performed in the same way. This prevents, in a toxicological approach, to do a comparison between *A. mellifera* and stingless bees. While *A. mellifera* has a progressive feeding, stingless bees have en mass food deposition, making impossible the same way of exposure in the food. Anyway, it is important to consider an ecological approach, which indicates, although by different methods, a LC_{50} for *S. postica* 50 times more sensitive to dimethoathe than *A. mellifera*. This highlights the importance of inclusion of a native Brazilian species as model-organism for risk assessments studies, which may be extended for other areas of the Neotropical region. Our results are very useful for a validation of method through developing of ring tests, in accordance to OECD.

2.5 Effects of chemical and biological Plant Protection Products on R&D colonies of the Buff-Tailed Bumblebee Bombus terrestris (2.5 Part 1)

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Abstract

Bumblebees (*Bombus terrestris*) are exposed daily to Plant Protection Products through their foraging and feeding activities. Through all possible means of contact with pesticides, consumption through sugarwater is the most severe. In the present study, lethal and sublethal effects of the consumption of sugarwater solutions with the pesticides Sivanto WG (flupyradifuron), Exalt SC (spinetoram) and Oikos EC (azadirachtin) were studied using a sequential dilution testing scheme of 1/1 and 1/10 of the maximum field recommended concentration (MFRC). For the weekly assessment, parameters such as the survival of the mother queen, of workers and drones, the formation of gynes, and the weight and volume of the colonies were recorded. Moreover, by the end of the colony's life, the total number of formed workers/drones, the number of newborn gynes and queen brood were also recorded. The IOBC side-effect classes for laboratory trials were applied in order for the results to be categorized and conclusions made. Both tested concentrations of Sivanto WG (flupyradifuron) were slightly harmful for queen, worker and drone populations, Exalt SC (spinetoram) was harmful at 1/1 dilution but only slightly harmful at the 1/10 dilution, and both concentrations of Oikos EC (azadirachtin) were slightly harmful for workers and drones but toxic for queens at both dilutions.

Keywords: *Bombus terrestris*, bumblebees, Sivanto WG (flupyradifuron), Exalt SC (spinetoram), Oikos EC (azadirachtin)

Introduction

To date, biopesticides and new age conventional pesticides are widely used with improved results in food production and environmental protection and studying the side-effects on non-target organisms is a necessary step. One of the most important non-target insects is the bumblebee, *Bombus terrestris*, which is contaminated daily by a number of pesticides through oral consumption or topical contact. Bumblebees nowadays are commonly exposed to the following widely used active ingredients: flupyradifuron (Sivanto 200 SL), spinetoram (Exalt 025 SC) and azadirachtin (Oikos 026 EC) and the study of their effects on these pollinators under practical conditions, imitating natural, field and glasshouse conditions is not yet extensively done.

Flupyradifuron (Sivanto) has not been tested on bumblebees before, but studies on honeybees (*Apis mellifera)* present a safe profile of the compound, at least for the tested conditions (Campbell *et al*. 2016; Hesselbach and Scheiner, 2018; Hesselbach *et al*. 2019).

On the other hand, many studies have been conducted for the effects of spinetoram on bumblebees: Hao *et al*. (2016) characterized spinetoram as a low risk compound to adult workers of *B. terrestris* as judged by the hazard quotient (HQ) value, while Besard *et al*. (2011) pointed out that the no observed effect concentration (NOEC) for spinetoram was 1/100 of the MFRC (25 mg AI L^{-1}).

Finally, studies concerning the effect of azadirachtin on bumblebees, such as from Barbosa *et al*. (2015) mentioned that the compound used (Insecticida Natural Neem, BioFlower) may affect *B. terrestris* with a range of sublethal effects, although Sterk *et al*. (2017) concluded that no toxic or

sublethal effects occur in practice with legally registered formulations of azadirachtin on *B. terrestris* after having been applied at the recommended and authorized dose rates.

Materials and methods

In the present study, specially designed *Bombus terrestris* R&D hives (IPM Impact, Belgium – Koppert, the Netherlands) were used, consisting of 80 callows and a mother queen from the same hibernation batch. All materials were provided by Koppert (Sterk et al. 2016). The main target is to form an experimental design with high comparability and to focus on the most important end point, as is generally the consensus: the formation of newborn queens, which are the only individuals from the colony that will hibernate and start a new colony the next spring (Sterk et al. 2016) as well as the evolution of the colony under the effect of the pesticide.

The bumblebees were fed with commercial sugar water (Koppert) and honey bee-collected pollen from different sources (Koppert). The bumblebee colonies were maintained in a room at 26-28°C and 60-70% relative humidity (RH) and continuous darkness. Assessments were carried out under red-light. Eight replicates were used for each object.

All PPP's were tested under two different concentrations, starting with the maximum recommended concentration in the field (MFRC) (1/1) and then diluted down to 1/10 of the MFRC. Details for the tested PPP are presented in Tab. 1. Side effects were only assessed via oral treatment of sugarwater. The treated sugarwater remained for four weeks and was then replaced with untreated. Plain sugarwater was used as a control treatment. Untreated pollen was provided ad libidum from day 0 onwards.

Tab. 1 Overview of the insecticides tested: commercial name, formulation type and maximum field recommended concentration (MFRC) in % of formulated compound

Active ingedient	Commercial name	Formulation[1]	MFRC (% formulated compound)
flupyradifuron	Sivanto	200 SC	0.15
spinetoram	Exalt	025 SC	0.24
azadirachtin	Oikos	026 EC	0.15

[1]SL Soluble Liquid, SC Suspension Concentrate, EC Emulsifiable Concentrate

Every week, the survival of the mother queen, the number of adults (workers and drones), the number of newly formed gynes, and the weight and volume of the colony was recorded. When the colony reached its' end, the number of queens (queen, gynes and queen cells) and the number of adults (workers, drones and the individuals with an unidentified gender) were recorded.

The lethal and sublethal effects on the bumblebees were scored in accordance with the classification of the International Organisation for Biological and Integrated Control of Noxious Animals and Plants (IOBC) for the laboratory studies: 'class 1' = <30% effect = Harmless; 'class 2' = 30–79% effect = slightly harmful; 'class 3' = 80–98% effect = moderately harmful; 'class 4' = >98% effect = harmful.

As mentioned, the estimation of the brood's volume was recorded every week. According to this new parameter, the development of the colony can be categorized according to the size/volume of the brood (Tab. 2) (IPM Impact-Koppert).

Tab. 2. Size (cm^3) of a bumblebee colony's brood and description

Code	Size (cm^3)	Description
A	30 cm^3	Basic colony in center of hive
B	235 cm^3	Expanding colony in center of hive
C	382 cm^3	Colony expanding, but not yet reaching the borders of the hive
D	655 cm^3	Colony expanding, and touching at least one side of the hive
E	1763 cm^3	Colony touching more than one side of the hive and growing in height
F	3489 cm^3	Colony covering the whole bottom of the hive and strongly expanding in height
G	4477 cm^3	Colony filling about half the hive
H	5373 cm^3	Colony almost filling the whole hive
I	6034 cm^3	Colony filling the whole hive. No space left for further expansion

Results

Weekly assessments

According to Fig. 1, Sivanto at 1/1 and 1/10 dilutions, and Exalt at 1/10 dilution caused a similar reduction in the population of adults, compared those that were untreated. When bumblebees were fed with Exalt at 1/1 dilution, all workers were dead after four weeks. Concerning Oikos, both 1/1 and 1/10 dilutions led to low numbers of workers and drones.

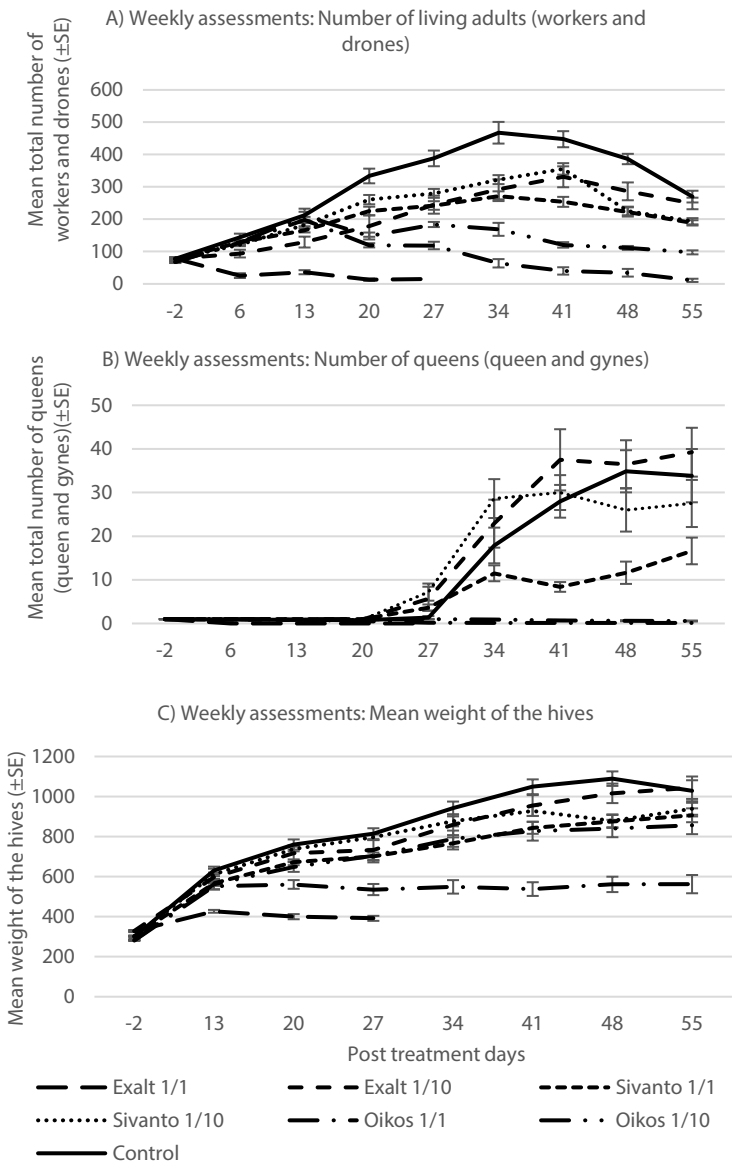

Fig. 1. Number of A) living adults (workers and drones), B) queens and gynes and C) mean weight of the hives at the weekly assessments, after applying Exalt (dilutions 1/1 and 1/10), Sivanto (dilutions 1/1 and 1/10) and Oikos (dilutions 1/1 and 1/10) through sugarwater application.

All queens fed with sugarwater with Exalt 1/1, Oikos 1/1 and Oikos 1/10 dilutions presented high mortality and therefore almost no gyne formation. Queens with access to Exalt 1/10 and Sivanto 1/10 sugarwater had high survival rates and gyne production similar to the control, while queens fed with sugarwater with Sivanto 1/1 also had high survival rates, but with gyne production lower than the control (Fig. 1).

Using weight as an indication of the development of the hives we can conclude that colonies with access to Sivanto 1/1, Sivanto 1/10, Exalt 1/10 and Oikos 1/10 dilutions to their sugarwater had a

slightly lower development than the untreated ones. Finally, colonies with Exalt 1/1 spiked sugarwater had no development and died four weeks after treatment (Fig.1). According to Tab. 3, which presents the development of the brood according to the estimation of its size (cm^3), all tested pesticides had an effect on the hives, which saw lower development than the untreated ones.

Tab. 3 Volume (size) of the colonies' brood, according to Table 2.

	Post treatnment days								
Product	**-2**	**6**	**13**	**20**	**27**	**34**	**41**	**48**	**55**
Control	C	C	D	E	E	F	F-G	F-G	G
Sivanto 1/1	C	C	C	D	E	E	E-F	E	E
Sivanto 1/10	C	C	C-D	D	E	E-F	F	E-F	E-F
Exalt 1/1	C	C	C	C					
Exalt 1/10	C	C	C	D	E	F	F	F	F
Oikos 1/1	C	C	C	D	D	D	D	D	D-E
Oikos 1/10	C	C	C	D	D-E	E	E	E	D

Final Assessment

According to the final assessment results (Tab. 4), all tested compounds caused minor or slight reductions in the number of workers and drones compared to control, as calculated according to the IOBC classification. It is worth mentioning that colonies with access to Exalt at the MFRC concentration of sugarwater died within four weeks and that the slight reduction (41.1%) in the population is due to the initial colonies' dynamic. Furthermore, queens that were fed with Exalt 1/1 dilution and Oikos at both tested dilutions, died and no gynes formed. The rest of the compounds had a slight negative effect on the number of queens (queen and gynes).

Tab. 4. Final total population of workers, drones and queens and mean weight of the hive at the final assessment.

Product	**Final total population of workers and drones**		**Final total population of queens**		**Mean (SE) weight (in gr)**
	Median (Min-Max)	**% reduction compared to control**	**Median (Min-Max)**	**% reduction compared to control**	
Control	480 (422-555)		63 (39-75)		1300.5±61.1
Sivanto 1/1	371 (320-402)	23.1	17 (13-28)	64.2	1156.3±10.7
Sivanto 1/10	322 (266-377)	32.4	37 (35-53)	38.5	1276.1±43.3
Exalt 1/1	273 (258-307)	41.4	1 (1-1)	98.2	674.9±10.7
Exalt 1/10	394 (345-438)	16.1	37 (15-47)	45.1	1273.5±42.9
Oikos 1/1	208 (175-221)	57.9	1 (1-1)	98.2	861.0±36.6
Oikos 1/10	179 (168-196)	60.3	1 (1-1)	98.2	1146.6±45.3

Conclusions

Flupyradifuron, when tested as Sivanto 200 SL, diluted at 1/1 and 1/10 of the MFRC in the sugarwater, was found to be harmless or only slightly harmful to the population (queens, gynes, workers, drones) of a *B. terrestris* colony. On the other hand, spinoteram (Exalt 025 SC) was toxic for bumblebee queen and adult populations when fed with the MFRC dilution in the sugarwater. However, the toxicity was reduced when the 1/10 dilution of Exalt was provided to the colonies. For avoiding the toxic effects of Exalt at the MFRC, closure of the hives' entrance before spraying and keeping the hives closed for 1-2 days after spraying is recommended. Finally, azadirachtin (Oikos 026 EC) was slightly harmful to the worker and drone populations, but toxic for queens in both solutions (1/1 and 1/10). The previous study for azadirachtin products by Sterk et al. (2017) showed the same results. Higher dilutions up to 1/100 of the MFRC may conclude in no or only slight negative effects in all parameters of the colonies and therefore further research is needed.

Nevertheless, the present study was extremely strict for the bumblebees, as compounds were provided at high concentrations with no alternative food source available for four weeks.

References

BARBOSA, W., L. DE MEYER, R. GUEDES, G. SMAGGHE. 2015. Lethal and sublethal effects of azadirachtin on the bumblebee *Bombus terrestris* (Hymenoptera: Apidae). Ecotoxicology 24:130-142.

BESARD, L., V. MOMMAERTS, G. ABDU-ALLAA, G. SMAGGHE. 2011. Lethal and sublethal side-effect assessment supports a more benign profile of spinetoram compared with spinosad in the bumblebee *Bombus terrestris.* Pest Management Science 67 (5): 541-547.

CAMPBELL, J. W., CABRERA, A. R., STANLEY-STAHR, C. & ELLIS, J. D. 2016. An evaluation of the honey bee (Hymenoptera Apidae) safety profile of a new systemic insecticide, flupyradifurone, under field conditions in Florida. Journal of Economic Entomology 109, 1967–1972.

HAO, Z., S. JUNAN, Z. YIFAN, W. GUANGAN, C. HAO, M. XINGYUAN, Y. YI, Z. LI. 2017. Toxicity and risk assessment of sulfoxaflor, spinetoram and their mixture to *Bombus terrestris* (Hymenoptera: Apidae). Acta Entomologica Sinica 60(7): 809-816.

HESSELBACH, H., R. SCHEINER. 2018. Effects of the novel pesticide flupyradifurone (Sivanto) on honeybee taste and cognition. Scientific Reports 8, Article number: 4954.

HESSELBACH, H., J. SEEGER, F. SCHILCHER, M. ANKENBRAND, R. SCHEINER. 2018. Chronic exposure to the pesticide flupyradifurone can lead to premature onset of foraging in honeybees (*Apis mellifera*). Journal of Applied Entomology,

STERK, G., B. PETERS, Z. GAO, U. ZUMKIEL. 2016. Large-scale monitoring effects of clothianidin-dressed OSR seeds on pollinating insects in Northern Germany: effects on large earth bumble bees (*Bombus terrestris*). Ecotoxicology, 2(9): 1666-1678.

STERK, G., J. CUYLAERTS, P. KOLOKYTHA. 2017. Lethal and sublethal effects of several formulations of azadirachtin on IPM IMPACT R&D colonies of the bumblebee *Bombus terrestris* (Hymenoptera: Apidae). Hazards of pesticides to bees – 13th International Symposium of the ICP-PR, October 18-20, 2017

2.5.1 Effects of *Bacillus thuringiensis* subsp. *aizawai* GC91 (Agree WG) on R&D colonies of the Buff-Tailed Bumblebee *Bombus terrestris* (2.5 Part 2)

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Abstract

Bacillus thuringiensis subsp. *aizawai*, a widely used biological plant protection product, was tested on buff-tailed bumblebee *Bombus terrestris*, using an updated laboratory method on full standardized R&D colonies. The maximum field recommended concentration (MFRC) was applied through topical, oral pollen and oral sugar water treatment. Parameters such as survival of the mother queen and workers, formation of gynes, weight and volume of the colonies were recorded during the study, while the total number of formed workers/drones, the number of newborn gynes and queen brood were taken also at the end of the colonies' life. For the evaluation of the results the data were calculated and categorized according to the IOBC side-effect classes, used for laboratory trials.

According to the results, no toxic effect was recorded for all parameters taken from the bumblebee colonies when they were exposed to *B. thuringiensis aizawai* GC91.

Keywords: *Bacillus thuringiensis* subsp. *aizawai* GC91, Agree WG, *Bombus terrestris*, bumblebees

Introduction

Pesticides introduced in Integrated Pest Managment programs are meant to have as little effect as possible on beneficial arthropods and pollinators. Over many years of research, the majority of the pesticides derived from natural sources appear to show low toxicity and persistence in the organisms and therefore may be involved in an IPM cultivation. Biopesticides are widely used plant protection products, which are not by definition harmless for pollinators, therefore testing is essential in order for such products to cooperate harmoniously with pollinators under an IPM program.

Up to now, *B. thuringiensis aizawai* was tested only on bumblebee microcolonies (Sterk *et al*., 2002, Mommaets *et al*. 2009, 2010) and only under the commercial product of Xentari WG. In our study, a new strain of *B. thuringiensis aizawai*, with the commercial brand name of Agree WG, was tested for toxicity on R&D colonies of the buff-tailed bumblebee *Bombus terrestris.* The R&D colonies are newly