

Abstracts: Oral Presentation

equipped and all involved are being trained to begin the first round of testing from September 2019. The Brazilian experience will be presented during the 13th SETAC Latin America for the exchange of experiences and discussion of more species-oriented methods from the tropical and subtropical regions of the Americas, with the aim of creating a network aimed at protecting local species.

2.4 Standardization of an *in vitro* rearing method for the stingless bee species *Scaptotrigona postica* larvae and its application for determining the toxicity of dimethoate on the larval phase

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Abstract

Currently, Brazil has a full framework for pesticide risk assessment established for *Apis mellifera*, based on North America's approach. However, the use of an exotic species as model-organism as a substitute for native species of Brazil (stingless bees) has been questioned. An *in vitro* larval rearing method has already been described for the Brazilian native *Melipona scutellaris* but, *Scaptotrigona postica* species has shown potential to be suitable for testing, mainly because its high number of individuals per hive comparing to the other stingless bee species and for do not belongs to the list of endangered species, like *M. scutellaris*. Thus, we aimed to establish an *in vitro* larval rearing method for *S. postica* and to apply it for determining the toxicity of dimethoate on larval phase. Larvae of 24 hours old were transferred to acrylic plates and five different procedures were carried out, considering the humidity control and the required fungus *Zygosaccharomyces* sp. as essential for the success of larval survivorship. Each replicate consisted of 100 larvae, totaling 4,800 larvae. Mortality and emergence parameters of the individuals, as well as the progress of the larval development were assessed, in order to check the efficiency of these methods. The intertegular distance, head width and wings asymmetry were assessed from the individuals emerged from the most efficient method. The same parameters were checked on individuals emerged from *in vivo* brood combs. The chosen method consisted of the deposition of the pure larval food followed by adding KCl and NaCl solutions 72 and 120 hours after the larval transference, respectively. This procedure was applied to determine the lethal concentration 50% (LC₅₀) of dimethoate, the standard active ingredient for toxicological tests, established by OECD. The active ingredient, obtained from Sigma-Aldrich (Pestanal), was directly diluted in the larval food, and successive subsequent dilutions were performed in the food, in order to reach the following concentrations to be offered to the larvae (in ng a.i./larva): 250, 200, 150, 100, 50 and 25. Each bioassay was carried out 4 times (20 larvae/concentration in triplicate). The negative control consisted of the pure larval food. The dose-response data were assessed with binomial generalized linear models, using the Cauchit function, for determining the LC₅₀ for 24 and 48 hours. The analysis was performed in the R software (R Core Team). The best procedure indicated emergence/larvae, emergence/pupae and mortality/larvae of 93.44, 97.6 and 2.85%. The mean of intertegular distance for the *in vitro* method was 136.5 mm and for *in vivo* of 127.7 mm. For the head width, *in vitro* showed 92.58 mm and *in vivo* was 89.88 mm. The t test indicated no significant difference between the *in vivo* and *in vitro* methods ($p > 0.05$). Regarding the wings asymmetry, the ANOVA Procrustes indicated a significant difference in the centroid size only in the "individual effect", on individuals emerged from both *in vitro* ($F = 11.33$; $p < 0.0001$) and *in vivo* ($F = 38.35$; $p < 0.0001$) treatments, and in the wing venation pattern in the "individual effect" *in vitro* ($F = 12.03$; $p < 0.0001$) and *in vivo* ($F = 12.13$; $p < 0.0001$), and in the "size effect" on individuals emerged from the *in vivo* treatment ($F = 0.50$; $p < 0.0005$). The tests with dimethoate indicated a LC₅₀ (in ng a.i./larva) of 172.48 and 156.33 for 24 and 48 hours, respectively. The main points for the success of the *in vitro* rearing were the humidity control, the non-use of eggs for transference, and to the use of acrylic plates manufactured which the size simulates the real dimensions of brood cells. The differences showed in some patterns of the wings asymmetry on individuals emerged from *in vitro* treatment are considered normal, since we can observe also on *in vivo* emerged individuals. These little variations in morphology are common in nature, especially because of environmental stresses. Thus, our results obtained *in vitro* may be used for representing *in vivo* conditions. According to the OECD, to be possible carry out a toxicological comparison by LC and/or LD values, is necessary that the experimental method has been

Abstracts: Oral Presentation

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 LC₅₀ for *S. postica* 50 times more sensitive to dimethoate 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