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.

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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 designed, while the number of queens and gynes, workers and drones as well as weight and volume of the R&D colonies have been the most important parameters for a bumblebee study.

Materials and methods

The tested insects, *Bombus terrestris*, were provided by Koppert Biological Systems (The Netherlands) and were especially selected in order to form R&D colonies, which, together with the method followed in this study, were developed by IPM Impact (Belgium) and Koppert. The colonies, in order to achieve a high comparability, consisted of a mother queen from the same hibernation batch and a number of 50 callows. The method is described in detail by Sterk *et al.* (2016). The present study was focused on the most important end point, as is generally the consensus: the formation of the newborn queens, as these are the only bumblebees 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 biopesticide.

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.

In this study, the Maximum Field Recommended Concentration was used (0.4%) through three different application methods: a) Topical application: approximately 50 ml water solution sprayed once on the whole colony with a Birchmeier hand spraying equipment (2 bars). The control colonies were sprayed with tap water. Untreated pollen and sugarwater were provided after the treatment. b) An oral sugarwater application: 1 liter of spiked sugar water, prepared in the same way as a spraying solution with the same concentration, (0.4%) was given to each colony. The spiked sugarwater remained for 4 weeks and was then replaced with untreated one. Plain sugarwater was used as a control treatment. Untreated pollen was provided from day 0 onwards. c) An oral pollen application: 100 grams of pollen in the form of a ball, saturated with the test compound was given ad libidum to each hive. The control colonies were fed with tap water treated pollen. Untreated sugarwater was provided from day 0 onwards.

For a weekly assessment, the surviving of the mother queen, the number of adults (workers and drones), the number of new formed gynes, the weight and the volume of the colony was recorded. When the colony reached its end, a final assessment was done where 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.

For the characterization of the biopesticide's lethal and sublethal effects on the bumblebees the IOBC classification system for laboratory side-effects was used (Tab. 1).

Tab. 1. Range (%) of effect and evaluation categories for laboratory side-effects studies, according to the IOBC

IOBC Class	Range % effect (mortality, reproduction)	Evaluation category
1	<30	Harmless
2	30-79	Slightly harmful
3	80-98	Moderately harmful
4	>98	Harmful

In the present study, the estimation of the brood's volume was recorded every week. According to this new parameter, which was introduced by IPM Impact and Koppert, the development of the colony can be categorized according to the size/volume of the brood (Tab. 2).

Tab. 2. Size (cm3) of a bumblebee colony's brood and description

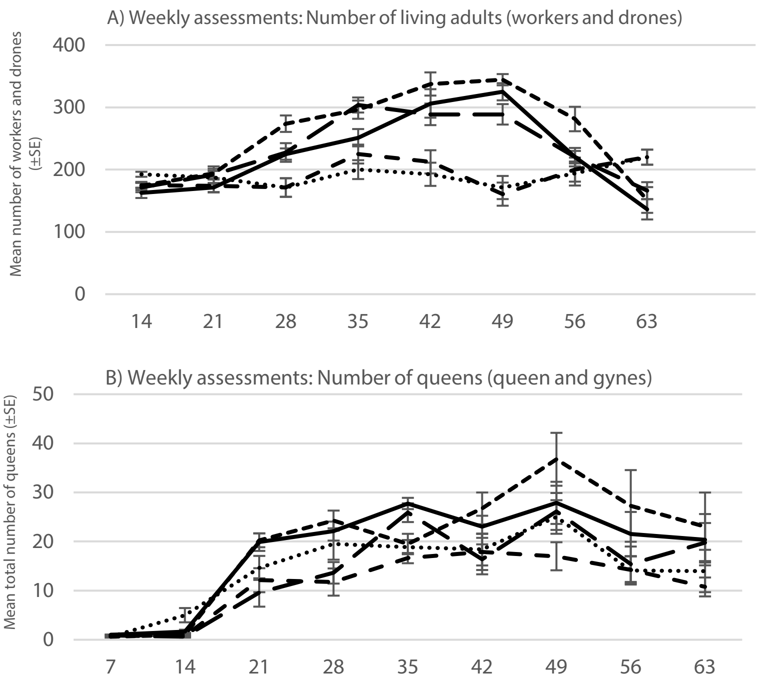
Code	Size (cm^3)	Description
A	30 cm^3	Basic colony in center hive
B	235 cm^3	Expanding colony in center hive
C	382 cm^3	Colony expanding, but not yet reaching the borders of the hive
D	655 cm^3	Colony expanding, and touching at least one side of the hive
E	1763 cm^3	Colony touching more than one side 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, the consumption of sugarwater and pollen treated with *B. thuringiensis aizawai* GC91 lead to a small decline of the workers' and drones' numbers. On the other hand, spraying of the colonies with the biopesticide lead to no differences compared to the control, on the workers' and drones' populations, as the development of the colonies with spiked sugar water and pollen seemed to continue after the 63rd day after treatment.

The number of queens (queen and gynes) was also lower than the control when the bumblebees were fed with Agree WG treated sugarwater and pollen. On the contrary, spraying with Agree WG caused no effect on the number of queens compared to the colonies sprayed with water (Fig. 1).



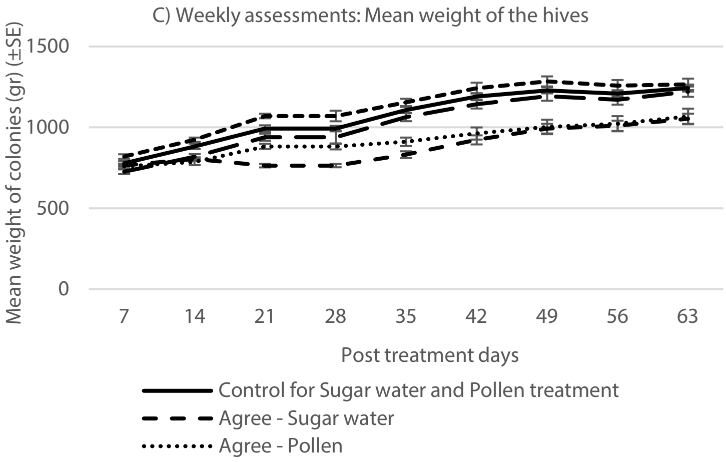


Fig. 1. Number of A) living adults (workers and drones), B) queens and gynes and C) mean weight of the hives at the weekly assessments, after applying Agree WG through sugarwater, pollen and spraying.

The mean weight of the treated hives followed the same development as the controls, but the sugarwater and pollen treated hives weighed less than the control (water sprayed and not treated) (Fig. 1).

Concerning the development of the brood and the estimation of its size (cm^3) (Tab. 3), the hives with access to treated sugarwater or pollen saw lower development than the untreated ones, but the growth of the brood size shows that development continues following the 63rd day after treatment**.**

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

	Post treatnment days								
Product	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**
Control – SW, Control - P	D	E	F	G	G	G	G	G	G
Agree - SW	D	E	F	E	E	E	E	D	F
Agree - P	D	E	F	F	F	F	E	F	F
Control (Water) - T	D	E	F	G	F	G	G	F	F
Agree - T	D	D	F	G	F	H	G	G	G

Final Assessment

After counting each colony's individuals and calculating the reduction compared to the untreated colonies (Tab. 4), the final assessment results show that there was no or only slight reduction in the numbers of workers, drones and gynes in all three applications of *B. thuringiensis aizawai* CG91 as calculated according to the IOBC classification (Tab. 1).

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 – SW	321 (293-357)		27 (13-32)		1160.3±32.7
Control – P					
Agree – SW	342 (327-367)	-6	16 (10-19)	26	1075.1±33.0
Agree – P	310 (241-341)	8	19 (13-26)	17	1059.9±51.8
Control (Water)–T	313 (261-360)		29 (12-35)		1121.3±43.2
Agree - T	353 (337-362)	14	34 (19-39)	-19	1175.9±37.1

Conclusion

When *B. thuringiensis aizawai* GC91 (Agree WG) was provided to R&D *B. terrestris* through all three treatments (topical treatment, oral application through pollen, oral application through sugarwater) at the MFRC (0.4%), there were hardly any significant differences in the formation of workers, drones and queens compared to the untreated or water treated colonies. Although *B. thuringiensis aizawai,* (Xentari WG) at the MFRC (0.1%) has been recorded in the past as toxic for workers when provided through sugarwater and pollen (Mommaerts *et al*. 2010), this new commercially available strain of *B. thuringiensis aizawai* is harmless and no specific measures are recommended when used together with bumblebees.

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2.6 Predicting wild bee sensitivity to insecticides utilizing phylogenetically controlled inter-species correlation models

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Abstract

Plant protection products (PPP), are a vital pillar of modern agricultural practice, but their potential adverse effect on bees has emerged as an intensively discussed topic. Historically, research on the effects of PPP on bees has focused on the honey bee (*Apis mellifera*), while non-*Apis* bee species remain largely understudied. This study is intended as a first step to address this obvious knowledge gap and hope that it may be used to facilitate the development and implementation of a scientifically sound wild bee risk assessment with limited additional testing needs. We have compiled a comparative data set on bee sensitivity (acute contact exposure) against acetylcholine esterase (AChE) inhibitors, pyrethroids, neonicotinoids, organochlorides and bee bodyweight, a trait likely influencing bee sensitivity to PPP exposure. In total, we collected sensitivity data for up to 24 bee