4.12 Effects of imidacloprid in combination with λ-cyhalothrin on the model pollinator *Bombus terrestris* at different levels of complexity

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1. Introduction

Bumblebees as *Bombus terrestris* are important pollinators for wild flowers and agricultural crops (Free, 1993; Kremen et al., 2007). In recent decades, declines of both managed and wild bee populations have been reported worldwide (Goulson, 2010; Potts et al., 2010). Loss of these pollinators deserves particular attention because of their ecological and economical importance.

Multiple anthropogenic pressures are responsible for the worldwide declines of bee populations (Vanbergen & the Insect Pollinator Initiative, 2013). The widespread use of insecticides in agriculture is speculated to be among the main causes. The last 15 years, both lethal and sublethal effects of insecticides on bumblebees have been studied (Mommaerts et al., 2010; Mommaerts & Smagghe, 2011; Gill et al., 2012). However, no unequivocal conclusions can be drawn concerning to what extent and in what way the use of insecticides affects bumblebee populations. In addition, most studies do not include testing of insecticide mixtures, nor do they include semi-field or field tests in order to evaluate risks at relevant field conditions. In the same context, the European Food Safety Agency (EFSA, 2013) proposed that risk assessment should be carried out in a stepwise approach with different 'tier' levels, i.e. linking laboratory tests with semi-field and field tests.

In this study we addressed the effects of insecticides on bumblebees of *B. terrestris* by 1) focusing on both lethal and sublethal effects of the neonicotinoid imidacloprid and the pyrethroid insecticide λ-cyhalothrin, 2) studying the effect of an insecticide mixture, and 3) linking laboratory and semi-field toxicity tests.

2. Material and methods

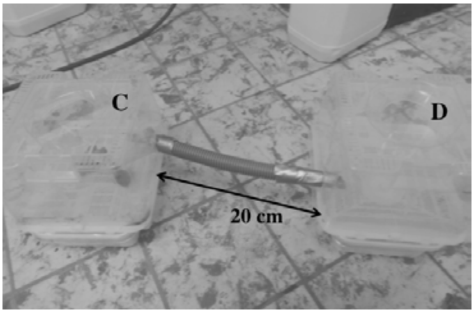
In the laboratory toxicity test, *B. terrestris* queenless microcolonies of five workers (Biobest, Westerlo, Belgium) were exposed for 7 weeks to a series of field realistic concentrations of imidacloprid, λ-cyhalothrin and corresponding mixtures (Table 1). The concentration range of imidacloprid was based on residue concentrations in nectar (Cresswell, 2011; EFSA, 2012). Due to a lack of residue concentrations, the concentrations for λ-cyhalothrin were based on the maximum recommended field concentration of 37.5 ppm (Syngenta Crop Protection, 2013). The methodology of the experimental setup is as developed before by Mommaerts & Smagghe (2011). Bumblebees had to walk 20 cm from a nest compartment to a feeding compartment to collect contaminated sugar water. This set up implies that the bumblebees have to forage for sugar water, which requires effort and coordination (Figure 1A**)**. Lethal effects on worker survival and sublethal effects on foraging behavior (as amount of consumed sugar water) and reproduction (number of drones) were monitored. Per treatment, 4 replicates were done.

Table 1 Concentration series of the different treatments. C = control treatment, I = imidaloprid treatment, LC = λ -cyhalothrin treatment and M = mixture treatment of both imidaloprid and λ-cyhalothrin.

Treatment	Concentration I (ppb)	Concentration LC (ppb)
C	0	0
I1	5	0
I2	10	0
I3	20	0
I4	40	0
LC1	0	469
LC2	0	938
LC3	0	1876
LC4	0	3752
M1	5	469
M2	10	938
M3	20	1876
M4	40	3752

In the greenhouse toxicity test, queen-right colonies with 20 to 25 workers and brood of *B. terrestris* (Biobest, Westerlo, Belgium) were exposed for 2 weeks to imidacloprid (40 ppb), λ-cyhalothrin (3750 ppb) and the corresponding mixture (Figure 1B). Bumblebees had to fly one meter in order to collect contaminated sugar water, which requires more effort and coordination. In this greenhouse setup, the bumblebees were subjected to more stringent conditions. Therefore, it is expected that the toxicity effects are stronger than in the laboratory test. Lethal effects on worker and queen survival and sublethal effects on foraging behavior were monitored. For each treatment 4 replicates were done.

For both the laboratory and the greenhouse toxicity test we tested statistical differences of all treatments compared to the control. Statistically significant interaction effects between both insecticides in the mixture treatments were also tested. Additionally, the risk of the different insecticide treatments to bumblebees was assessed with a PEC/PNEC (Predicted Environmental Concentration/Predicted No Effect Level) approach according to Halm et al. (2006).



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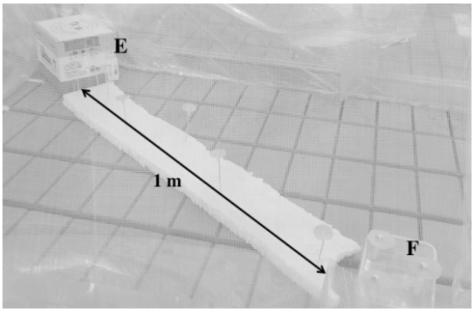

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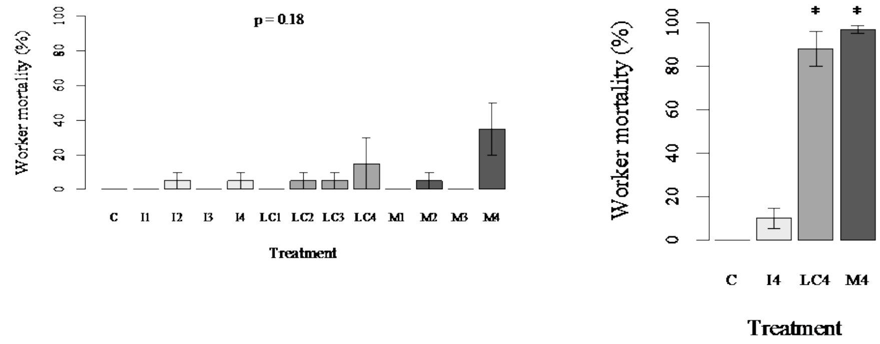
Figure 1 Experimental setup of the chronic toxicity test including foraging behaviour in the laboratory (A) and in the greenhouse (B). Arrow depicts foraging distance, C = nest compartment with pollen, D = food compartment with sugar water, E = A = a queen-right colony and B is the sugar water container with three adjusted wicks resembling the artificial flowers.

3. Results

The treated colonies in the laboratory experiment showed no significant ($p > 0.05$) worker mortality (Figure 2A). Reduced reproductive performance was detected in both the single and mixture treatments ($p < 0.05$), while the foraging behavior was only affected by imidacloprid (p <

0.05) (Figure 3 & Figure 4A). In the greenhouse experiment significant worker and queen mortality were detected in the λ-cyhalothrin and mixture treatments (Figure 2B). Both the single as well as the mixture treatments negatively impaired foraging behavior (Figure 4B). Insecticide exposure of 3750 ppb λ-cyhalothrin at higher levels of complexity (greenhouse vs. lab test) increased the susceptibility of bumblebee colonies to insecticides with effects occurring both faster and more severely (Figure 2 & Figure 4).



A B

Figure 2 Worker mortality with standard error of the laboratory (A) and greenhouse (B) test (p = level of significance, * = significance at the level of 0.05, C = control, I = imidacloprid, LC = λ-cyhalothrin and M = mixture).

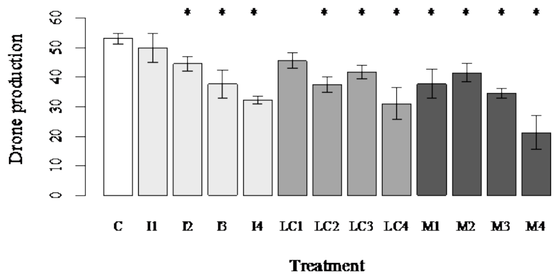


Figure 3 Drone production with standard error of the laboratory test (* = significance at the level of 0.05, C = control, I = imidacloprid, LC = λ-cyhalothrin and M = mixture).

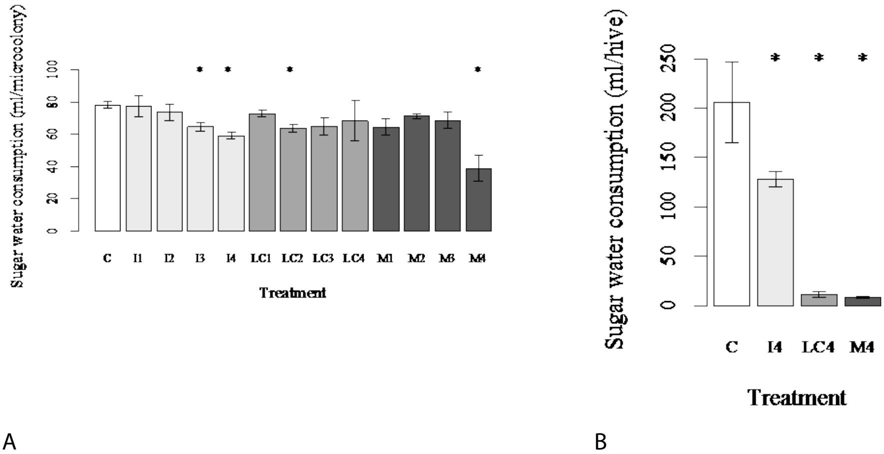


Figure 4 Sugar water consumption with standard error of the of the laboratory (A) and greenhouse (B) test (* = significance at the level of 0.05, C = control, I = imidacloprid, LC = λ-cyhalothrin and M = mixture).

4. Risk assessment

The possible risk was evaluated by the risk quotient (RQ), which was obtained by the PEC/PNEC ratio (Halm et al., 2006). A ratio greater than 1 indicates that the concentration of the insecticide poses a risk, whereas a ratio smaller than 1 indicates that there is no risk.

The PEC was calculated as the product of the residue concentration that was found in literature and the daily worker consumption of sugar water (EFSA, 2013). The PNEC was calculated as the product of the NOEC and the daily worker consumption of sugar water. NOEC's were detected for reproduction, i.e. 5 ppb for imidacloprid and 469 ppb for λ-cyhalothrin (Figure 2). As no significant interaction was detected, the individual RQ's can be summed up to assess the risk of the mixture (Backhaus & Faust, 2012). The obtained RQ's can be considered as a first indication of possible risks. To refine the assessment, empirical assessment factors (AF) are used. Such AF make it possible to estimate these concentrations taking uncertainties into account due to a lack of data and lack of resemblance of the complexity of the field situations in the experiment (Halm et al., 2006: Backhaus & Faust, 2012). We found three AF's to apply on the PNEC and non to apply for the PEC:

- an AF of 5 was used for the extrapolation from laboratory to field effects and for possible differences for subspecies (EFSA, 2013)
- an AF of 5 was used since bumblebees are potentially more susceptible to worker loss than honeybees and because the first AF is assumed for honeybees (EFSA, 2013).
- an AF of 3 is used because our experimental setup is not validated (EFSA, 2013)

To adjust the PNEC's and the RQ's for the AF's, the PNEC's are divided by each of the AF's. The obtained RQ's with and without AF's are listed in Table 2.

Table 2 Derived risk quotient (RQ) for the single insecticides and for the mixture, with and without application of the assessment factors (AF).

Treatment	RQ without AF	RQ with AF
Imidacloprid	7.07	530.4
λ-cyhalothrin	0.08	6.3
Mixture	7.15	536.7

5. Discussion

Single and combined insecticide exposure of imidacloprid and λ-cyhalothrin clearly affected bumblebee behavior and performance. Whereas no lethal effects were detected in the laboratory test, clear lethal effects occurred with exposure to λ-cyhalothrin and the mixture in the greenhouse test. Foraging behavior was also affected more severely in the greenhouse test. Therefore, a more complex and stringent setup (greenhouse vs. laboratory test) results in a more sensitive test as is in accordance with the findings of Mommaerts et al. (2010). To our knowledge no other study than that of Gill et al. (2012) has studied the effect of combined insecticide exposure to bumblebees. Like Gill et al. (2012), our study showed that combined exposure was more harmful than exposure to the single insecticides, resulting in more severe lethal and sublethal effects. Two of the four replicates of the mixture treatment even lead to colony failure in the greenhouse test. Yet, we did not detect any significant interactive effects between both insecticides in the mixture treatment in the laboratory test, nor in the greenhouse test. Nevertheless, our preliminary risk assessment suggests that single as well as combined exposure to environmentally realistic concentrations of imidacloprid and λ-cyhalothrin may affect bumblebee behavior and performance and may pose a risk of reduced reproduction. An important remark and working point here is the shortage of data and assessment factors to perform the risk assessment more adequately.

6. Conclusion

In conclusion, our study addresses limitations of previous research by 1) exposing bumblebees to mixtures of insecticides and 2) indicating the significance of linking semi-field and laboratory toxicity tests. Consequently, concentrations of insecticides that seem harmless in laboratory tests might lead to lethal and/or sublethal effects in semi-field conditions, either alone or in combination with other insecticides. These findings are very useful to improve current risk assessment practices for pollinators as they show the need to include semi-field studies in order to quantify the effects at a relevant level of complexity. In the field, foraging bumblebees experience combined exposure of different insecticides and other agrochemicals (Osborne, 2012). Therefore, our data suggest that the effects of combined insecticide exposure need to be addressed further and should be considered when updating the guidelines for pesticide registration and use.

7. References

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