



Survivorship and food consumption of immatures and adults of *Apis mellifera* and *Scaptotrigona bipunctata* exposed to genetically modified eucalyptus pollen

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Received: 21 November 2022 / Accepted: 14 March 2023 / Published online: 7 April 2023
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Abstract *Eucalyptus* comprises the largest planted area of cultivated production forest in Brazil. Genetic modification (GM) of eucalyptus can provide additional characteristics for increasing productivity and protecting wood yield, as well as potentially altering fiber for a diversity of industrial uses. However, prior to releasing a new GM plant, risk assessments studies with non-target organisms must be undertaken. Bees are prominent biological models since they play an important role in varied ecosystems, including for *Eucalyptus* pollination. The main goal of this study was to evaluate whether a novel event (*Eucalyptus* 751K032), which carries the *cp4-epsps* gene that encodes the protein CP4-EPSPS and *nptII* gene that encodes the protein NPTII, might adversely affect honey bees (*Apis mellifera*) and stingless bees (*Scaptotrigona bipunctata*). The experiments were performed in southern Brazil, as follows: (i) larvae and adults were separately investigated, (ii) three or four different pollen diets were offered to bees, depending on larval or adult status, and (iii) two biological

attributes, *i.e.*, survivorship of larvae and adults and food intake by adults were evaluated. The diets were prepared with pollen from GM *Eucalyptus* 751K032; pollen from conventional *Eucalyptus* clone FGN-K, multifloral pollen or pure larval food. The insecticide dimethoate was used to evaluate the sensitivity of bees to toxic substances. Datasets were analyzed with Chi-square test, survival curves and repeated measures ANOVA. Results indicated no evidence of adverse effects of *Eucalyptus* pollen 751K032 on either honey bees or stingless bees assessed here. Therefore, the main findings suggest that the novel event may be considered harmless to these organisms since neither survivorship nor food consumption by bees were affected by it.

Keywords Biotechnology · Biosafety · GMO · Pollinator · Risk assessment · Social bees

Introduction

Biotechnology in the form of genetic engineering has been widely employed into multiple commercial plantations around the world (Park et al. 2011). Such improvements have been targeted at meeting growing demands for timber, cellulose, paper, and biofuel (Harfouche et al. 2011; Meilan et al. 2012; Chang et al. 2018; Arpaia et al. 2021). One of the most important cultivated tree that has being targeted with genetic engineering is the *Eucalyptus*

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genus (Grattapaglia and Kirst 2008; Harfouche et al. 2011; Lucas et al. 2017; Silva et al. 2017). *Eucalyptus* covers the largest planted area of cultivated production forest in Brazil (de Oliveira et al. 2020; Assad et al. 2022). The current area of 7.5 million hectares represents approximately 75% of the entire planted forest area in the country (IBGE 2020; IBA 2021). Genetic modification of *Eucalyptus* provides an addition to conventional tree breeding approaches for significantly increasing and protecting growth, as well as potentially providing improved fiber for a diversity of industrial uses. Currently, *Eucalyptus* provides raw materials for diverse economic sectors (paper/cardboard/packaging, cellulose, furniture, civil construction, resins, and bio-products), while also generating energy for agriculture and steel production (de Oliveira et al. 2020; Assad et al. 2022).

Prior to releasing genetically modified plants in the environment, a risk assessment to identify and evaluate exposure of non-target organisms to the new GM crop along its life cycle must be conducted (Romeis et al. 2008, 2011; Hilbeck et al. 2011; Ricroch et al. 2018). In Brazil, the legislation specifying safety norms and inspection mechanisms for activities involving genetically modified organisms (GMOs) is presented in the law no. 11.105/2005, and in the normative Resolutions of the National Biosafety Technical Commission (CTNBio). CTNBio is responsible to advise the federal government on issues related to biosecurity of GMOs. One of the functions of CTNBio is evaluating whether there would be harm to non-target animals like bees before commercial release of GMOs.

Bees play an important role in pollination of wild and cultivated plants (Heard 1999; Kevan 1999; Klein et al. 2007; Winfree et al. 2007; Mitchell et al. 2009). Furthermore, bees also provide an important source of income and livelihood around the world (Kwapong et al. 2010; Formato and Smulders 2011; Devkota et al. 2016; dos Santos et al. 2021), especially for rural beekeepers. Since bees are dominant visitors to *Eucalyptus* flowers, where they obtain nectar and pollen in abundance (Heard 1999; Braga et al. 2012; Hilgert-Moreira et al. 2014; Gostinski et al. 2018), potential effects of novel substances inserted in these plants require risk assessments on bees. Such evaluation is critical, as bees appear to be sensitives to environmental contaminants. As result, they are good candidates for ecotoxicological analysis (Rose et al.

2007; Gill et al. 2012; Uhl et al. 2016; Cham et al. 2017; Krupke et al. 2017; Newhouse et al. 2021).

Among bees, the honey bee, *Apis mellifera* Linnaeus, 1758 stands out due to its wide geographic distribution and its ecological and economical value, being used as an experimental model in risk assessments of pesticides and GMOs (Malone and Pham-Delègue 2001; Desneux et al. 2007; Duan et al. 2008; Medrzycki et al. 2013; Sanchez-Bayo and Goka 2014; Ricroch et al. 2018). However, *A. mellifera* does not fully represent the diversity of bees in terms of susceptibility, which implies that native bees should be also explored in this context (Barbosa et al. 2015; Cham et al. 2017; Bernardes et al. 2018; Dorneles et al. 2021).

In the tropics, a wide diversity of social bees defined as stingless bees exist (Apidae: Meliponini) (Sakagami 1982). Recently, several species of stingless bees have been included in risk assessments of pesticides and other potential compounds like GMOs (Lima et al. 2013; Barbosa et al. 2015; dos Santos et al. 2016; Bernardes et al. 2018; Seide et al. 2018; Dorigo et al. 2019; Dorneles et al. 2021; Viana et al. 2022). The risk assessments with stingless bees is slightly distinctive from the one conducted with *A. mellifera*. Like honey bees, the stingless bees contribute to pollination in tropical agriculture (Heard 1999; Slaa et al. 2006) and for honey production, generating profitable income for smallholders (dos Santos et al. 2021). One prominent stingless bee species that has been selected as an indicator of the fauna of Brazilian native pollinators is *Scaptotrigona bipunctata* (Lepeletier, 1836). This species has populous colonies, with individuals adapted to experimental conditions (Dorneles et al. 2017, 2021; Diniz et al. 2021) and their natural geographic distribution has been registered in, at least, 13, out of 26 administrative Brazilian states (dos Santos et al. 2021).

To achieve a sustainable environment, risk assessments evaluate how the novel plants (genetically modified *Eucalyptus* trees) may affect non-target arthropods like bees. Thus, in order to contribute to the evaluation of a GMO event prior to its release for commercial use, the main goals of this study were to assess the survivorship (temporal attribute) and food consumption (behavioral attribute) of immatures and adults of two species of bees (*A. mellifera*, *S. bipunctata*) ingesting a diet containing pollen grains of genetically modified *Eucalyptus*.

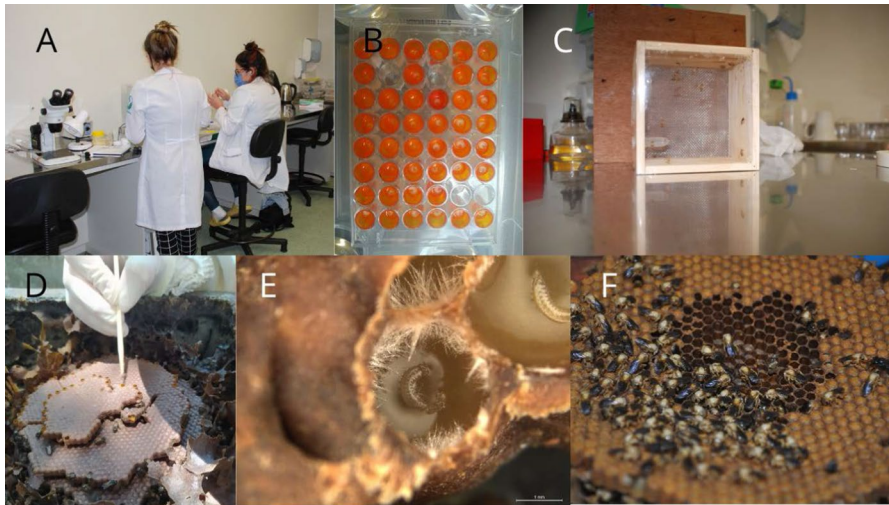


Fig. 1 Pictures showing different moments of study. **A** Researchers (FGC, JDR) revising protocols prior conducting collects in beehives. **B** A rearing plate used to expose *Apis mellifera* (Apidae, Apini) larvae to different pollen diet. **C** A patterned wood box for exposing adults of *A. mellifera* to dif-

ferent pollen diets. **D** A comb of *Scaptotrigona bipunctata* (Apidae, Meliponini) used to provide bee larvae and larval food in the experiments. **E** A detail of two larvae of *S. bipunctata*. **F** Newly-emerged workers of *S. bipunctata* used for the experiments

Materials and methods

Bees

Immature and adult bees came from standardized and healthy hives. Individuals of *A. mellifera* were obtained from three colonies kept in the apiary of the Federal University of Rio Grande do Sul (UFRGS). Specimens of the stingless bees *S. bipunctata* were obtained from three colonies maintained in the scientific stingless apiary (CTF475062/Cat20-45-MMA/IBAMA) of the Pontifical Catholic University of Rio Grande do Sul (PUCRS). Both locations, UFRGS and PUCRS, are located in the municipality of Porto Alegre, southern Brazil. All experiments were performed at Center for Experimental Biological Models (CeMBE, PUCRS) in an authorized biosafety structure (Biosafety Quality Certificate, CQB 390/15), Fig. 1A–E.

Pollen

The pollen from *Eucalyptus* 751K032 (10 g), and conventional clone individuals FGN-K (10 g) were obtained from manual collections in the flowers within greenhouse cultivation of Suzano (Futura-Gene Division), located in Itapetininga/ Brazil (CQB

325/11). Commercial, multifloral, dehydrated pollen grains (of honey bees) were included in treatments representing negative control. The choice of *Eucalyptus* 751K032 represents a landmark achievement in the world of agriculture and forestry, as it is the first genetically modified eucalyptus approved for commercial use that possesses tolerance to the herbicide glyphosate. This innovative technology offers a number of crucial benefits to the Brazilian forestry industry. It enables the implementation of effective and environmentally friendly weed control methods, eliminates the risk of damage to young plantlets from herbicide drift, leads to reduced operational costs and improved working conditions for forestry operators through the use of advanced machinery. In short, the adoption of this genetically modified eucalyptus event represents a major step forward in sustainable and efficient forestry management.

Exposing immatures of *Apis mellifera* to different pollen diets

For exposures, (i) 10 mg of *Eucalyptus* pollen 751K032 was added in 30 µL of larval food (Steijven et al. 2016). Similarly, the control groups had the addition of, (ii) 10 mg of conventional *Eucalyptus* clone FGN-K (1st negative control) and (iii) 10 mg of

multifloral pollen (2nd negative control) in 30 μL of larval food. The last treatment was (iv) diet without pollen addition (hereafter, WPA). The active ingredient dimethoate (99.3%, Ehrenstorfer GmbH) was used to evaluate the sensitivity of bees to toxic substances. It was added to the larval food, at the corresponding value of 8.8 μg a.i./larva for *A. mellifera* (OECD 2013).

Apis mellifera larvae were obtained from brood combs from three colonies, each one representing one replicate. Larvae aged 2 to 3 days were transferred to in vitro breeding plates and placed in breeding chambers with controlled temperature (32 ± 1 °C), relative humidity ($95 \pm 1\%$) and absence of light. The larvae were exposed to a single dose of the product on the 4th day of the test (OECD 2013). Each treatment (triplicate) was conducted with 12 bees. Therefore, there were 36 individuals per treatment, and a total of 144 honey bees were analyzed (triplicates $\times$ four treatments). After the onset of exposure, the survival rate was verified and recorded on 24 h, 48 h, and 72 h or, respectively, 5th, 6th and 7th days.

Exposing immatures of *Scaptotrigona bipunctata* to different pollen diets

The amount of larval food offered to offspring of *S. bipunctata* within their natural colonies is 35 μL (Rosa et al. 2015) and the amount of pollen in such a volume of larval food is around 1.9 mg/larva (Rosa et al. 2015). The treatments were composed by 35 μL aliquot of larval food with addition of (i) 1.9 mg of *Eucalyptus* pollen 751K032; (ii) 1.9 mg of conventional clone *Eucalyptus* FGN-K pollen (1st negative control); (iii) 1.9 mg of multifloral pollen (2nd negative control); and the fourth treatment was (iv) WPA. To evaluate the sensitivity of bees to toxic substances, 172 ng a.i./larva of the active ingredient dimethoate (99.3%, Ehrenstorfer GmbH) that is LD_{50} for *S. postica* (Dorneles et al. 2021) was added to the larval food.

Scaptotrigona bipunctata larvae were obtained from brood combs from three colonies, each one representing a replicate. Larvae aging 1 to 3 days were transferred to in vitro rearing plates. The feeding process for *S. bipunctata* larvae, like all stingless bees, is unique and distinct from that of *A. mellifera*. Unlike *A. mellifera*, where the larvae are fed progressively by nurse workers over several days, *S. bipunctata*

larvae receive all the food they need in a single feeding. This method ensures that the larvae receive the proper nutrients in a timely manner to support their development (OECD 2013, 2017; Crailsheim et al. 2013), stingless bee species are fed massively, i.e., larvae receive the entire amount of food to develop to the adult stage (Sakagami 1982). Accordingly, *S. bipunctata* larvae were placed in breeding chambers with controlled temperature (28 ± 1 °C) and relative humidity ($98 \pm 1\%$) during the feeding period within following 1st to the 5th day. After that, saturated sodium chloride solution was added within in vitro rearing plates until the end of the experiment since it keeps relative humidity around 75% (Dorneles et al. 2021). Three replicates with 11 bees each were performed. Therefore, 33 individuals per treatment, totaling 132 stingless bees were analyzed (triplicates $\times$ four treatments). The survival of individuals was recorded daily until the 14th day, when they enter pre-pupa stage, with the larval development only.

Exposing adults of *Apis mellifera* to different pollen diets

Mature brood combs of *A. mellifera* were collected from three colonies. The combs had no contact with each other. Each material was collected individually, the material was sterilized, and changed (when necessary) for each colony collection. Both the collection material and combs were individually placed in identified Petri dishes. They were kept in a brood chamber until adult emergence. The newly emerged workers, at a maximum of two days old, were transferred to wooden boxes ($12 \times 12 \times 7$ cm) for the tests. Prior to the beginning of the tests, the bees were adapted for 24 h, being fed with syrup only (1:1 sucrose solution). During the entire experiment, the bees were kept in rearing chambers with controlled temperature (32 ± 2 °C), relative humidity ($70 \pm 2\%$) and absence of light. Pollen was added to the syrup to facilitate consumption (Rose et al. 2007).

For exposing, the treatments were: (i) 13 mg of pollen of *Eucalyptus* 751K032 / mL of food (Yi et al. 2018); (ii) 13 mg of pollen from conventional clone *Eucalyptus* FGN-K (1st negative control); (iii) 13 mg of multifloral pollen (2nd negative control). Additionally, the active ingredient dimethoate (99.3%, Ehrenstorfer GmbH) CL_{50} for *A. mellifera*, which is 7.96 ng ai/ μL of diet (Barbosa 2015) was evaluated

to corroborate its toxicity to bees. Each experimental group was tested in triplicate, with 15 bees. Therefore, 45 individuals per treatment, totaling 135 honey bees were analyzed (triplicates $\times$ three treatments).

The bees were monitored daily in order to account for the mortality of individuals and the amount of diet consumed. The food was offered to the bees *ad libitum* during the exposure period of 10 days (chronic) and the food was changed daily. The parameters evaluated are in accordance with protocol 245—honey bee (*A. mellifera*), chronic oral toxicity test—10 day feeding (OECD 2017).

Exposing adults of *Scaptotrigona bipunctata* to different pollen diets

Brood combs of *S. bipunctata* were collected from three colonies of the PUCRS stingless bee apiary. They were kept in a brood chamber until the emergence of adults. The newly emerged workers (max. 2 days) were collected and transferred to wooden boxes (12 $\times$ 12 $\times$ 7 cm) for testing. Prior to the beginning of the tests, the bees were adapted for 24 h, being fed with syrup only (1:1 sucrose solution). During the entire experiment, the bees were kept in rearing chambers with controlled temperature (28 $\pm$ 2 °C), relative humidity (70 $\pm$ 2%) and absence of light. Pollen was added to the syrup to facilitate consumption (Rose et al. 2007). The exposure treatments were identical for both honeybees and *S. postica*. Only that the sensitivity test using the CL₅₀ of the active ingredient dimethoate, which was 3.24 ng ai/ μ L in the diet for *S. postica* (Tofili et al. 2018).

Data analysis

All statistical analyzes were conducted in R programming language (Ihaka and Gentleman 1996; R Core Team 2021).

Eucalyptus pollen 751K032 effect on immatures of *Apis mellifera*

The possible effects of *Eucalyptus* pollen 751K032 on immatures of *A. mellifera* was analysed with a Chi-square test with multiple comparisons for each time point (24 h, 48 h, 72 h). For this, the ‘chisq.multip-comp’ function of the *RVAideMemoire* (Hervé 2020)

was used. The *p*-values were adjusted with the Benjamini–Hochberg method.

Effect of *Eucalyptus* pollen 751K032 on the survival of immatures and adults of honey bees and stingless bees

The evaluation of the possible effects of *Eucalyptus* pollen 751K032 on immatures of *S. bipunctata* and both adults of *A. mellifera* and *S. bipunctata* was performed by analyzing and comparing the survival curves for the larval development period of the immatures and for a pre-established period of consumption by the adults, respectively. In the first case, this period covered the first days of the larvae (1–3 days) until the phase in which they all became pupae, which is the most critical stage of bee development, where most mortality is commonly observed (Santos et al. 2015; Dorneles et al. 2021). In the second case, the exposure of adults occurred for a period of 10 consecutive days.

The Kaplan–Meier survival curves were generated using the ‘Surv’ function and the difference between them was tested using the ‘survdiff’ function both from the *survival* (Therneau 2020). Here, the development time (*right censored*) and the status, i.e., the event indicating 1 = death or 0 = survival of the organisms were evaluated by the treatments as predictor variable. This test was stratified by replicates to control for possible inconsistencies within each of them. Finally, the *log-rank* test was selected as a scalar parameter of the *G-rho* family, which prioritizes the differences at the end of two or more survival curves. After that, multiple comparisons were made between the survival curves to investigate which curves would be different according to the corresponding treatment. This procedure was done with *p*-values adjusted by the Benjamini–Hochberg method using the ‘pairwise_survdiff’ function of the *survminer* (Kassambara et al. 2020). The survival curve plots were constructed using the ‘survfit’ function from the *survival* (Therneau 2020), ‘ggsurvplot’ from the *survminer* (Kassambara et al. 2020) and *ggplot2* (Wickham 2016).

Eucalyptus pollen effect 751K032 on food consumption by adults of honey bees and stingless bees

Finally, we performed repeated measures ANOVA to assess the daily food consumption by adults of *A. mellifera* and *S. bipunctata*. In this model, consumption was the response variable and treatments the predictor (fixed) variable, with the random variable assigned as the replicates per treatment. This analysis was done using the ‘aov’ function in R. Pairwise comparison was extracted with the ‘emmeans’ function of the *emmeans* (Lenth 2022) using Tukey method.

Results

Mortality of immatures of *Apis mellifera* after 24 h, 48 h and 72 h

Practically all *A. mellifera* immatures exposed to dimethoate died in the first hours of observation.

Yet, the deaths under statistical analysis of immatures of *A. mellifera* during the experiments of exposure to different treatments through the diet showed no significant difference (Table 1, Fig. 2A). Therefore, the groups treated with *Eucalyptus* pollen 751K032 and with the other diets did not differ significantly.

Survival curves of immatures of *Scaptotrigona bipunctata*

The immatures of *S. bipunctata* did not survive for more than five days after exposure to dimethoate. On the other hand, data indicate that there was no difference between treatments using any types of pollen and the diet without pollen addition; however ($\chi^2 = 1.00$, p -value = 0.80, Table 2, Fig. 2). Treatments using pollen from *Eucalyptus* 751K032 and conventional clone *Eucalyptus* FGN-K tended to have a slightly higher survival ($\geq 81\%$) than the control without pollen addition (75%), although this difference is not significant (Table 2; Fig. 2B).

Table 1 Results of the Chi-square test with paired comparisons on the mortality of immatures of *Apis mellifera* (Hymenoptera: Apidae, Apini) treated with different diets, including

eucalyptus pollen 751K032. The acronyms given in the treatments column are the same as those used in paired comparisons

Periods	χ^2	Degrees of freedom	p -values
24 h	0.57	3	0.90
48 h	0.27	3	0.96
72 h	3.33	3	0.34
	WPA	GME	ECC
<i>Pairwise comparisons (p-values)</i>			
24 h			
GME	1.00	–	–
ECC	0.92	0.92	–
MFP	0.92	0.92	0.92
48 h			
GME	1.00	–	–
ECC	1.00	1.00	–
MFP	1.00	1.00	1.00
72 h			
GME	0.48	–	–
ECC	0.47	0.65	–
MFP	0.47	0.48	0.65

WPA=diet without pollen addition; GME=eucalyptus pollen 751K032; ECC=conventional clone eucalyptus pollen FGN-K; MFP=multifloral pollen

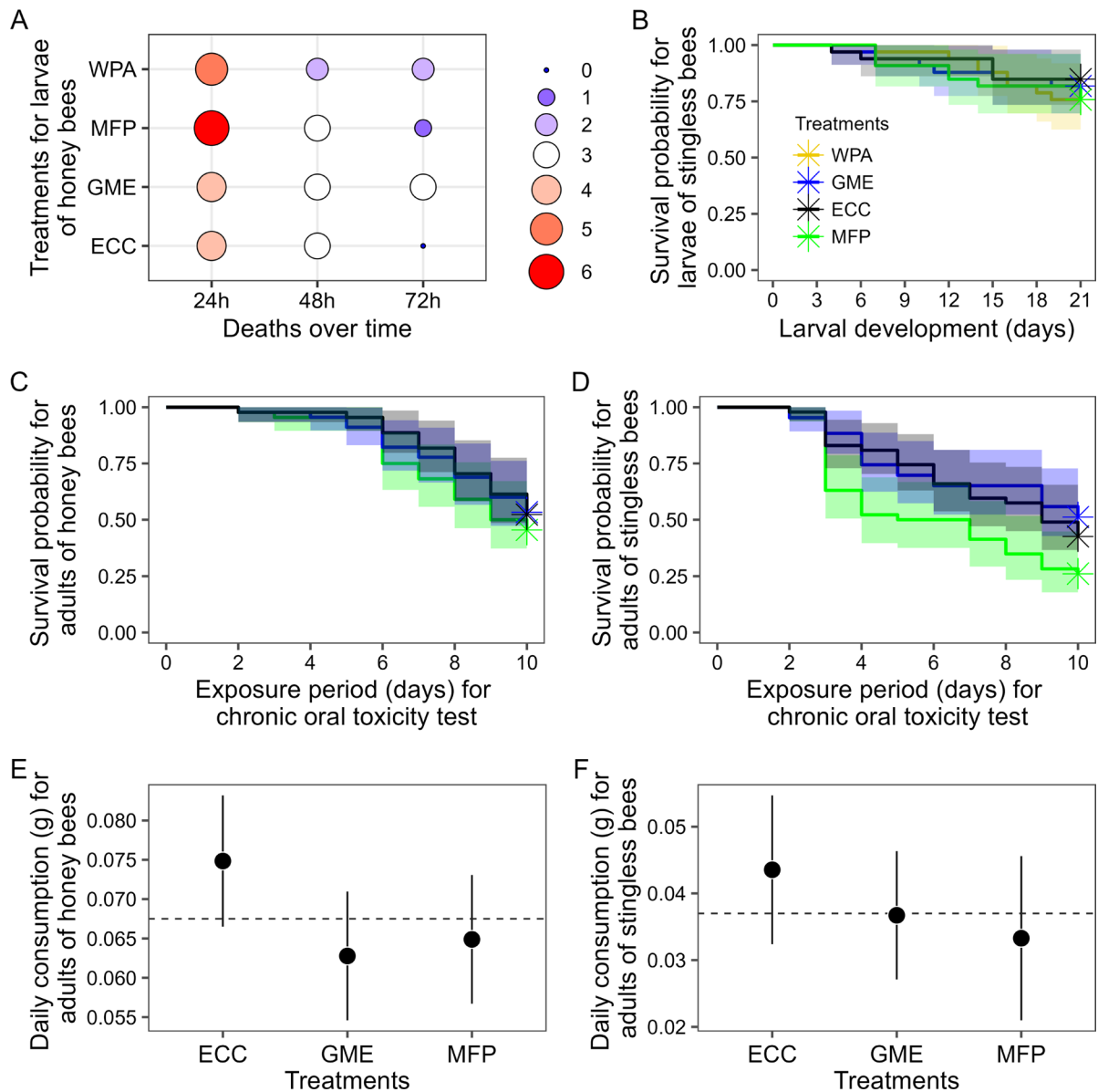


Fig. 2 **A** Ballonplot showing results of Chi-square test. The size of points indicate the proportional mortality values of larvae of *Apis mellifera* (Apidae, Apini) exposed to different pollen diets. Note: The legend on the right shows the values in size scale and by color scale, the hottest being the biggest losses. The acronyms used in y-axis are applicable to panels B up to F, they are: WPA=diet without pollen addition; GME=*Eucalyptus* pollen 751K032; ECC=conventional clone eucalyptus pollen FGN-K [1st negative control]; MFP=Mul-tifloral pollen [2nd negative control]. **B** Kaplan–Meier survival curves for larvae of *Scaptotrigona bipunctata* (Apidae, Meliponini). Notes: 95% confidence intervals are provided as

colored shadows. Asterisks mean right censored (end of experiment). It is also applicable for panels C and D. **C** Kaplan–Meier survival curves for adults of *A. mellifera* exposed to different types of pollen diets. **D** Kaplan–Meier survival curves for adults of *S. bipunctata* exposed to different types of pollen diets. **E** and **F** Daily consumption of food offered to both adults of *A. mellifera* and *S. bipunctata*, respectively, containing different pollen diets. Notes: Points mean average values, while vertical lines indicate 95% confidence intervals; Horizontal dashed lines show the overall average of consumption. It was inserted to facilitate interpretation. (Color figure online)

Table 2 Results of survival curve analysis by log-rank test (upper) and test of pairwise comparisons between each treatment of the experiments of possible effects of eucalyptus pol-len 751K032 on immature *Scaptotrigona bipunctata* (Apidae, Meliponini). The acronyms given in the treatments column are the same as those used in pairwise comparisons

Treatments	The amount	Observed	Expected	(OE)^2/E	(OE)^2/V
WPA	33	8	6.94	0.16	0.22
GME	33	5	6.70	0.43	0.58
ECC	33	6	6.74	0.08	0.11
MFP	33	8	6.62	0.28	0.38
$\chi^2 = 1.00$, degrees of freedom = 3, p -value 0.80					
WPA		GME		ECC	
<i>Pairwise comparisons (p-values)</i>					
GME	0.88	–		–	
ECC	0.88	0.88		–	
MFP	0.95	0.88		0.88	

WPA=diet without pollen addition; GME=eucalyptus pollen 751K032; ECC=conventional clone eucalyptus pollen FGN-K; MFP=multifloral pollen

Table 3 Results of survival curve analysis by log-rank test (upper) and test of pairwise comparisons between each treatment of the experiments of possible effects of eucalyptus pol-len 751K032 on adult worker bees of *Apis mellifera* (Hymenoptera: Apidae, Apini). The acronyms given in the treatments column are the same as those used in pairwise comparisons

Treatments	The amount	Observed	Expected	(OE)^2/E	(OE)^2/V
GME	45	21	23.0	0.17	0.29
ECC	44	24	20.1	0.74	1.20
MFP	44	21	22.9	0.15	0.26
$\chi^2 = 1.2$, degrees of freedom = 2, p -value = 0.50					
GME					ECC
<i>Pairwise comparisons (p-values)</i>					
GME		0.64			–
MFP		0.64			0.96

GME=eucalyptus pollen 751K032; ECC=conventional clone eucalyptus pollen FGN-K; MFP=multifloral pollen

Survival curves of adults of both *Apis mellifera* and *Scaptotrigona bipunctata*

No adult *A. mellifera* exposed to dimethoate survived for more than two days. The survival curves of *A. mellifera* adults showed no difference between treatments with *Eucalyptus* pollen 751K032, conventional clone *Eucalyptus* pollen FGN-K and multifloral pollen ($\chi^2 = 1.2$, p -value = 0.50, Table 3, Fig. 2C). In descending order, the survival of *A. mellifera* adults was: *Eucalyptus* pollen 751K032 (53%), multifloral pollen (52%), conventional clone *Eucalyptus* pollen FGN-K (45%), Fig. 2C.

Similarly, no adult *S. bipunctata* exposed to dimethoate survived for more than four days. The survival curves of *S. bipunctata* adults indicate differences between treatments ($\chi^2 = 7.3$, p -value < 0.03, Table 4, Fig. 2D). In the pairwise analysis, conventional clone *Eucalyptus* FGN-K pollen differed of *Eucalyptus* pollen 751K032, Table 4. In this case, the survival rate of adults fed with conventional pollen was practically half of that observed for bees treated with *Eucalyptus* pollen 751K032. However, there was no difference between *Eucalyptus* pollen 751K032 and multifloral pollen, Table 4. In descending order, the survival of *S. bipunctata* adults was: *Eucalyptus* pollen 751K032 (51%), multifloral pollen (42%), and

conventional clone *Eucalyptus* FGN-K pollen (26%), Fig. 2D.

Food consumption by adults of both *Apis mellifera* and *Scaptotrigona bipunctata*

There was no difference in food consumption by *A. mellifera* adults (*repeated measures* ANOVA, $F_{(2, 73)} = 2.47$, p -value = 0.09), Table 5, Fig. 2E). The same was observed, that is, no difference in food consumption by adults of *S. bipunctata* (*repeated measures* ANOVA, $F_{(2, 67)} = 0.83$, p -value = 0.43). Evidently, therefore, in the pairwise comparisons there are no differences between the pairs of treatments (Table 6, Fig. 2F).

Discussion

The findings suggest that the survivorship and the food consumption of immatures and adults of *A. mellifera* and *S. bipunctata* were not affected by genetically modified eucalyptus pollen (*Eucalyptus* pollen 751K032). Overall, the treatments with *Eucalyptus* pollen 751K032 and conventional clone *Eucalyptus* and multifloral pollen (negative controls) demonstrated very similar survival curves and food consumption, according to the bee species being tested. Subsequently, as food offered to larvae included a diet without pollen addition, results were concordant with other treatments (novel event and negative controls). Parallel, it was observed that most immatures

Table 4 Results of the survival curve analysis by the log-rank test (upper) and the test of pairwise comparisons between each treatment of the experiments of the possible effects of 751K032 eucalyptus pollen on adult worker bees of *Scap-*

totrigona bipunctata (Hymenoptera: Apidae, Meliponini). The acronyms given in the treatments column are the same as those used in pairwise comparisons

Treatments	The amount	Observed	Expected	(OE) ² /E	(OE) ² /V
GME	43	21	28.6	2.00	3.53
ECC	46	34	24.1	4.11	6.80
MFP	47	27	29.4	0.19	0.35
$\chi^2 = 7.30$, degrees of freedom = 2, p -value < 0.03					
ECC					GME
<i>Pairwise comparisons (p-values)</i>					
GME	0.02				–
MFP	0.06				0.50

GME = eucalyptus pollen 751K032; ECC = conventional clone eucalyptus pollen FGN-K; MFP = multifloral pollen

Table 5 Results of the repeated measures ANOVA showing the paired comparisons on the daily food consumption by adult bees of *Apis mellifera* (Hymenoptera: Apidae, Apini) related to the experiments of possible effects of eucalyptus pollen

751K032 on these bees. The acronyms given in the treatments column are the same as those used in pairwise comparisons with Tukey method

	Degrees of freedom	Sum of squares	F-value	<i>p</i> -value
Treatments	2	0.002	2.47	0.09
Residuals	73	0.029		
	Estimate	SE	<i>p</i> -values	
<i>Pairwise comparisons</i>				
GME × ECC	0.012	0.005	0.09	
ECC × MFP	0.009	0.005	0.19	
GME × MPF	−0.002	0.005	0.92	

GME = eucalyptus pollen 751K032; ECC = conventional clone eucalyptus pollen FGN-K; MFP = multifloral pollen; SE = Standard Error

Table 6 Results of the repeated measures ANOVA showing the paired comparisons on the daily food consumption by adult bees of *Scaptotrigona bipunctata* (Hymenoptera: Apidae, Meliponini) related to the experiments of the possible effects

	Degrees of freedom	Sum of squares	F-value	p-value
Treatments	2	0.001	0.83	0.43
Residuals	67	0.045		
	Estimate	SE		p-value
<i>Pairwise comparisons</i>				
GME × ECC	0.006	0.007		0.66
ECC × MFP	0.009	0.007		0.41
GME × MPF	0.003	0.007		0.90

GME=eucalyptus pollen 751K032; ECC=conventional clone eucalyptus pollen FGN-K; MFP=multifloral polle; SE=Standard Error

and adults of both bee species exposed to dimethoate, a well-known toxic insecticide for bees (Crailsheim et al. 2013; Pirk et al. 2013), died faster than in treatments under statistical analysis or they tended to consume an extremely low amount of food, if any.

We highlight that despite the fact that these newly expressed proteins are known to be non-toxic to many insect species, including bees, it was necessary to perform bioassays to exclude possible unintended effects. This approach is consistent with regulatory requirements in Brazil and EU legislation, as well as scientific best practices since it provides important information for stakeholders about safety and potential benefits for pollinators.

Commonly, risk assessment studies structure their experimental designs with one positive control and a single negative control (Malone and Pham-Delègue 2001; Desneux et al. 2007; Duan et al. 2008; Crailsheim et al. 2013; Medrzycki et al. 2013; Pirk et al. 2013; Sanchez-Bayo and Goka 2014). Here, in order to augment our decision power in inferring whether the novel substance under evaluation (*Eucalyptus* pollen 751K032) might or might not cause adverse effects on the bees being evaluated, the experiments were carried out with two negative controls, namely the conventional clone *Eucalyptus* FGN-K (1st negative control) and the multifloral pollen (2nd negative control). Consequently, suitable and more consistent evidence demonstrated that the target GMO had no effect during the development larval of *A. mellifera* and *S. bipunctata*. Similarly, adults

of eucalyptus pollen 751K032 on these bees. The acronyms given in the treatments column are the same as those used in pairwise comparisons with Tukey method

of both bee species showed equivalent rates of food intake.

The major goal of these experiments was to compare the protein diet containing *Eucalyptus* pollen 751K032 against mainly conventional clone *Eucalyptus* FGN-K and multifloral pollen. As they were more similar to each other exhibiting average values and confidence intervals close to the overall mean, all treatments with any type of pollen were congruent per se.

To add more reliability in the risk assessment, this study was carried out with two bee species. Therefore, not only a traditional bee specie was tested, i.e., *A. mellifera* (Malone and Pham-Delègue 2001; Duan et al. 2008; Romeis et al. 2008, 2011; Hilbeck et al. 2011; Arpaia et al. 2021), but also a native and surrogate stingless bee species (*S. bipunctata*), as recommended by Brazilian norms (Cham et al. 2017). Also, different pollen diets were offered and they were evaluated in two stages of the life cycle of both bee species (immatures and adult workers). Finally, survivorship was investigated for individuals over time during their larval development as well as survival of adults consuming different pollen diets. Aggregating the results, it may be considered that the genetically modified eucalyptus pollen (*Eucalyptus* pollen 751K032) is harmless to the organisms assessed.

The measurement of food consumption is only one aspect of the numerous sublethal effects that are relevant to pollinators (Arpaia et al. 2011). This is

why a more comprehensive approach is needed to understand the potential impact of genetically modified agro-ecosystems on bee populations. A multifaceted approach, including examination of reproduction, behavior, and overall health, provides a more comprehensive understanding of the potential impact on pollinators. For a sustainable environment, it is important to constantly review the exposure of bees to genetically engineered crops (Seide et al. 2018). However, recent literature reviews have indicated that most of GM crops are harmless to bees under laboratory conditions or in field/semi-field trials (Ricroch et al. 2018; Arpaia et al. 2021). Accordingly, this work which demonstrated a lack of adverse effects on two bee species during the ingestion by immatures and adults of the *Eucalyptus* pollen 751K032, adds to this growing body of safety data.

Based on a comprehensive analysis of previous research on the impact of GM crops on pollinators, studies have shown that the impact on bees and other pollinators has been either neutral or minimal. Thus, although some studies have found limited evidence of a significant impact on bee health, the negligible effects find of GM crops and outcomes from lab, semi-field, and field on bees cannot be overemphasized. It is imperative to continue the discussion and further investigate the effects of GM crops on pollinators, which is crucial in making well-informed decisions about their use in agriculture and ensuring the sustainable health of pollinator populations.

Author contribution All authors planned the biological questions and the sampling design. JDR, FGC and AD collected the biological data, performed the experiments, and organized dataset for statistical analysis. CFS executed the data analysis. All authors contributed to conceptual development of this work and they wrote the original draft. All authors discussed the main topics and reviewed the last version of the manuscript.

Funding This study was supported by a postdoctoral fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Process Number: 309542/2020-0) linked to FAPERGS/CNPq/SEBRAE 08/2019 (Programa Doutor Empreendedor—PDEmp, Process Number: 20/2551-0000203-9) provided to CFS. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Availability of data and materials The datasets generated and analyzed during the current study are deposited in GitHub repository in Zenodo.

Code availability Not applicable.

Declarations

Competing interests During the development of activities, the authors were working at Suzano S/A, FuturaGene or were third-party contractors of Suzano S/A, which develops genetically modified (GM) eucalyptus.

Ethics approval This work complied with the laws governing animal research in Brazil.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest During the development of activities, the authors were working at Suzano S/A, FuturaGene or were third-party contractors of Suzano S/A, which develops genetically modified (GM) eucalyptus.

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References

- Arpaia S, De Cristofaro A, Guerrieri E, Bossi S, Cellini F, Di Leo GM, Germinara GS, Iodice L, Maffei ME, Petrozza A, Sasso R, Vitagliano S (2011) Foraging activity of bumblebees (*Bombus terrestris* L.) on Bt-expressing eggplants. *Arthropod-Plant Inter* 5:255–261. <https://doi.org/10.1007/s11829-011-9144-5>
- Arpaia S, Smaghe G, Sweet JB (2021) Biosafety of bee pollinators in genetically modified agro-ecosystems: current approach and further development in the EU. *Pest Manag Sci* 77:2659–2666. <https://doi.org/10.1002/ps.6287>
- Assad ED, Zanatta JA, Rachwal MFG et al (2022) Impact of climate change on eucalyptus plantations in southern Brazil. *Colombo: Embrapa Florestry*
- Barbosa WF, Tomé HVV, Bernardes RC et al (2015) Biopesticide-induced behavioral and morphological alterations in

- the stingless bee *Melipona quadrifasciata*. Environ Toxicol Chem 34:2149–2158. <https://doi.org/10.1002/etc.3053>
- Barbosa RA (2015) Avaliação dos efeitos de concentrações subletais de dimetoato no cérebro e ventrículo de *Apis mellifera* africanizada. Universidade Estadual Paulista
- Bernardes RC, Barbosa WF, Martins GF, Lima MAP (2018) The reduced-risk insecticide azadirachtin poses a toxicological hazard to stingless bee *Partamona helleri* (Friese, 1900) queens. Chemosphere 201:550–556. <https://doi.org/10.1016/j.chemosphere.2018.03.030>
- Braga JA, Sales EO, Neto JS et al (2012) Floral sources to *Tetragonisca angustula* (Hymenoptera: Apidae) and their pollen morphology in a Southeastern Brazilian Atlantic forest. Rev Biol Trop 60:1491–1501
- Cham KO, Rebelo RM, Oliveira RP et al (2017) Manual de avaliação de risco ambiental de agrotóxicos para abelhas. Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. Ministério do Meio Ambiente, Brasília/Brazil
- Chang S, Mahon EL, MacKay HA et al (2018) Genetic engineering of trees: progress and new horizons. Vitro Cell Dev Biol - Plant 54:341–376. <https://doi.org/10.1007/s11627-018-9914-1>
- Crailsheim K, Brodschneider R, Aupinel P et al (2013) Standard methods for artificial rearing of *Apis mellifera* larvae. J Apic Res 52:1–16. <https://doi.org/10.3896/IBRA.1.52.1.05>
- de Oliveira AL, Borges LAC, Junior MGC et al (2020) Forest replacement in Brazil: a fundamental policy for forestry. Floresta e Ambient 27:1–12. <https://doi.org/10.1590/2179-8087.002118>
- Desneux N, Decourtye A, Delpuech J-M (2007) The sublethal effects of pesticides on beneficial arthropods. Annu Rev Entomol 52:81–106. <https://doi.org/10.1146/annurev.ento.52.110405.091440>
- Devkota K, Dhakal SC, Thapa RB (2016) Economics of beekeeping as pollination management practices adopted by farmers in Chitwan district of Nepal. Agric Food Secur 5:6. <https://doi.org/10.1186/s40066-016-0053-9>
- Diniz TO, Pereira NC, da Silva BG et al (2021) Effects of combined fungicide in stingless bees *Scaptotrigona bipunctata*. Res Soc Dev 10:e53710112029. <https://doi.org/10.33448/rsd-v10i1.12029>
- Dorigo AS, Rosa-Fontana AS, Soares-Lima HM et al (2019) In vitro larval rearing protocol for the stingless bee species *Melipona scutellaris* for toxicological studies. PLoS ONE 14:e0213109
- Dorneles AL, Rosa AS, Blochtein B (2017) Toxicity of organophosphorus pesticides to the stingless bees *Scaptotrigona bipunctata* and *Tetragonisca fiebrigii*. Apidologie. <https://doi.org/10.1007/s13592-017-0502-x>
- Dorneles AL, Rosa-Fontana AS, dos Santos CF, Blochtein B (2021) Larvae of stingless bee *Scaptotrigona bipunctata* exposed to organophosphorus pesticide develop into lighter, smaller and deformed adult workers. Environ Pollut 272:116414. <https://doi.org/10.1016/j.envpol.2020.116414>
- dos Santos CF, Acosta AL, Dorneles AL et al (2016) Queens become workers: pesticides alter caste differentiation in bees. Sci Rep 6:1–9. <https://doi.org/10.1038/srep31605>
- dos Santos CF, Raguse-Quadros M, Ramos JD et al (2021) Diversidade de abelhas-sem-ferrão e seu uso como recurso natural no Brasil: permissões e restrições legais consorciadas a políticas públicas. Rev Bras Meio Ambiente 9:2–22
- Duan JJ, Marvier M, Huesing J et al (2008) A meta-analysis of effects of Bt crops on honey bees (Hymenoptera: Apidae). PLoS ONE. <https://doi.org/10.1371/journal.pone.0001415>
- Formato G, Smulders FJM (2011) Risk management in primary apicultural production. Part 1: Bee health and disease prevention and associated best practices. Vet Q 31:29–47. <https://doi.org/10.1080/01652176.2011.565913>
- Gill RJ, Ramos-Rodriguez O, Raine NE (2012) Combined pesticide exposure severely affects individual- and colony-level traits in bees. Nature 490:105–108. <https://doi.org/10.1038/nature11585>
- Gostinski LF, Oliveira FF, Contrera FAL, Albuquerque PMC (2018) Trophic niche and floral resources partition between two species of *Melipona* (Hymenoptera, Apidae) in the Eastern Amazon. Oecologia Aust 22:449–462. <https://doi.org/10.4257/oeco.2018.2204.08>
- Grattapaglia D, Kirst M (2008) *Eucalyptus* applied genomics: from gene sequences to breeding tools. New Phytol 179:911–929. <https://doi.org/10.1111/j.1469-8137.2008.02503.x>
- Harfouche A, Meilan R, Altman A (2011) Tree genetic engineering and applications to sustainable forestry and biomass production. Trends Biotechnol 29:9–17. <https://doi.org/10.1016/j.tibtech.2010.09.003>
- Heard TA (1999) The role of stingless bees in crop pollination. Annu Rev Entomol 44:183–206
- Hervé M (2020) RVAideMemoire: Diverse basic statistical and graphical functions
- Hilbeck A, Meier M, Römbke J et al (2011) Environmental risk assessment of genetically modified plants - concepts and controversies. Environ Sci Eur 23:13. <https://doi.org/10.2478/ffp-2014-0011>
- Hilgert-Moreira SB, Fernandes MZ, Marchetti CA, Blochtein B (2014) Do different landscapes influence the response of native and non-native bee species in the *Eucalyptus* pollen foraging, in southern Brazil? For Ecol Manag 313:153–160. <https://doi.org/10.1016/j.foreco.2013.10.049>
- IBA (2021) Relatório anual 2021. Accessed 8 Feb 2022
- IBGE (2020) As florestas plantadas – Painel interativo. Accessed 20 Jul 2020
- Ihaka R, Gentleman R (1996) R: a language for data analysis and graphics. J Comput Graph Stat 5:299–314
- Kassambara A, Kosinski M, Bieck P (2020) survminer: Drawing survival curves using “ggplot2”
- Kevan PG (1999) Pollinators as bioindicators of the state of the environment: species, activity and diversity. Agric Ecosyst Environ 74:373–393. [https://doi.org/10.1016/S0167-8809\(99\)00044-4](https://doi.org/10.1016/S0167-8809(99)00044-4)
- Klein A-M, Vaissière BE, Cane JH et al (2007) Importance of pollinators in changing landscapes for world crops. Proc R Soc B Biol Sci 274:303–313. <https://doi.org/10.1098/rspb.2006.3721>
- Krupke CH, Holland JD, Long EY, Eitzer BD (2017) Planting of neonicotinoid-treated maize poses risks for honey bees and other non-target organisms over a wide area without consistent crop yield benefit. J Appl Ecol 54:1449–1458. <https://doi.org/10.1111/1365-2664.12924>

- Kwapong P, Kwame A, Rofela C, Afia K (2010) Stingless bees: Importance, management and utilisation (a training manual for stingless beekeeping). Unimax MacMillan
- Lenth R (2022) emmeans: Estimated marginal means, aka least-squares means. <https://CRAN.R-project.org/package=emmeans>
- Lima MAP, Pires CSS, Guedes RNC, Campos LAO (2013) Lack of lethal and sublethal effects of Cry1Ac Bt-toxin on larvae of the stingless bee *Trigona spinipes*. *Apidologie* 44:21–28. <https://doi.org/10.1007/s13592-012-0151-z>
- Lucas AM, Pasquali G, Astarita LV, Cassel E (2017) Comparison of genetically engineered (GE) and non-GE Eucalyptus trees using secondary metabolites obtained by steam distillation. *J Essent Oil Res* 29:22–31. <https://doi.org/10.1080/10412905.2016.1187674>
- Malone LA, Pham-Delègue M-H (2001) Effects of transgene products on honey bees (*Apis mellifera*) and bumblebees (*Bombus* sp.). *Apidologie* 32:287–304
- Medrzycki P, Giffard H, Aupinel P et al (2013) Standard methods for toxicology research in *Apis mellifera*. *J Apic Res* 52:1–60. <https://doi.org/10.3896/IBRA.1.52.4.14>
- Meilan R, Harfouche A, Nehra N (2012) Risk assessment of Biotech trees: a path forward. *J* for 110:171–172
- Mitchell RJ, Irwin RE, Flanagan RJ, Karron JD (2009) Ecology and evolution of plant-pollinator interactions. *Ann Bot* 103:1355–1363. <https://doi.org/10.1093/aob/mcp122>
- Newhouse AE, Allwine AE, Oakes AD et al (2021) Bumble bee (*Bombus impatiens*) survival, pollen usage, and reproduction are not affected by oxalate oxidase at realistic concentrations in American chestnut (*Castanea dentata*) pollen. *Transgenic Res* 30:751–764. <https://doi.org/10.1007/s11248-021-00263-w>
- OECD (2013) Test No. 237: Honey bee (*Apis mellifera*) larval toxicity test, single exposure. OECD Guidel Test Chem 23:1–10. <https://doi.org/10.1787/9789264203723-en>
- OECD (2017) Test No. 245: Honey bee (*Apis mellifera* L.), chronic oral toxicity test (10-day feeding). OECD Guidel Test Chem 1–7. <https://doi.org/10.1787/9789264284081-en>
- Park JR, McFarlane I, Hartley Phipps R, Ceddia G (2011) The role of transgenic crops in sustainable development. *Plant Biotechnol J* 9:2–21. <https://doi.org/10.1111/j.1467-7652.2010.00565.x>
- Pirk CWW, Miranda JR, Kramer M et al (2013) Statistical guidelines for *Apis mellifera* research. *J Apic Res* 52:1–24. <https://doi.org/10.3896/IBRA.1.52.4.13>
- R Core Team (2021) R: A language and environment for statistical computing. The R Foundation for Statistical Computing, Vienna, Austria
- Ricroch A, Akkoyunlu S, Martin-Laffon J, Kuntz M (2018) Assessing the environmental safety of transgenic plants: Honey bees as a case study
- Romeis J, Bartsch D, Bigler F et al (2008) Assessment of risk of insect-resistant transgenic crops to nontarget arthropods. *Nat Biotechnol* 26:203–208. <https://doi.org/10.1038/nbt1381>
- Romeis J, Hellmich RL, Candolfi MP et al (2011) Recommendations for the design of laboratory studies on non-target arthropods for risk assessment of genetically engineered plants. *Transgenic Res* 20:1–22. <https://doi.org/10.1007/s11248-010-9446-x>
- Rosa AS, Ferreira MZ, Blochtein B et al (2015) Quantification of larval food and its pollen content in the diet of stingless bees—subsidies for toxicity bioassays studies. *Braz J Biol* 75:771–772. <https://doi.org/10.1590/1519-6984.22314>
- Rose R, Dively GP, Pettis J et al (2007) Effects of Bt corn pollen on honey bees: emphasis on protocol development. *Apidologie* 38:368–377
- Sakagami SF (1982) Stingless bees. In: Hermann HR (ed) Social insects. New York Academic Press, New York, pp 361–423
- Sanchez-Bayo F, Goka K (2014) Pesticide residues and bees—a risk assessment. *PLoS ONE* 9:e94482. <https://doi.org/10.1371/journal.pone.0094482>
- Santos CF, Santos PDS, Blochtein B (2015) In vitro rearing of stingless bee queens and their acceptance rates into colonies. *Apidologie* 47:539–547. <https://doi.org/10.1007/s13592-015-0398-2>
- Seide VE, Bernardes RC, Pereira EJG, Lima MAP (2018) Glyphosate is lethal and Cry toxins alter the development of the stingless bee *Melipona quadrifasciata*. *Environ Pollut* 243:1854–1860. <https://doi.org/10.1016/j.envpol.2018.10.020>
- Silva PHM, Sebbenn AM, Grattapaglia D, Conti JLF (2017) Realized pollen flow and wildling establishment from a genetically modified eucalypt field trial in Southeastern Brazil. *For Ecol Manag* 385:161–166. <https://doi.org/10.1016/j.foreco.2016.11.043>
- Slaa EJ, Sánchez Chaves LA, Malagodi-Braga KS, Hofstede FE (2006) Stingless bees in applied pollination: practice and perspectives. *Apidologie* 37:293–315
- Steijven K, Steffan-Dewenter I, Härtel S (2016) Testing dose-dependent effects of stacked Bt maize pollen on in vitro-reared honey bee larvae. *Apidologie* 47:216–226. <https://doi.org/10.1007/s13592-015-0392-8>
- Therneau T (2020) A package for survival analysis in R
- Tofili GM, Grella TC, Miotelo L et al (2018) Determinação da concentração letal média (CL50) do inseticida dimetoato para *Scaptotrigona postica*. In: Congresso de Iniciação Científica Universidade Estadual Paulista. p 1
- Uhl P, Franke LA, Rehberg C et al (2016) Interspecific sensitivity of bees towards dimethoate and implications for environmental risk assessment. *Sci Rep* 6:34439. <https://doi.org/10.1038/srep34439>
- Viana TA, Barbosa WF, Jojoa LLB et al (2022) A genetically modified anti-*Plasmodium* bacterium is harmless to the foragers of the stingless bee *Partamona helleri*. *Microb Ecol* 83:766–775. <https://doi.org/10.1007/s00248-021-01805-9>
- Wickham H (2016) ggplot2: Elegant graphics for data analysis. Springer, New York
- Winfree R, Griswold T, Kremen C (2007) Effect of human disturbance on bee communities in a forested ecosystem. *Conserv Biol* 21:213–223. <https://doi.org/10.1111/j.1523-1739.2006.00574.x>
- Yi D, Fang Z, Yang L (2018) Effects of Bt cabbage pollen on the honeybee *Apis mellifera* L. *Sci Rep* 8:1–6. <https://doi.org/10.1038/s41598-017-18883-w>

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