

biological pesticides while they are foraging. Biological pesticides may contain biological active materials that could grow on or in the insect. Therefore possible adverse effects on beneficial pollinators must be evaluated as pollination must be guaranteed.

This study has examined the potential adverse effects of commercial biological pesticides that contain bacteria, fungi, yeasts and viruses on the bumblebee *Bombus terrestris*. Worker bees were exposed under laboratory conditions to the maximum field recommended concentration (MFRC) of each compound via three different routes of exposure: dermal contact and oral feeding via the consumption of treated sugar water and pollen. In general all tested MCAs were found safe for workers of *B. terrestris*, with the exception of Botanigard (*Beauveria bassiana* GHA) via dermal contact treatment that caused 90% worker mortality at its MFRC after 12 weeks. Even at half of the MFRC, 50% mortality was observed, but there was no mortality with a lower dose of 1/10 of the MFRC.

Apart of to acute toxicity also sublethal effects on nest reproduction were examined. Here none of the tested compounds did exert detrimental effects as the production of drones after 12 weeks appeared to be not significantly different from the control nests ( $39.5 \pm 6.7$ ) ( $P > 0.05$ ).

Overall, the results demonstrated that most of the biological pesticides tested can be considered as safe for *B. terrestris*, but some can be harmful. Therefore it is recommended that before any use in combination with pollinators all should be tested. In this context it is also advised that these compounds should be evaluated for potential effects on the foraging behavior in more field related tests.

### **Side effects of commercial *Bacillus thuringiensis* insecticides on micro-colonies of *Bombus terrestris***

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#### **Abstract**

*Bacillus thuringiensis* (*Bt*) is a natural soil bacterium that is used worldwide for the control of pest insects as its protein crystals possess insecticidal activity. Due to the intensive use of *Bt* in different crops like vegetables, ornamentals, flowers and fruiting plants, the question has raised whether *Bt* is safe for non-target organisms. Nowadays cultivators are using beside honeybees also bumblebees for the pollination of their crops such as tomatoes.

In this study the risk of two different strains of commercial *Bt* insecticides, *B. thuringiensis kurstaki* (Dipel® WG) and *B. thuringiensis aizawai* (Xentari® WG) on the biology of the bumblebee *Bombus terrestris* was assessed. In order to evaluate potential lethal and sublethal effects on the reproduction, micro-colonies of worker bumblebees were exposed to 0.1% of each compound, representing the maximum field recommended concentration (MFRC), and this via three different routes of exposure: dermal contact and oral feeding via treated sugar water and treated pollen.

For both *Bt* compounds no loss of survival was scored after dermal contact treatment. Via treated sugar water, Xentari® at 0.1% killed all worker bumblebees, but with a lower dose of 0.01% (1/10 of the MFRC) mortality was zero. With Dipel® at 0.1% in the sugar water and in the pollen, no mortality was scored.

Next to lethal effects, also sublethal effects were evaluated. In the nests exposed to Xentari® at 0.1% via the pollen a significantly lower number of drones was produced ( $P < 0.05$ ). However, no detrimental effects were seen with a lower dose of 0.01% ( $P > 0.05$ ). For the treatments with Dipel®, the reproduction in the micro-colonies was normal ( $37.6 \pm 5.5$  drones per nest) as in the controls ( $39.5 \pm 6.7$  drones per nest).

Then in a next step in our risk assessment study on side effects we evaluated the impact of sublethal concentrations of Xentari® (0.01% via the sugar water and the pollen) on the foraging behavior of bumblebees with a new experimental setup in the laboratory. Here no change in the behavior of the workers was seen.

Overall the results showed that the tested Bt insecticides cause an effect on the biology of *B. terrestris*. However, more information about relevant environmental concentrations is necessary before making final conclusions about the compatibility of these compounds with *B. terrestris*.

## Can pesticide acute toxicity for bumblebees be derived from honeybee LD50 values?

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### Abstract

Pesticide acute toxicity towards animals is commonly assessed using lethal doses (LD<sub>50</sub>). The LD<sub>50</sub> can be generated with two routes of exposure: when animals ingest the pesticide (oral LD<sub>50</sub>) or when it is in contact with it (contact LD<sub>50</sub>). Toxicity values for honeybees are usually used in ecotoxicological risk assessment inferring that honeybees represent the pollinating insects. LD<sub>50</sub> values are also measured for bumble bees but to a lesser extend.

The first step of this exercise was to collect known LD<sub>50</sub> (contact and oral) values measured for both honey bees and bumble bees.

Based on the LD<sub>50</sub> values of 20 pesticides, the relationship between oral LD<sub>50</sub> values of honey bees and bumble bees was calculated with the regression formula. The same calculation was done with contact LD<sub>50</sub>. Results showed that there was an approximate relationship; toxic active ingredients for honey bees were also toxic for bumble bees. However, when honey bee LD<sub>50</sub> values in the toxic range (LD<sub>50</sub> < 1 µg/bee) and less toxic range (LD<sub>50</sub> > 1 µg/bee), were compared to bumble bee LD<sub>50</sub>, the relationship was very much less statistically significant. This both counted for the oral and contact LD<sub>50</sub> values. It is concluded that the known LD<sub>50</sub> values of honey bees could indicate broadly a range of LD<sub>50</sub> values for bumble bees. However, for toxic and less toxic substances, the LD<sub>50</sub> for bumble bees cannot be derived from known honey bee LD<sub>50</sub> values. It must be noticed furthermore that the LD<sub>50</sub> values for honey bees, presented in literature and databases of universities and legislation offices vary significantly.

## IV. Test methodology (laboratory, cage, field, sub-lethal, etc.)

### Influence of the brood rearing temperature on honey bee development and susceptibility to intoxication by pesticides

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### Abstract

The brood rearing temperature is one of the most precisely controlled physiological parameters in a honey bee colony. Adult bees keep the brood area centre at 35 ± 1°C. In order to maintain the temperature within this narrow range, the high or low external temperature is contrasted by thermoregulation behaviours. Thus, normally only slight deviations from the optimal level may occur. Nevertheless, in particular situations the brood may be subject to conditions of suboptimal temperature. For example, a slight bee poisoning, causing