1.12 Selection matrix for Brazilian bee species to risk assessment of pesticides

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Abstract

Many countries are using honeybee (*Apis mellifera*) as a surrogate to evaluate the risk of pesticides to all bee species. However, there is uncertainty regarding the extent honey bees can be used as surrogates for non-*Apis* species in pesticides risk assessment. A selection matrix for Brazilian bee species was built to support the selection process. To be considered as a candidate representative species in the Brazilian agricultural scenario a bee should have a wide geographic distribution, and be recorded in at least 4 agricultural crops. The selection matrix provides a foundation to elect meliponines (stingless bees) as a priority group. Therefore, in the near future Ibama intends to assess the need for changes in the risk assessment procedure for bees, eventually including a stingless bee as a representative species.

Keywords: selection matrix, non-*Apis* bees, risk assessment, pesticides.

Introduction

Bees are considered the main pollinator group due to its close relationship with plants both on collecting food resources (pollen and nectar) and on resources to build or protect their nests (leaves, resin and seeds)[1]. Globally there are increasing concerns about possible declines in pollinators and environmental authorities and research groups point out that the health status of bees is affected by many factors such as destruction of their habitats, pesticides, climate changes, nutrition, diseases, and improper management of the hives.

Many countries consider the honey bee (*Apis mellifera*) as a surrogate for all bee species in their pesticides risk assessment schemes for pollinators[2,3,4]. Honeybees are used worldwide as a standardized species due to its wide geographical distribution, well-known biology and because it can be easily dealt with in laboratory conditions. Brazil is also using *A. mellifera* for pesticide risk assessment purposes[5]. Early in 2017, it was published the Normative Instruction No. 02 (NI 02/2017)[6] that establishes procedures to pesticides risk assessment to pollinators, which is the first Brazilian specific regulation based on a risk approach. However, there is uncertainty regarding the extent to which honeybees can serve as surrogates for Brazilian non-*Apis* bee species.

About 5,000 bee species have been described for the Neotropical region[7] and approximately 1,600 of these species occur in Brazil[8]. Since many plants grown in Brazil are good sources of pollen, nectar or both, it is expected that hundreds of bee species will be found in Brazilian agroecosystems[9] and even more species are expected to occur in natural habitats[10]. Like the honeybee, stingless bees also can be used for pollination of native or cultivated plants. However, few studies have addressed the importance of these bees as pollinators.

Since 2015 Ibama have coordinated a Working Group (WG) on risk assessment of pesticides to bees in Brazil, composed of members from government, academia and industry, to discuss and develop clear and scientifically-based schemes of risk assessment to pollinators. Given the uncertainty on the use of *A. mellifera* to cover all the other native species of bees - which have significant biological differences when compared to *Apis* -, in 2016 the WG decided to focus on native non-*Apis* bees.

Considering this scenario, and due to the impracticality of evaluating the risk to all species, it is necessary to choose one or a few species that may be representative of the others. Hence, a selection matrix for Brazilian bee species was proposed for electing native species to be potentially used in pesticide risk assessment.

Materials and methods

In order to select species for which more data could be gathered or produced, a bibliographical survey was carried out. The selection matrix was constructed following the approach proposed by Hilbeck et al. (2006)[11] and detailed for pollinators by Arpaia et al. (2006)[12]. In this approach, the species are selected based on technical-scientific criteria and considering the degree of exposure to pesticides.

This process uses a long list of species present in agricultural environments that can be later ranked according to different criteria and scores, resulting in a matrix showing which species should be prioritized for further research.

Considering the absence of toxicity data on non-*Apis* bees and the lack of information about pollination service es provided by them to crops in Brazil, this survey aimed to identify which non-*Apis* bees species have a higher occurrence in the brazilian agroecosystems, and, therefore, it is assumed to have an increased likelihood of direct exposure to pesticides.

To construct the list of bee species were selected the agricultural crops of economic importance to Brazil[13] and also those for which there are requests for insecticides registration, resulting in data collection for 40 crops from open literature. Table 1 summarizes the crops for which data on visitors was found. From this point, a list of species was created for each crop[14].

Criteria and order of priority were defined to evaluate the degree of exposure of different bee species to pesticides based on its occurrence in agricultural environments. Table 2 describes the criteria and its importance. The main criteria included, among other factors, the geographic distribution of the species and their occurrence and abundance in the crops.

Table 1 Agricultural crops for which data on visitors was gathered.

Agricultural crops			
Açai berry	Cassava	Macadamia nut	Pumpkin
Annatto	Castor oil plant	Mango	Soybean
Apple	Citrus	Melon	Star fruit
Avocado	Coffee	Mulberry	Strawberry
Barbados cherry	Cotton	Okra	Sugar cane
Bean	Cucumber	Onion	Sunflower
Brazil nut	Eggplant	Passion fruit	Suriname cherry
Canola	Gliricidia	Peach	Tomato
Carrot	Guava	Pepper	Watermelon
Cashew	Jatropha	Pomegranate	Wheat

Table 2 Criteria and its importance for the selection matrix of native bee species.

Main criterion	**Secondary criterion**	**Importance**
Geographical distribution		Assessing the degree of distribution in the 26 Brazilian states and Federal District. The wider the geographical distribution a species has, the greater the chance it will be a good surrogate.
Association with agricultural environments	Occurrence in crops	Evaluating the number of records of the species in the 40 crops. It assumes that a species present in various crops has a higher probability of being exposed to pesticides.
	Abundance	Evaluating the abundance in: - agricultural crops; - weeds around the crop; - natural vegetation, i.e., other plants outside the crop area; The more abundant a species is, the higher is the probability of exposure to pesticides.

Importance as pollinator	For the crop	Evaluating the degree of dependence on pollination or the increase in crop productivity when pollinators are present.
	For the natural vegetation	Evaluating the degree of dependence on pollination service for natural vegetation maintenance.
Collected resources	Nectar	Evaluating the main resources collected.
	Pollen	
	Floral oils	
	Resin	
Biological aspects	Nidification inside the collecting area	Evaluating the exposure by other routes, such as contact with contaminated soil.
	Is it a managed species?	Evaluating the possibility of that species being reared in laboratory conditions.
	Size of the colonies	Evaluating the availability of individuals for trials.
Economic importance	Production of honey, propolis, pollen and royal jelly	Evaluating the economic gain that could be obtained with hive products.

Scores were assigned for each of the criteria, with 0 and 4 corresponding to the lowest and highest values, respectively[14]. The final score conferred to each species of bee was the sum of the scores assigned according to the different criteria.

Results and discussion

A total of 386 non-*Apis* species were identified, among social and solitary bees. Considering only the species observed in 4 or more crops it was identified 20 social species and 28 solitary species. Distinguishing the bees as either social or solitary is crucial for evaluating how each group is affected by exposure to pesticides, since each of these groups show their own behavioral traits in either the agroecosystems or in the natural environments, factors which can impinge on the risk assessment for these organisms.

The top 5 species of social bees identified, according to the selection criteria, are summarized in Table 3. The top 7 species of solitary bees identified are also summarized in Table 3.

Table 3 Top 5 species of social bees and top 7 species of solitary bees identified by the selection matrix.

Social bee species	**Final score**
Trigona spinipes	28
Tetragonisca angustula	24
Nannotrigona testaceicornis	22
Melipona scutellaris	21
Melipona quadrifasciata	20
Solitary bee species	Final score
Xylocopa frontalis	20
Xylocopa grisescens	19
Eulaema nigrita	18
Centris aenea	17
Centris tarsata	
Exomalopsis analis	16
Epicharis flava	

Table 4 summarizes the negative and positive aspects of each species pre-selected as potential surrogates for risk assessment purposes. Solitary bee species have yet gaps of data on biology and routes of exposure in agricultural scenarios.

After the final classification, the criterion "species management" was considered as a qualifying factor because it is important that methods to rear and handle colonies in laboratory conditions are available to provide organisms for use in risk assessment. Despite *T. spinipes* has received the higher final score for social bee species, this species is not available commercially and, therefore, was eliminated.
Size of the colonies is also an important criterion since risk assessment requires a large amount of individuals for doing the trials *in vitro* and *in situ*.

A point to reflect is the inclusion on the list of endangered species of the most promising species selected in the matrix according to the pros and cons identified so far. This fact can be a barrier to propose this organism as a test species, but at the same time, it highlights the importance to assess its exposure to pesticides in agricultural environments.

Table 4 Species selected for more investigation and related pros and cons of their use for risk assessment purposes.

Species	Pros	Cons
Trigona spinipes	- Colonies with large number of individuals (can reach 180.000 individuals per colony); - Wide geographic distribution in Brazilian territory; - Representative and extremely abundant (found in 32 of 40 crops, *Apis mellifera* found in 36 of 40 crops); - Collect different types of nest materials (mud, leaves, feces, resins).	- Lack of data on life traits; - Can pollinate effectively several important crops but may also behave in a way that damages the flowers as they search for nectar, being also considered a pest in some crops; - Not available commercially, very aggressive bee; - No methods to handle colonies in laboratory conditions; - Protocols for adult acute toxicity tests available, but not standardized[15]; - No protocols for semi-field or field tests.
Tetragonisca angustula	- Colonies with large number of individuals; - Wide geographical distribution in Brazilian territory; - Relatively representative (found in 19 of 40 crops); - Easy to rear and manipulate; - Commercially available; - Very small bee.	- Lack of data on life history traits; - No protocols for laboratory toxicity tests nor semi-field and field tests.
Nannotrigona testaceicornis	- Hives available commercially; - Easy to rear and manipulate; - Very small bee.	- Geographical distribution in northeast, southeast and south, but not in the states considered part of Amazon biome; - No methods to manage colonies in laboratory conditions; - No protocols for laboratory toxicity tests, semi-field nor field tests.
Melipona quadrifasciata	- Easy to rear and manipulate; - Toxicity can be tested using standardized protocols available; - Hives commercially available (but not in large scale).	- Geographical distribution in northeast, southeast and south, but not in in the states considered part of Amazon biome; - Colonies moderately populated.
Melipona scutellaris	- Biology well known; - Easy to rear and manipulate; - Colonies with large number of individuals; - Toxicity to adults can be tested using standardized protocols available (laboratory/field); - Hives commercially available in a large scale.	- Geographical distribution restricted to Northeast; - Method for larvae toxicity testing available but not standardized.

Table 4 (cont.). Species selected for more investigation and related pros and cons of their use for risk assessment purposes.

Species	Pros	Cons
Xylocopa frontalis	- Easy to rear and manipulate; - Wide geographical distribution; - Medium occurrence in crops (found in 13).	- Hives not available commercially in a large scale.
Xylocopa grisescens	- Easy to manipulate; - Medium geographical distribution; - Medium occurrence in crops (found in 11).	- Method for rearing not standardized.
Eulaema nigrita	- Wide geographical distribution; - Medium occurrence in crops (found in 13).	- Lack of knowledge on how to managed the colonies.
Centris aenea	- Medium geographical distribution.	- Lack of knowledge on how to manage colonies.
Centris tarsata		- Restricted geographical distribution; - Lack of knowledge on how to manage colonies.
Exomalopsis analis	- Medium geographical distribution; - Relatively representative (found in 18 crops); - Nests on soil.	- Lack of knowledge on how to manage colonies.
Epicharis flava	- Wide geographical distribution.	- Lack of knowledge on how to manage colonies.

Conclusions

The selection matrix proved to be a useful tool since even in the absence of data for some of the parameters and species, it was possible to select five social and seven solitary bee species out of 386, based on scientific criteria, which could be used in pesticide risk assessments.

According to this survey, the most abundant bee species in agricultural environments belong to the tribe Meliponini which have different biology and different routes of exposure compared to *Apis mellifera*. A species would need to meet several requirements in order to be a good surrogate: a) be commercially reared so that sufficiently large managed populations are available; b) be easily handled in laboratory, semi-field and field conditions; c) show behavioral and life history traits representative of other species of the same taxonomic or ecological group. Meeting such requirements is challenging and even harder when considering the lack of data needed for the risk assessment process.

In addition, it is extremely important to consider that the matrix is a dynamic tool, the knowledge gaps can be filled as studies on the biology and ecology of native bees advance and thus species that have been excluded until now can be considered in the near future, contributing for a better and more robust process of risk analysis. As science evolves, methods and studies using non-*Apis* bees can be considered and incorporated into risk assessment. Therefore, in the near future Ibama intends to assess the need of changes in the risk assessment procedure for bees, eventually including a stingless bee as a representative species.

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1.13 Using respiratory physiology techniques in assessments of pesticide effects on bees

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Abstract

The determination of sub-lethal effects of pesticides on beneficial insects is challenging topic because the vast number of different possible endpoints. Traditionally measured endpoints reflect the basic outcome but do not give any information about the mode of actions or the real non-harming dosages of the studied toxicants. Physiological changes, however, reflect even small deviations from normal state. The gas exchange patterns are sensitive cues to determine the sub-lethal toxicosis in insects. Methods of respiratory physiology have been used to detect sub-lethal toxic effects of many chemicals, but information for biological preparations is also needed, especially when bees are used in entomovectoring task.

The aims of this study were i) to clarify which are the effects of three microbiological preparations on two bee species, honey bees *Apis mellifera* L. and bumble bees *Bombus terrestris* L. and ii) could we compare the effects of the same preparations on different bee species. We saw that honey bees and bumble bees react similarly on microbiological preparations, however the reaction strength differed. We found that kaolin affects the survival of bumble bees and honey bees as much as did entomopathogenic preparations, whereas pure spores of a non-hazardous fungus and wheat flour did not. Bumble bees seem to be more tolerant to microbiological preparations than honey bees.

Keywords: measuring sub-lethal effect, honey bee, bumble bee, microbiological preparation

Introduction

Pesticide residues in environment are told to be among the reasons contributing to decreasing pollinator populations.[1] Establishment of lethal dosages or concentrations to both target and non-target organisms is demanded by legislation process of pesticides, but sublethal effects have gained much less attention. However, the sub-lethal effects of pesticides may affect insects

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Pieter A. Oomen (Bumble bee *Bombus lapidarius* on thistle)

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