the MR decreased significantly (t=7.18 df=5 p<0.001), whereas the WLR stayed unchanged (t=1.36 df=5 p=0.23) (see also Karise et al., 2016).

None of the biopreparations nor inert materials affected the metabolic rate of either of the species (F(4,42)=0.32, p=0.86), although the variation of the change rate was larger in honey bees compared to bumble bees (F(1,42)=7.39, p=0.009). There was no co-effect of species and treatment (F(4,42)=0.40, p=0.81).

Water loss rate, however, was significantly affected by treatment in both species (honey bee: F(6,29)=35.54; p<0.001; bumble bee: F(4,20)=6.75; p=0.001). We saw that kaolin and Prestop-Mix increased the water loss rate of either of bee species, BotaniGard increased it in honey bees, whereas powder of *G. catenulatum* spores, Met52 and wheat flour did not (Figure 2).

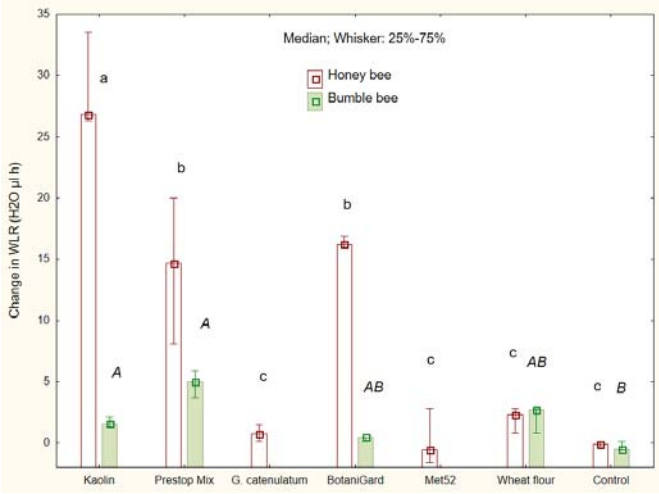

Figure 2 Mean change in water loss rate (WLR) after treatment with microbial biopesticides and inert powders. Letters indicate statistically significant ($p<0.05$) differences between treatments

Discussion

Measuring sub-lethal effects by means of respiratory physiology is effective and precise, however the technique has its limitations. The initial acquirement costs of the equipment would be high, however running the experiments would not cost much. Positive is that the technique allows to measure processes in a living intact organism and several characteristics in parallel, but demands individual measurements, which makes the process time-consuming.[6] In addition, the large variability of individuals makes detecting significant changes less achievable.

Honey bees and bumble bees are both social bee species, however their individual traits and species specific behaviour may differ largely. Bumble bees are considered as primitively eusocial, which differs by queen developmental pathway from advanced eusociality present in honey bees and ants.[12] We saw that bumble bees have lower metabolic rate than honey bees. This may be due to physiological properties or behavioural peculiarity. We saw, that bumble bees are able to calm down much faster. When forced to limited space, they stop struggling and eventually enter to deep resting state,[13,14] which is recognizable through presence of discontinuous gas exchange cycles in their respiratory patterns.[15,16] By honey bees we did not record discontinuous respiration cycles nor during 3h of pre-treatment period neither during the 3h course after the treatment. Treatment itself causes rapid increase of the activity level, which passes faster in bumble bees than in honey bees. We explain the difference in natural respiratory patterns and with the variable nature of bee species. Honey bee foragers are meant to fulfil the highly demanding foraging task for rapidly growing colonies, whereas for bumble bees this intrinsic pressure is lower. In addition, when it is too cold, honey bees use to cluster and heat themselves collectively,[17] when bumble bees are able to stay overnight alone out of hives.[18] Bumble bees´ ability to survive in unpleasant conditions is much better. This was seen also in our experiment. The measurements of MR in honey bees have shown, that in more favourable conditions they start respire discontinuously, too (unpublished observations of the authors). It is suggested that discontinuous respiration aids to diminish respiratory water loss.[15]

We saw variable effects of different microbial preparations on the studied bee species. Typically, honey bees´ reaction on treatments was stronger, however the trends were similar. Both entomopathogenic preparations affected honey bee and bumble bee survival. Biological fungicide Prestop-Mix, however affected significantly only honey bees and not bumble bees. The kaolin, an inert component of Prestop-Mix, affected significantly both bumble bee and honey bee

survival at the rate comparable with bioinsecticides. Kaolin and some other mineral powders are also used as insecticides against warehouse pest insects or to protect leaf and fruit surfaces from damages made by sucking insects.[19] We saw that the mineral powder may affect also bees, when they are delivering biological preparations to crops. Kaolin has been shown to change the lipid structure[20] on insect cuticle thus increasing the cuticular water permeability.[11] In our experiment the fine wheat flour did not affect the mortality, MR or WLR in either of bee species, which points out, that the mineral composition of kaolin rather affects insects than powder itself. The non-toxic microorganisms themselves do not affect the physiological processes of bees: no effect of pure *G. catenulatum* spores was detected on honey bee WLR, neither of Met52 which contains corn as carrier material. BotaniGard however contains mineral powder and affected honey bee WLR at the same rate than Prestop-Mix. The effect of treatments on bee WLR indicates that any preparation with corn as inert material is causing less stress to bees used in entomovectoring.

Conclusion

We saw that honey bees and bumble bees react similarly on microbiological preparations, however the reaction strength differed. Entomopathogenic preparations do affect the longevity of both bee species, in addition the inert powders also can do it. This should be taken into account when developing novel microbial preparations for entomovectoring systems. Comparison of these two bee species under stress from microbiological preparations revealed that bumble bees seem to suffer less. In addition, bumble bees suite better in analysing changes in respiratory patterns of bees.

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1.14 New working group – Testing side effects of microbials

Shannon Borges, Emily McVey, Jacoba Wassenberg

For the developments with this working group, see these proceedings:
Thomas Steeger - Working groups of the ICPPR Bee Protection Group – Developments and progress.

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Group photo of all symposium participants, standing in front, from left:
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Jens Pistorius (new chairman),
Françoise & Pieter Oomen with award (editor & former chairman),
Guy Smagghe (organiser, symposium host and new board member),
Job & Margreet van Praagh with award,
Anne Alix (secretary of the board)

Foto
Pieter A. Oomen (Bumble bee *Bombus lapidarius* on thistle)

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