Inconsistent negative impacts of azoxystrobin-based fungicide on bumblebee colonies in a dose-response experiment mimicking fungicide decline

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Highlights

- *Bombus terrestris* colonies were fed different concentrations of the azoxystrobin fungicide Ortiva
- Fungicide degradation was mimicked and multiple factors applied to create a realistic dose-response experiment
- Negative impacts on colony growth were only found for 4-times the field-typical concentration, but not for lower or higher concentrations
- Inconsistent impacts in this study and the literature might suggest that at very high concentration faster degradation is triggered and that the exposure route matters

Introduction

Fungicides are often applied to flowering crops despite their potential to negatively affect bees. Azoxystrobin is a systemic fungicide that is applied to a wide variety of crops globally[1,2]. Recent studies suggest that azoxystrobin can harm bees at field-realistic concentrations but effects were inconsistent between bee species, crops and studies[3–7]. Contrary results may in part be due to subtle differences in exposure levels.

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Methods

To assess the potential impacts of the azoxystrobin-based fungicide Ortiva on the colony development of bumblebees (*Bombus terrestris*), we conducted a **highly replicated dose-response experiment** with a unique study design. Bumblebee colonies with the possibility to forage freely were fed with syrup spiked with different concentrations of Ortiva for 10 days. **To mimic pesticide degradation, the concentrations declined in the first 4 days and then a constant dose was used.** One set of colonies received a field-typical sequence of doses (1170 ppb, 656 ppb, 70 ppb, 16 ppb on days 0-3, respectively and 5 ppb on days 4-9) and additional sets of colonies received either a multiple or a fraction of these concentrations (concentration factors: 0, 0.5, 1, 2, 4, 8; Fig. 1). Per concentration factor, seven commercial B. *terrestris* colonies were used (i.e., 42 in total).

Figure 1 | Fungicide exposure.
Syrup spiked with Ortiva (except control) placed inside nest and often exchanged

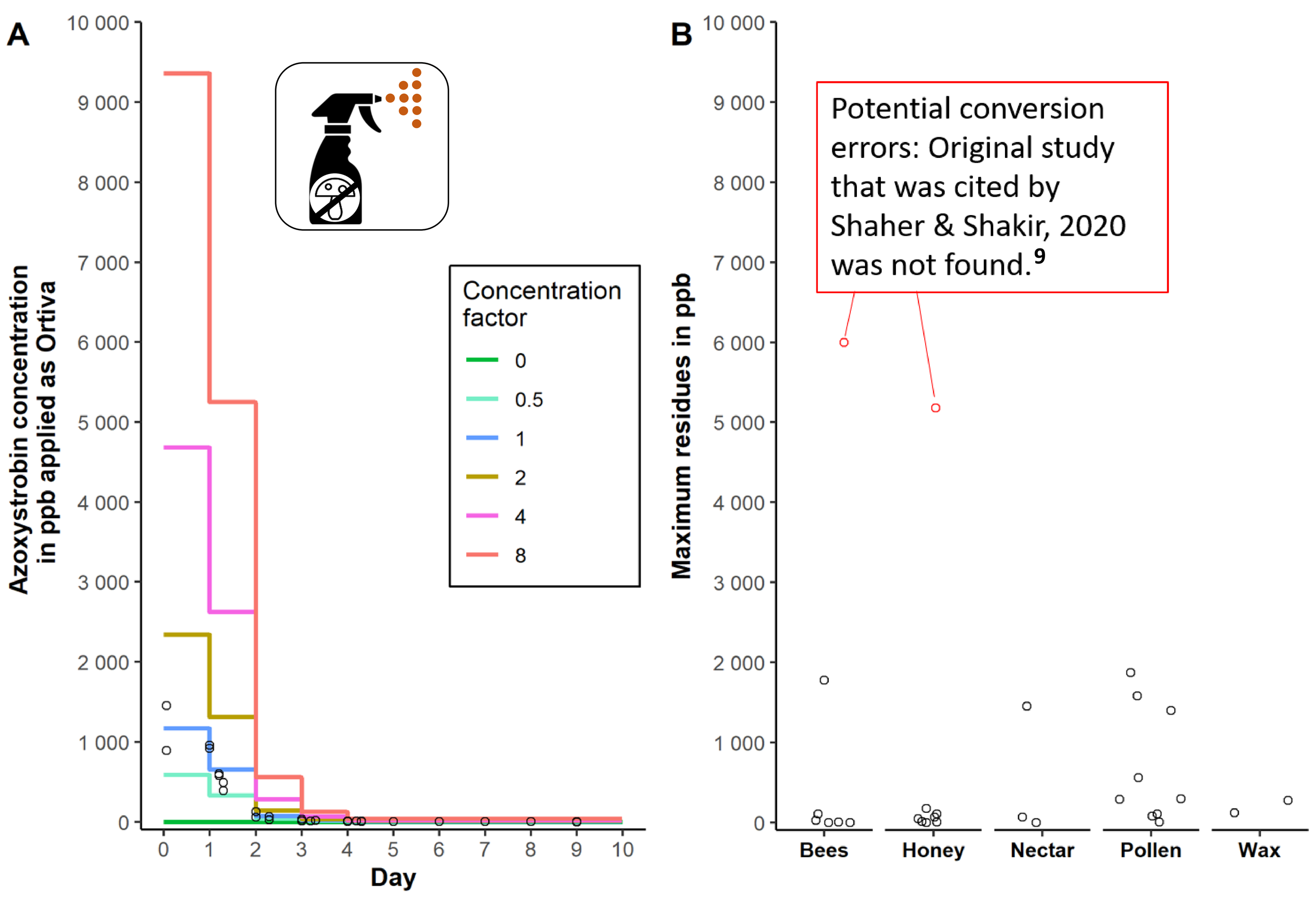

Figure 1 | A: Azoxystrobin concentration administered to syrup samples placed in bumblebee nests. Circles indicate measured azoxystrobin concentrations in nectar collected from honeybees of two colonies foraging on oilseed rape treated with Ortiva[8]. Factor 1 represents a field-typical sequence of concentrations that was determined by taking the daily mean azoxystrobin concentration of these observed values within the first 4 days. Afterwards a constant concentration was used. All other doses are multiples of this set of field-typical doses as indicated by the factor. B: Maximum azoxystrobin residue concentrations reported per study until mid-2021.

Discussion and conclusion

Exposure to the azoxystrobin-based fungicide Ortiva showed **negative effects on *Bombus terrestris* colonies but only in colonies feeding on syrup spiked with 4-times the field-typical concentrations.** Field-typical concentrations were based on residues in honeybee-collected nectar, where Ortiva was applied on oilseed rape with the Dropleg method that typically results in lower residue levels than conventional spray methods[8]. Nonetheless, based on residue levels reported in the literature (Fig. 1B), it **seems unlikely that bees often encounter oral residue levels 4-times the maximum field-typical concentration**. Also, no effects were determined on colonies receiving the highest concentration (factor 8). Previously, azoxystrobin down-regulated genes in honeybees whereby effects at the highest dose were more short-lived than at an intermediate dose[5], suggesting **that high doses potentially trigger quicker degradation** processes. Even the intermediate dose, was above field-realistic levels, but field-realistic semi-field studies found negative effects of Amistar, which is identical in composition to Ortiva, on bumblebees[3,4] – effects varied however between food resources[4]. Our findings illustrate **the importance of dose-response experiments** and a need to **study mechanisms of pesticide effects** depending on the exposure route.

Results

Preliminary results show:

- **No difference in overall syrup consumption across the experiment** between Ortiva-exposed and control colonies (P>.07, Dunnett-test; Fig. 2A).
- Colonies exposed to **4-times the field-typical concentration decreased their syrup consumption over time** (P=.03; Fig. 2A), **gained less weight** (factor 4 vs 0: P=.01; Fig. 2C) and ended the experiment with **3 times as many (i.e. c. 8 more) dead bees** than control colonies (P=.01, Dunnett-test; Fig. 2B)
- **Control colonies consumed initially less syrup** than some Ortiva-exposed colonies (factor 1 vs factor 0: P=.04, factor 4 vs 0: P=.02; Dunnett-test), but syrup consumption of control colonies increased over time (P=.01).
- Ortiva-exposed colonies, did not differ from control colonies in initial or final number of alive bees (P>.3, Dunnett-test; Fig. 2D).
- On day 1, azoxystrobin residues were found in bees sampled from within nests receiving at least twice the field-typical concentration (factor 2: 14 ppb; factor 4: 16; factor 8: 26 ppb), while in groups exposed to lower concentrations no azoxystrobin was found (limit of detection/quantification = 10 ppb).

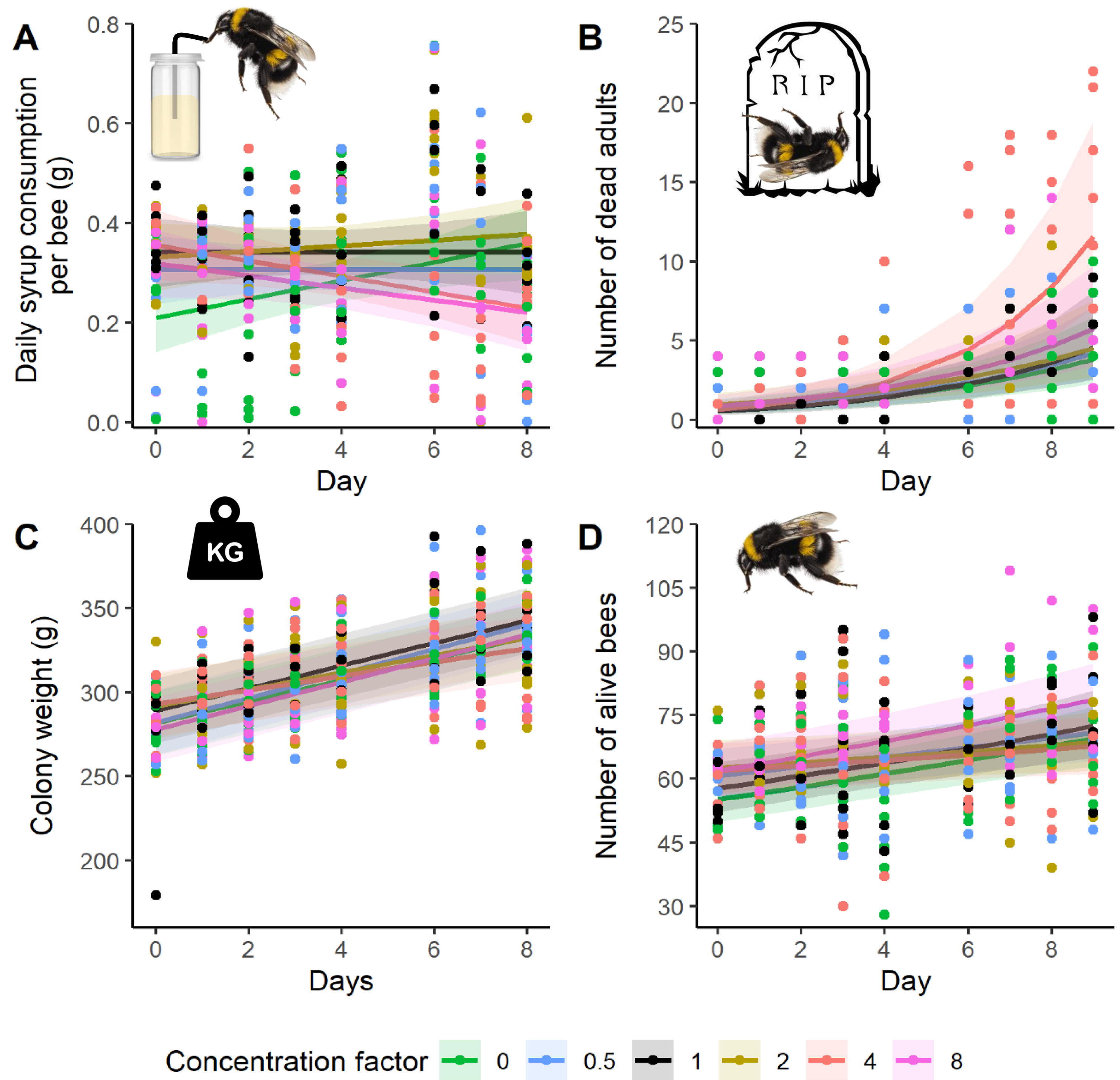

Figure 2 | A: Daily syrup consumption per bee, B: the number of alive bees per colon, C: colony weight, and D: cumulative number of dead adults in relation to time and the concentration factor (multiple of a field typical fungicide degradation curve). Dots indicate observations, lines show estimated marginal means obtained from (G)LMMs and shaded areas depict 95% confidence intervals.

References

1. Bartlett, D. W. et al. The strobilurin fungicides. Pest Manag. Sci. 58, 649–662 (2002).
2. Leadbeater, A. J. Plant Health Management: Fungicides and Antibiotics. Encyclopedia of Agriculture and Food Systems vol. 4 (Elsevier Ltd., 2014).
3. Tamburini, G. et al. Fungicide and insecticide exposure adversely impacts bumblebees and pollination services under semi-field c onditions. Environ. Int. 157, 106813 (2021).
4. Wintermantel, D. et al. Flowering resources modulate the sensitivity of bumblebees to a common fungicide. Sci. Total Environ. 829, 154450 (2022).
5. Christen, V., Krebs, J. & Fent, K. Fungicides chlorothanolin, azoxystrobin and folpet induce transcriptional alterations in genes encoding enzymes involved in oxidative phosphorylation and metabolism in honey bees (*Apis mellifera*) at sublethal concentrations. J. Hazard. Mater. 377, 215–226 (2019).
6. Fisher, A., Coleman, C., Hoffmann, C., Fritz, B. & Rangel, J. The synergistic effects of almond protection fungicides on honey bee (Hymenoptera: Apidae) forager survival. J. Econ. Entomol. 110, 802–808 (2017).
7. Tamburini, G. et al. Sulfoxaflor insecticide and azoxystrobin fungicide have no major impact on honeybees in a realistic-exposure semi-field experiment. Sci. Total Environ. 778, 146084 (2021).
8. Schatz, F. Pflanzenschutzmittelapplikation in blühenden Raps (*Brassica napus*) und deren Auswirkungen auf die Rückstandssituation in Honig, Nektar und Pollen der Honigbiene (*Apis mellifera* L.). (2009).
9. Shaher, K. W. & Manjy, M. S. Degradation of honey bees and environmental pollution: A review. Plant Arch. 20, 339–345 (2020).

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