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ORIGINAL PAPER

Effects of dietary lambda-cyhalothrin exposure on bumblebee survival, reproduction, and foraging behavior in laboratory and greenhouse

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Abstract Bumblebees of *Bombus terrestris* are indispensible pollinators for ecosystems and for various agricultural crops. Unfortunately, bumblebees are challenged by various stress factors including insecticide applications. Today sublethal effects of various insecticides need to be thoroughly investigated to allow their combined use with pollinators and other beneficial organisms. In this study, we used lambda-cyhalothrin as a model pyrethroid insecticide and investigated lethal and sublethal effects by different dilutions, ranging from 1/10 to 1/100 of its maximum field recommended dosage (MFRC, 37.5 ppm), with the use of a chronic toxicity tests in the laboratory and in flight cages in the greenhouse. In the laboratory, small microcolonies with five bumblebee workers with one being pseudo-queen were used, while in the greenhouse we used queen-right mini-hives where the bumblebees need to fly for pyrethroid-contaminated food. We observed strong sublethal effects in the laboratory with treatments of 1/10 and 1/20 of the MFRC: the nest reproduction was reduced by 49 and 32 %, respectively, and the sugar water consumption by 36 %. With free-flying bumblebees, the toxic effects at 1/10 of the MFRC were more pronounced. A mortality of 88 ± 8 % was observed after only 2 weeks, being twice the mortality in the laboratory microcolonies test (43 ± 11 %). Besides, it should be mentioned that in the greenhouse experiment all queens were dead and most of the workers showed signs of incoordination and convulsion and gradually became apathetic. In conclusion, our results demonstrated the pyrethroid lambda-cyhalothrin with a range of lethal and sublethal effects, both crucial for the development and survival of the *B. terrestris* colonies. Moreover, this study supports the demand to test insecticide compounds on their safety, especially when the bees have to perform complex tasks such as foraging for their food.

Keywords *Bombus terrestris* · Chronic toxicity tests · Pyrethroid · Sublethal effects

Key messages

- Bumblebees of *Bombus terrestris* were exposed to lambda-cyhalothrin via sugar water at 1/10–1/100 of the MFRC (37.5 ppm).
- Chronic dietary pyrethroid exposure caused severe decreases in survival, food consumption, and reproduction.
- Differences in effects were investigated when flight behavior was included.
- Mortality was more pronounced in flight conditions with all queens being dead.

Introduction

Bumblebees such as *Bombus terrestris* L. provide important pollination services to wild flowers and agricultural crops (Kremen et al. 2007; Goulson 2010). As the use of

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different agrochemicals is a common practice in agriculture, contact of bees with these compounds is often inevitable (Velthuis and van Doorn 2006; Osborne 2012). Moreover, it is discussed that next to habitat loss and fragmentation, the toxic effects of these chemicals can be among the main causes for the declines of both diversity and abundance of bumblebees and other bee species observed during the last decade (Potts et al. 2010; Hatfield et al. 2012). Toxicity effects of agrochemicals are often expressed as a lethal concentration estimate or LC_{50} values (concentration that induces 50 % lethality), and to evaluate the sensitivity of the bio-assay, a no effect concentration (NOEC) is often defined (Devillers and Pham-Delègue 2002). Despite previous research efforts and the development of new insights for more accurate bio-assays (Biondi et al. 2012), the lethal and sublethal effects of agrochemicals on bumblebees are not completely understood yet (Mommaerts and Smagghe 2010; EFSA 2013). To obtain a comprehensive understanding about whether or not and in what way such chemicals can affect bumblebees, thorough risk assessment studies—preferably on the long term—are still needed. Risk assessment guidelines have been composed by the European Food Safety Authority (EFSA 2013), the European and Mediterranean Plant Protection Organisation (EPPO 2001), and the International Commission on Plant-Bee Relationships (ICPBR 2000), but these guidelines apply mainly to honeybees and procedures for pesticide registration need to be revised (Decourtye et al. 2013). In addition, because sublethal effects of agrochemicals on beneficial arthropods can occur at multiple levels, a complete analysis of their impact (reproduction, survival, learning and foraging behavior, neurophysiology, etc.) is required (Desneux et al. 2007).

Pyrethroids are non-systemic insecticides and synthetic chemical analogs of natural pyrethrins. Pyrethroids are used to control several important pest insects such as coleopterans, lepidopterans, and aphids on a range of agricultural crops as well as in household settings (Palmquist et al. 2012; Fishel 2014; Pansa et al. 2015). They are often sprayed during the flowering period of a range of crops, but they can also be applied as granules or treated nets (He et al. 2008; Biondi et al. 2015). In this study, we used lambda-cyhalothrin as a representative pyrethroid insecticide to assess the effects of this group of compounds on *B. terrestris*. Lambda-cyhalothrin is highly effective against several insect pests (Desneux et al. 2007; Palmquist et al. 2012). It disrupts the normal functioning of the insects' nervous system by acting on the voltage-gated sodium channels, and additionally also on calcium and chloride channels (Burr and Ray 2004), resulting in hyperactivity, uncoordinated behavior, paralysis, and eventually death (Du et al. 2009). Different types of sublethal effects on beneficial arthropods including effects on learning performance, behavior, and neurophysiology have been reported (Desneux et al. 2007). Nevertheless, most studies mainly focused on honeybees and on acute lethal toxicity effects based on LD_{50} values, while data concerning long-term sublethal effects over a range of pesticide concentrations, including pyrethroids, and tier levels remain relatively understudied (EFSA 2013).

In the present study, *B. terrestris* bumblebees were exposed in a laboratory microcolony setup (Mommaerts et al. 2010) to lambda-cyhalothrin at different dilutions ranging from 1/10 to 1/100 of its maximum field recommended concentration (MFRC), concentrations which are considered to be found in the field. The objective was to investigate chronic lethal and sublethal effects of exposure to field realistic concentrations after a period of 7 weeks by focusing on worker survival, reproduction, and foraging performance (Baron et al. 2014). Secondly, we examined the effects under a higher level of complexity, i.e. on queen-right, free-flying bumblebee hives in flight cages in the greenhouse. Here we investigated whether the exposure to pyrethroid at 1/10 of the MFRC resulted in stronger negative effects, when the bees have to fly for their food. Under the latter conditions, the bumblebee workers perform the complex task of foraging.

Materials and methods

Insects

The experiments were carried out with *B. terrestris* bumblebees, as obtained from a continuous mass-rearing program at Biobest (Westerlo, Belgium), and they were fed ad libitum with sugar water (50 % BioGluc®, Biobest) and floral pollen mixture (Biobest) as reported before (Mommaerts et al. 2006).

Chronic toxicity test in the laboratory with microcolonies

Here we used the experimental setup with queenless microcolonies to evaluate lethal and sublethal effects including foraging behavior under laboratory conditions of 28–30 °C and continuous darkness, as reported before (Mommaerts et al. 2010; Besard et al. 2011). In brief, five newly hatched workers were placed in an artificial plastic nest box (15 cm × 15 cm × 10 cm) and fed ad libitum with pollen and sugar water. Upon start of nest building, the nest box was connected by a 20 cm tube to a second box wherein untreated sugar water was provided, so that bumblebee workers had to walk from the nest compartment to the feeding compartment to collect sugar water. After 2 weeks with pseudo-queen establishment and first egglaying, the bumblebees were exposed via the sugar water for a period of 7 weeks to a series of concentrations of lambda-cyhalothrin (Karate Zeon©; Syngenta, Oosterzele, Belgium), namely 1/10, 1/20, 1/40, and 1/100 of the MFRC (corresponding to 3.75, 1.88, 0.94, and 0.38 ppm, respectively). These concentrations were based on the MFRC of 37.5 ppm for use in oilseed rape in Europe. In the control series, the bumblebees were fed with untreated sugar water during the whole experiment. The experiment was done with 8 replicates for each concentration and the untreated control.

We scored worker mortality, sugar water consumption per bumblebee worker, number of drones produced (hatching started at week 3), and mean drone weight on a weekly basis in the microcolonies. Newly hatched drones and dead individuals were discarded each day. Significant differences at the end of the testing period of 7 weeks compared to the control treatment were analyzed by one-way analysis of variance (ANOVA) (Mommaerts et al. 2010). Mean ± SEM was separated using a post hoc Tukey HSD test using the software Statistica 12 (StatSoft®, Tulsa, OK). In addition, the medium response concentration (LC_{50}) that is causing 50 % mortality in treated bumblebees, was calculated with a probit analysis (Prism®, GraphPad, La Jolla, CA) of the worker mortality data of the four treatments, and the goodness of fit of the data to the curve model was evaluated based on R^2 values, as reported before (Mommaerts et al. 2010).

Chronic toxicity test in the greenhouse with flight cages

In this greenhouse experiment, the effect of oral exposure to 1/10 of the MFRC was tested on a higher level of complexity, more specifically in greenhouse conditions including flight behavior. The test was done in May 2014 at the greenhouse complex of ILVO-Ghent University at Melle in Belgium. The experiment was conducted with separate flight cages (1.5 m × 1.2 m × 1.2 m) with one queen-right mini-hive (1 queen, 25 workers and brood; Biobest, Westerlo, Belgium) per cage, as reported before (Mommaerts et al. 2010) with some minor modifications. In brief, the mini-hives were provided with sufficient pollen, while the sugar water solution was placed ad libitum in a container at a distance of 1 m from the entrance of the mini-hives. In this way, the workers needed to fly over a distance of minimally 1 m in order to obtain sugar water, in contrast to walking a distance of 20 cm in the laboratory test as described above. Four mini-hives were fed with treated sugar water, while the control consisted of 4 mini-hives fed with untreated sugar water. Temperature and light conditions in the greenhouse followed a diurnal pattern with a minimum of 12 °C and a maximum of 30 °C. Queen and worker survival were monitored during 2 weeks, and the sugar water consumption was used as a proxy of the foraging activity.

Significant differences in worker mortality between treatment and control in the greenhouse experiment were analyzed using a Mann–Whitney *U* test.

Results

Chronic toxicity test in the laboratory with microcolonies

For the lethal effects, colonies treated with 1/10 and 1/20 of the MFRC showed a significantly ($p < 0.001$ and $p < 0.01$ resp.) higher worker mortality (ANOVA: $F = 24.3$, $df = 4$, $p < 0.001$) with 80 ± 5 % and 45 ± 3.5 %, respectively, after a period of 7 weeks compared to the control (Fig. 1a). Hence, as for the sublethal effects, the mean sugar water consumption was significantly ($p < 0.001$) reduced by 36 % (ANOVA: $F = 12.2$, $df = 4$, $p < 0.001$) in the treatment with 1/10 of the MFRC (Fig. 1b). In the treatments with 1/10 and 1/20 of the MFRC, the reproduction was strongly reduced by 49 % ($p < 0.05$) and 32 % ($p = 0.074$), respectively (Fig. 1c) (ANOVA: $F = 6.3$, $df = 4$, $p < 0.001$). Furthermore, the drone weight was also significantly lower ($p < 0.001$) with a decrease of 35 % with 1/10 of the MFRC (Fig. 1d) (ANOVA: $F = 23.5$, $df = 4$, $p < 0.001$).

In contrast, 1/40 and 1/100 of the MFRC did not pose a significant lethal and sublethal effect ($p > 0.05$) after 7 weeks compared to the control treatments (Fig. 1a–c). Therefore, the concentration of 1/40 of the MFRC can be considered as the "no observed effect concentration" (NOEC). In addition, probit analysis with the cumulative mortality values of the four different treatments resulted in LC_{50} value for lambda-cyhalothrin of 2.06 ppm ($R^2 = 0.9927$), which corresponds to 1/22 of the MFRC.

Chronic toxicity test in the greenhouse with flight cages

In the greenhouse experiment, we scored clear lethal effects with a high cumulative worker and queen mortality after 2 weeks in the greenhouse at 1/10 of the MFRC. The worker mortality was 68 ± 9 % and 88 ± 8 % for the first and second week, respectively. Furthermore, we observed that the queen was dead in 3 of the 4 mini-hives within the first week of exposure to 1/10 of the MFRC, and after 2 weeks, all queens were dead. In contrast, all queens were alive in the controls. Hence, in the treatment with 1/10 of the MFRC, many dead bumblebees were found outside the mini-hive, reaching about 62 % of total number of dead

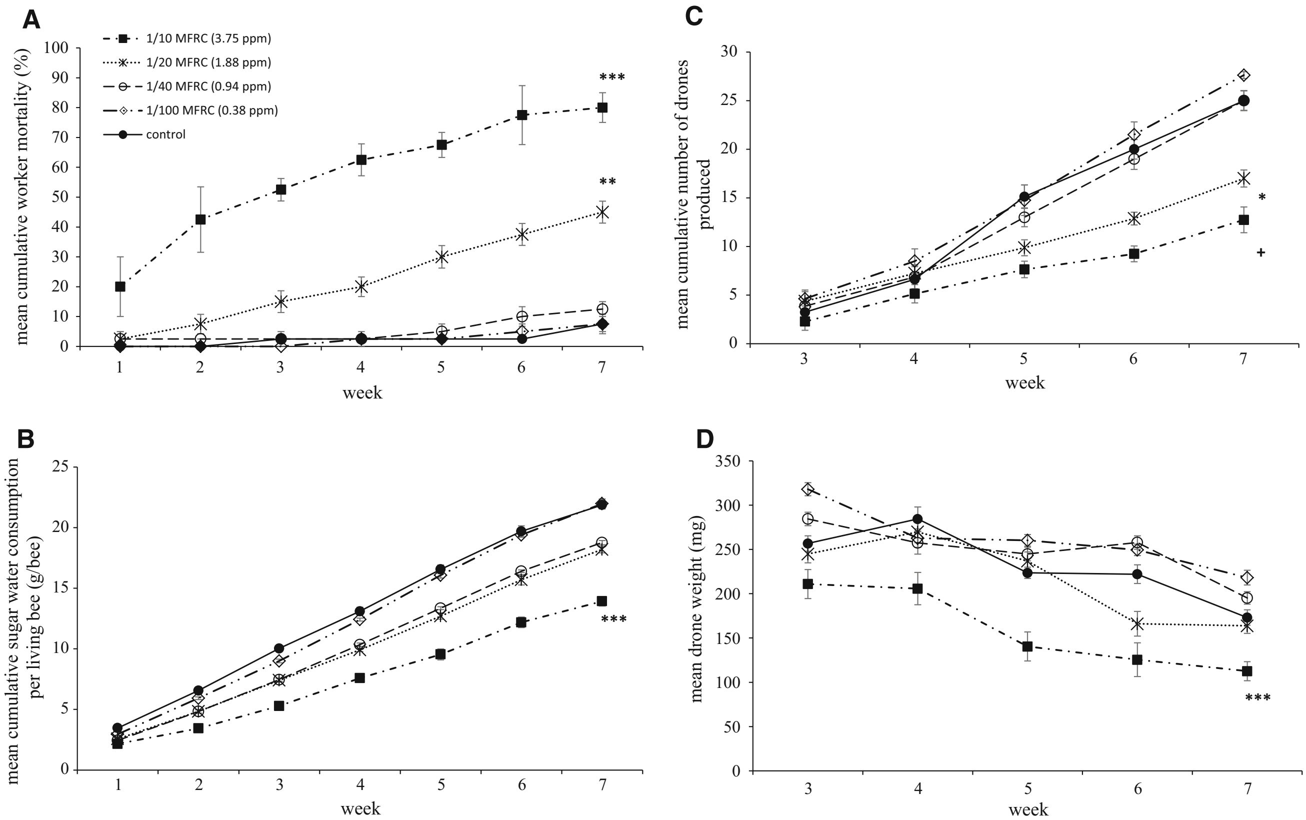

Fig. 1 Chronic toxicity effects of lambda-cyhalothrin at concentrations of 1/10, 1/20, 1/40, and 1/100 of the MFRC on **a** worker mortality, **b** sugar water consumption, **c** nest reproduction with drone production, and **d** drone fresh weight during an exposure period of 7 weeks. *Error bars* denote the standard errors. For the data after a period of 7 weeks, significant differences with the control treatment are indicated with an *asterisk* (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; + $0.5 < p < 0.1$)

bumblebees. Taken all together, the cumulative worker mortality with the concentration of 1/10 of the MFRC in the greenhouse experiment in the first 2 weeks was 3 and 2 times higher compared to the cumulative mortality at the same concentration in the microcolony laboratory experiment (20 ± 10 % in week 1, and 43 ± 11 % in week 2).

Finally, we report that the bumblebee colonies in both the laboratory and greenhouse experiment clearly suffered from the insecticide treatment at 1/10 of the MFRC after their respective treatment period, as most of the workers showed signs of incoordination and convulsion, and gradually became apathetic. In contrast, the bumblebee workers of the control treatments foraged efficiently and remained vital.

Discussion

The demand for classical insecticides such as pyrethroids is likely to increase in the next years, especially because of the recent EU restrictions on neonicotinoid usage for crops pollinated by bees, and as well as on cereals (Garthwaite et al. 2010). In this project, we demonstrated that pyrethroids can have a significant impact on bumblebees of *B. terrestris*, which are representative examples of essential wild pollinators and managed pollinators. Especially in practical, more complex conditions including flight behavior, the effects are significant. Chronic toxicity was observed in those nests that were exposed to 1/10 and 1/20 of the MFRC. Moreover, these effects included impacts on bumblebee mortality, reproduction, and foraging activity which accumulated in time. However, it should also be remarked that our results seem to be in contrast with the findings of Baron et al. (2014), where lambda-cyhalothrin exposure at a concentration of 37.5 ppm (i.e., MFRC) via contaminated pollen did not cause differential worker survival. A possible explanation is that insecticide intake via sugar water has stronger adverse effects than intake through pollen, as Smagghe et al. (2013) observed with chlorantraniliprole in bumblebees. Because sugar water is the main energy source, the adult bumblebee's consumption rate of sugar water is higher than for pollen, especially for pollinators performing energy-demanding activities such as flight. A higher energetic demand implicates a higher consumption of sugar water, and accordingly a higher intake of pyrethroid insecticide. This was clearly observed in the flight cages, where allowing flight behavior resulted in 88 ± 8 % worker mortality and 100 % queen mortality within a short period of 2 weeks. In fact, worker mortality of free-flying bumblebees in the greenhouse experiment was about 2 to 3 times higher than in the experiment in the laboratory at the same exposure of 1/10 of the MFRC of lambda-cyhalothrin.

Not only worker survival was impaired, but also reproduction was quantitatively and qualitatively affected with a lower number of drones and smaller drones. This negative effect was very clear, although we did not treat the pollen that is important as protein source for reproduction and larval development. According to Desneux et al. (2004), pyrethroids as lambda-cyhalothrin can reduce the oviposition activity in insects resulting in a smaller number of progeny. Accompanied by an interference with larval development and adult emergence, pyrethroids can lead to a reduction of brood size (Desneux et al. 2007). The effects against the offspring fitness with a lower body mass of the drones also agree with Baron et al. (2014). Based on Goulson (2002), indeed bumblebee worker size is related to colony productivity and performance, as larger workers can collect food resources more efficiently. Small impairments of reproduction have also been proven to prevent the production of new queens and survival of the colony (Müller and Schmid-Hempel 1992). Therefore, we support the belief that exposure to pyrethroids as lambda-cyhalothrin, and potentially pesticides in general, can therefore make the colony more vulnerable, especially in the young stages of colony development (Goulson 2010). Furthermore, the size of adult workers depends on the larval feeding during their development (Sutcliffe and Plowright 1988). A reduced feeding of larvae by the adult workers could indeed have occurred since sugar water consumption of treated colonies was significantly lower.

The reduced sugar water consumption can be attributed to a repellent activity by the pyrethroid molecules (Thompson and Wilkins 2003). Hence, differences in sugar water consumption may also have been provoked by an anti-feedant effect of the pyrethroid, as He et al. (2013) observed with a whitefly pest on pyrethroid-treated plants. Likewise, a disruption in the ability to locate food, potentially caused by a reduced olfactory capacity, might interfere with the feeding behavior of the exposed bees (Decourtye and Pham-Delègue 2002). Detrimental effects on foraging activity have been observed in honeybees and bumblebees after exposure to several other pesticides (Gill et al. 2012; Henry et al. 2012; Schneider et al. 2012). In addition, it was reported that these disturbances are a consequence of the chemicals on the motor neuron transmission involved in navigation and orientation capacity (Thompson 2003; Desneux et al. 2007). As we observed in the greenhouse experiment with free-flying bumblebees, indeed the behavioral changes had dramatic effects on foraging and thus whole colony performance, and in turn, this increased the likelihood of the colonies to fail.

The conditions in our experimental setup represent a scenario where adult workers had only access to a continuous source of contaminated sugar water, which is unlikely to occur in the field. However, the possibility to encounter pyrethroids at higher doses outside the colony is real, for instance, through direct contact during spraying or while foraging on several (adjacent) crops with different application times, and the drinking of guttation water on sprayed plants. Hence, pyrethroids can be sprayed multiple times on the same crop during the season, causing potential frequent exposure to higher dosages. Nevertheless, little is known about the prevalence of residues of pesticides in contaminated pollen and nectar, and therefore more data on residue amounts are required to make final conclusions (Desneux et al. 2007; Baron et al. 2014).

Our study indicated that exposure to bumblebees that need to fly for (contaminated) food is resulting in stronger detrimental effects. This suggests that the present laboratory microcolony setup including behavior may underestimate the risks for side-effects. Although this setup in the laboratory already provided a strong stress wherein workers need to orientate toward and forage for sugar water in a second compartment, the food was provided in the near proximity of the bumblebees. When real flight behavior to obtain food was included, the effects were more pronounced. The latter experimental setup in the greenhouse is more relevant for field conditions, where bumblebees have an average foraging distance of 267 m (Wolf and Moritz 2008). The increased sensitivity that we observed when the bumblebees were subjected to more complex and environmental realistic conditions, highlights the need for semi-field testing in appropriate risk assessment studies. Furthermore, it should also be taken into account that bumblebee colonies in natural field conditions also encounter other stress factors that can re-enforce the effects of pyrethroids. Baron et al. (2014) did not found any increased susceptibility of *B. terrestris* to the parasite *Crithidia bombi* in combination with lambda-cyhalothrin, but, in contrast, Aufauvre et al. (2012) reported higher synergistic effects in honeybees with the parasite *Nosema ceranae* and a pyrethroid insecticide. Moreover, the application of multiple pesticides at different times and on different crops in regions of intensive agriculture implies that foraging bees can encounter agrochemicals that possibly act synergistically, as Gill et al. (2012) observed with imidacloprid and lambda-cyhalothrin. This increased combinatorial effect of the two chemicals clearly negatively affected bumblebee colony performance. Together with other stressors such as nutritional limits, temperature fluctuations, competition, etc., it is likely that bumblebees experience a higher risk in realistic field conditions. Our results emphasize that in more complex situations, the susceptibility of bumblebees to lambda-cyhalothrin is elevated. It is therefore recommended to include more complex situations in long-term toxicity testing, together with relevant pesticide residue concentrations in order to detect cumulative lethal and sublethal effects rather than acute ones on beneficial pollinators such as *B. terrestris.*

Authors contribution

BC, ME, and GS conceived and designed research; BC and ME conducted experiments; TV, GC, and IRR contributed reagents or analytical tools; BC and ME analyzed data; BC, ME, and GS wrote MS; and all authors read and approved MS.

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