Comparative Toxicity of Pesticides to Stingless Bees (Hymenoptera: Apidae: Meliponini)

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ABSTRACT   Stingless bees are potential pollinators of commercial tropical crops and their use may increase in the short term. However, studies comparing the toxicity of pesticides to different individuals and species are lacking, making it difficult to evaluate their short- and long-term effects on colonies and populations of these insects. In this work, we tested the lethality of compounds from the main pesticide groups on stingless bees of the species *Melipona beecheii* Bennett, *Trigona nigra* Provancher, and *Nannotrigona perilampoides* Cresson. The LD_{50} (in micrograms per bee) for each pesticide was calculated for callow workers and foragers of the three species as well as for gynes and drones of *M. beecheii*. The results showed that all species were highly susceptible to the evaluated compounds. Nicotinoid pesticides were the most toxic, followed in descending order by permethrin, diazinon, and methomyl. We found evidence of a relationship between the body weight of the species and their LD_{50} for permethrin and methomyl ($r = 0.91$ and 0.90, respectively) but not for diazinon ($r = -0.089$). An analysis of contingency tables showed that within each species, callow workers had higher mortalities than foragers ($P < 0.01$). In *M. beecheii* at similar pesticide dose more males died compared with females [$\chi^2_{(0.01),1} = 10.16$]. However, gynes were less resistant than workers [$\chi^2_{(0.01),1} = 8.11$]. The potential negative consequences of pesticides to native stingless bees are discussed considering the reproductive biology of these insects. It is important to take actions to prevent damage to these key species for the ecology and agriculture of México and Latin America.

KEY WORDS   stingless bee, pesticide, LD_{50}, México

Modern agriculture relies on chemicals of different kinds for insect pest control that may be toxic for beneficial crop-visiting insects such as bees (Kevan et al. 1990, Desneux et al. 2007). In temperate areas, native bees are experiencing a decline of their populations, which is mirrored in a loss of bee pollinated plants (Biesmeijer et al. 2006), and pesticides may have had a significant effect on such reductions (De la Rúa et al. 2009).

In the tropics, no long-term studies are available on the population dynamics of native bees, but some species are already considered in danger (Kerr 2002). Although the effect of such losses on tropical agriculture and ecosystems remains unclear, they could be significant because bees are key pollinators in such habitats (Ackerman 1983, Freitas et al. 2009, Macías-Macías et al. 2009).

Stingless bees (tribe Meliponini) are among the most common pollinators in tropical environments and are the dominant bees visiting many crop species (Macías-Macías et al. 2009). These insects are a diverse group that includes >400 species that show high variability in physiology, morphology, and size, ranging from 0.2 mm in the genus *Trigonisca* to almost 20 mm in some *Melipona* species (Michener 2000, Moure et al. 2007). Stingless bees can intensively exploit floral resources due to their collective foraging system at colony level as honey bees do (Roubik 1989). Pesticides of different kinds can directly affect foragers causing alterations in the dynamics of resource collection and impair colony fitness (De la Rúa et al. 2009). Thus, it is likely that colonies of stingless bees could be affected by their direct contact with pesticides when visiting treated crops.

Pioneering studies in Brazil have evidenced the toxicity of insecticides to workers of stingless bees (Malaspina and Stort 1983, Macieira and Hebling-Beraldo 1989, Moraes et al. 2000). However, comparison between species has not been conducted and the role of factors such as age and size in relation to the relative

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resistance remains unclear. In social species, other than the honey bee, the effect of sex and caste on the susceptibility to insecticides remains undocumented, too. This is worth examining as sexually mature male stingless bees live outside their nests (Velthuis et al. 2005) where they could be more readily exposed to insecticides. Moreover, stingless bees are potential pollinators of commercial tropical crops and their use may increase in the short term (Slaa et al. 2006); thus, evaluating the effect of chemicals used in these systems is necessary.

In this article, we evaluated the toxicity of commonly used pesticides to three local species of stingless bees that are representative to this area of Mexico. We chose *Melipona beecheii* Bennett because of economic importance as one of the main species used in stingless bee keeping in Mesoamerica. Its feral populations are declining, and it is considered endangered (Kerr 2002, González-Acereto et al. 2006). Most *M. beecheii* remnant populations in southern Mexico are under domestic management in backyards where pesticides are of common use. We also selected *Nannotrigona perilampoides* Cresson and *Trigona nigra* Provancher. Both of these species are commonly found across the Yucatan Peninsula, they are good pollinators in enclosed cultivars and frequent visitors of commercially important crops (Cauich et al. 2004, Can-Alonzo et al. 2005, Palma et al. 2008).

The aim was to obtain basic information on the lethal toxicity of pesticides to these bees that could be used as reference to design better schemes for pesticide applications and in future field studies to evaluate their direct and sub lethal effects on individuals and colonies.

Materials and Methods

The experiments were conducted in March–June when colonies of *M. beecheii, T. nigra,* and *N. perilampoides* were more populous. Colonies of stingless bees were obtained from the stingless bee yard at the Department of Apiculture, Universidad Autónoma de Yucatán (UADY) in Xmatkuil, México. The evaluations were conducted at UADY and the Laboratorio de Entomología del Instituto Tecnológico de Conkal, Conkal, México.

In the experiments, we used callow workers (3–5 d old) and forager bees of all the species plus gynes and males of *M. beecheii.* To obtain the different individuals, combs of each species were kept in an incubator at 70% of humidity and 31°C (Moo-Valle et al. 2004). When workers, gynes, and males emerged they were introduced in an adjacent section (separated with a mesh) of two colonies in observation hives (20 by 12 by 6) at room temperature in the lab. They were maintained with a supply of honey and pollen until the experiments were conducted. Forager bees were obtained from the population entering the hives from the field.

Technical grade pesticides of the main classes were used in the tests: permethrin (pyrethroid), diazinon (organophosphorous), and methomyl (carbamate).

In addition, the effect of the neocotinoid pesticides imidacropid, thiamethoxan, and thiacloprid was evaluated in foragers of *N. perilampoides.* The pesticides were diluted in acetone to reach doses of 0.01, 0.1, 0.5, and 1 μg per bee after the application of 2 μl to each individual. The toxicity of the pesticides was evaluated by means of their LD_{50} on groups of 10 individuals of each type, with two replicates per dose. Each dose of pesticide was applied to the dorsal part of the thorax of each individual after anesthetizing them at low temperatures in a freezer (-8°C for 20 s). After the application of the pesticide, each group of 10 bees was confined to a Plexiglas container (11.5 by 8.5 by 7.5 cm). To correct for natural deaths, there were two control containers with 10 bees that were anesthetized and received application of 2 μl of acetone per individual. In the gynes and drones, one group of 10 individuals per dose and two control groups were used. The containers with the different treatment (doses) and control bees were provided with 2 ml of sugar syrup (1:1) in Eppendorf tubes and were kept together in a large chamber (Lab-Line Biotronette) at 70% of humidity and 31°C (Moo-Valle et al. 2004). In each trial, one bee species with all the doses, and individuals were run at the same time. Mortality of individuals per treatment was evaluated in a period of 24 h.

To estimate the values of LD_{50}, Probit regression analysis was used (Finney 1971). LD_{50} were calculated in micrograms per bee. Adjustments were made for control mortality by using Abbott's correction (Abbott 1925). Confidence intervals were calculated using standard procedures (Finney 1971). A chisquare test was used to test the fit between the slope (b) in the calculated model and the data. To test whether the occurrence of deaths due to pesticide application was independent of the age of the individuals within a species, we used analyses of two (callow and forager) × two (alive or dead) contingency tables on the standardized residuals of all pooled data (Manly 1992). For *M. beecheii*, we also compared males versus females (workers and gynes together) and workers versus gynes separately. The effect of the body weight on the susceptibility of each species to permethrin, diazinon, and methomyl was evaluated by means of Pearson's correlations between the average weight of workers of the three bee species plus that of gynes and males of *M. beecheii* ($n = 20$ weighed per class) and their respective LD_{50}s for those pesticides.

Results

The results of the Probit showed that all the evaluated insecticides were highly toxic to all types of individual of the three stingless bee species in accordance to the criteria of <2 μg per bee to reach the LD_{50} (Atkins et al. 1981) (Table 1). The most toxic insecticides for any of the species were the nicotinoids. Among the nicotinoids, imidacloprid was the most toxic, with only 0.001 μg needed to achieve 50% mortality in foragers of *N. perilampoides* followed by thiamethoxan (0.004 μg) and thiacloprid (0.007 μg).

Table 1. LD_{50} values of different pesticides topically applied to individuals of three species of stingless bees

Insecticide	Species	Type of individual	Probit LD_{50} (μg/bee)	95% CL (μg/bee)	Slope (b)	χ^2 for b	r
Permethrin	*M. beecheii*	♀	0.066	0.054–0.076	7.04	2.34	0.91**
		♂	0.072	0.064–0.081	6.92	4.97	
		Callow	0.091	0.084–0.098	7.23	1.14	
		Forager	0.098	0.090–0.11	5.1	3.58	
	T. nigra	Callow	0.021	0.017–0.028	9.21	4.67	
		Forager	0.031	0.025–0.037	8.3	2.98	
	N. perilampoides	Callow	0.010	0.006–0.015	10.22	5.11	
		Forager	0.014	0.01–0.018	10.1	3.21	
Diazinon	*M. beecheii*	♀	0.19	0.15–0.2	3.21	1.98	−0.089
		♂	0.185	0.15–0.21	6.91	2.17	
		Callow	0.19	0.16–0.24	5.11	3.21	
		Forager	0.24	0.17–0.29	5.13	3.6	
	T. nigra	Callow	0.18	0.12–0.23	4.45	1.64	
		Forager	0.24	0.19–0.31	2.91	1.93	
	N. perilampoides	Callow	0.12	0.09–0.15	4.11	2.64	
		Forager	0.19	0.15–0.25	2.21	2.9	
Methomyl	*M. beecheii*	♀	0.29	0.23–0.33	3.802	1.42	0.90*
		♂	0.25	0.20–0.29	4.21	1.58	
		Callow	0.34	0.28–0.41	6.16	3.98	
		Forager	0.41	0.35–0.49	4.76	3.56	
	T. nigra	callow	0.08	0.05–0.13	5.72	3.85	
		Forager	0.15	0.1–0.19	3.11	2.65	
	N. perilampoides	Callow	0.05	0.03–0.08	3.62	1.42	
		Forager	0.12	0.08–0.16	2.42	2.87	
Imidacloprid	*N. perilampoides*	Forager	0.0011	0.0008–0.0015	1.15	5.65	
Thiamethoxan	*N. perilampoides*	Forager	0.004	0.003–0.006	2.03	22.32**	
Thiacloprid	*N. perilampoides*	Forager	0.007	0.004–0.01	0.715	4.40	

The values of the chi-square test for each slope (b) are presented as well as the Pearson's correlations between wt and LD_{50}. **, significant at $P < 0.01$; *, significant at $P < 0.05$.

For *M. beecheii* and *T. nigra*, the toxicity of pesticides was in descending order permethrin > diazinon > methomyl. For *N. perilampoides*, the toxicity was imidacloprid > thiamethoxan > thiacloprid > permethrin > diazinon > methomyl.

The slope (b) values were generally higher for permethrin and lower for the nicotinoids, with diazinon and methomyl being intermediate. The value of b will in general reflect the variability of response of the individuals to the different doses expressed as mortality within the group: the higher the value of b the steeper the slope of the curve (Seefeld et al. 1995). Because the age of callow workers was controlled (2-d range), the variation in mortality resulted in a large value of b probably reflecting a more defined and uniform response of this age group compared with that of foragers. In addition, a large b means that if increased in dose, that pesticide could be potentially more detrimental to such age group (Macieira and Hebling-Beraldo 1989). The chi-square tests for b indicated that the models fitted the data, except for thiametoxan in which there were significant differences between the model and the calculated slopes; thus, the results for this compound should be interpreted with caution.

For the three species and for the pooled data for permethrin, diazinon, and methomyl, callow workers died in higher numbers compared with foragers [values of the calculated $\chi^2_{(0.01),1}$ were 6.93, 7.31, and 7.85 for *M. beecheii, T. nigra,* and *N. perilampoides,* respectively]. Accordingly, the b values of all the insecticides were higher for callow workers than for forager bees

due to their more homogenous response in this regard (Table 1). For *M. beecheii*, the females (workers and gynes) were comparatively more resistant than the males [$\chi^2_{(0.01),1} = 10.16$]. However, gynes were less resistant than workers [$\chi^2_{(0.01),1} = 8.11$].

The average weights registered for the different bee species were for *M. beecheii* gynes, 0.048 g; for males, 0.052 g; and for workers, 0.057 g. In *T. nigra* and *N. perilampoides* workers, average weights were 0.0099 and 0.0082 g, respectively. There were significant correlations between the average weight of each species and the amount of permethrin ($r = 0.91$) and methomyl ($r = 0.90$) to achieve the LD_{50} but not for diazinon (-0.089) for which the correlation was not significant ($P > 0.01$) (Table 1).

Discussion

Our results revealed that all the evaluated pesticides were highly toxic to native stingless bees in México. Moreover, all the stingless bee species studied showed higher susceptibility to the evaluated pesticides compared with *A. mellifera.* The calculated LD_{50} for the Western honey bee to permethrin, diazinon, and methomyl were 0.159, 0.372, and 1.29 μg per bee, respectively (Atkins et al. 1981), which are significantly higher than the lethal doses that we found for the same chemicals on the three studied stingless bees. Our results and the findings by Moraes et al. (2000) in *Scaptotrigona tubiba* (Smith) indicate that stingless bees are highly susceptible to some pesticides. Nevertheless, it is reported that species such as *Scapto-*

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trigona postica (Latreille) and *Melipona quadrifasciata* Lepeletier were more resistant than the honey bee to DDT (Malaspina and Stort 1983). Interestingly, *T. spinipes* is reported as more susceptible than the honey bee to permethrin and methomyl, like the three species in our study; but it was more resistant to organophosporous insecticides (malathion, dicrotophos, and parathion) and to carbaryl (Macieira and Hebling-Beraldo 1989). Thus, it seems that as a group, stingless bee species could be more susceptible than the honey bee to some pesticides (namely, permethrin and methomyl) but comparatively more resistant to other compounds.

When comparing the susceptibility of the three species in our study, we found that body weight was related with response to permethrin and methomyl: the larger *M. beecheii* were less susceptible to both compounds compared with the smaller *T. nigra*, and this species was more resistant than *N. perilampoides.* However, there was no evidence for the susceptibility due to body weight for diazinon. For the first two pesticides, the ratio of surface area to body weight could explain the different susceptibility between species (Johansen 1972). There is evidence indicating that the larger bees' intake of insecticide is proportionally lower as a consequence of their increased volume with respect to their body surface (Johansen et al. 1983). However, in small bees the resistance could be explained by other factors. For example, the type and development of detoxifying enzymes, levels of fat deposits and the pH of the hemolymph vary between bee groups (Ahmad and Johansen 1973). Such differences may affect the stability of the pesticides within the body, which could explain the resistance in stingless bees independent of body size as has been reported in other bee groups (Ahmad and Johansen 1973, Yu et al. 1984).

We found evidence for the effect of age on the susceptibility of workers. Callow workers were more susceptible than foragers, which is in agreement with findings in the honey bee (Graves and Mackensen 1965, Meled et al. 1998). In social bees, physiological changes occur in worker bees as a consequence of age and hormonal status (Devillers 2002). Such changes may also encompass differences in the development of enzymes or pesticide receptors that explain the different susceptibility of bees of different ages within a species (Meled et al. 1998, Guez et al. 2001).

An interesting finding was that drones of *M. beecheii* were more susceptible to the insecticides than the females (gynes and workers) and that the workers were more resistant than gynes. Males are acknowledged as more susceptible to pesticides in other bees, too (Tasei 2002). In the solitary bee *Megachile pacifica* (Panzer), males were more susceptible than the females to pesticides and their susceptibility varied with age (Guirguis and Brindley 1975). However, in the honey bee, *Apis mellifera* L., drones and queens were more resistant than workers to DDT (Graves and Mackensen 1965). Nevertheless, these authors acknowledged that the larger size of both sexuals may explain their higher resistance compared with the

workers. In accordance with this, stingless bee gynes are smaller than both males and workers, which could make them more susceptible to the evaluated pesticides, but biochemical differences between castes could also be involved. Drones of *M. beecheii* are larger than gynes, and they were more susceptible to insecticides than the latter. We used young drones and this may in part explain their lower resistance. Stingless bee drones undergo a maturation process within the colony after which they live a solitary life. In *M. beecheii* this process takes between 14–15 d (Pech-May and Q.-E., unpublished data); thus, it may be possible that older males could show different levels of resistance than immature males similar to what happened with the workers of this species and to males of other bees (Guirguis and Brindley 1975). This hypothesis would be worth evaluating because mature stingless bee males live a solitary life outside their colonies that would expose them to pesticides in the environment. There are no studies on the effect of pesticides on the population of male native bees. However, it is important to consider whether pesticides may also influence the reproduction of colonies and their genetic diversity via male decline.

Our results predict that direct lethal effects are expected on stingless bees visiting crops where pesticides have been applied. In honey bees it is argued that highly toxic pesticides may be less harmful for the colonies because most foragers would immediately die in the field (Atkins 1993). However, in Meliponini, apparently moderate forager losses may represent a substantial part of the colony because most species are not populous (Michener 2000). Moreover, the individuals of stingless bees take a longer time to develop from egg to adult (51 d in *M. beecheii*) compared with 21 d in the honey bee and the queen's egg laying rate is not higher than 40 per day (Moo-Valle et al.2004). If a decline of the worker population occurs, the colonies may not be able to gather sufficient resources and their opportunity for swarming also would be greatly reduced (Slaa 2006). Thus, any loss of foraging workers may affect more severely the survival of stingless bee colonies than in honey bees.

Stingless bees are increasingly being used in beekeeping, pollination and are kept nearby human settlements across southern Mexico (González-Acereto et al. 2006). In these regions, permethrin is used for mosquito control to prevent dengue outbursts during the rainy season (Valdovinos-Núñez et al. 2003); this is the pesticide that we found as most toxic for the stingless bees. A study in the Yucatan found that permethrin at doses used for aerial mosquito control was highly poisonous for stingless bees (Valdovinos-Núñez et al. 2003). Moreover, permethrin and related pyrethroids are common ingredients of several household insecticides in Mexico. Thus, insecticide use not only for agricultural purposes may put stingless bees in more danger compared with other bee species. The sublethal effects of pesticides on colonies of stingless bees are also important to consider because they may be more damaging to their populations over the long term (De la Rúa et al. 2009).

Our study makes evident that pesticide use can be highly detrimental to stingless bees. Foragers seem prone to their direct effect but our results indicate that males also can be affected increasing the negative consequences at colony and population levels. An organized scheme for the use of agrochemicals by farmers is needed to apply them in hours of the least bee activity and protecting domestic colonies when possible (Atkins 1993). Such measures are needed to ameliorate damage to the ecosystems and agriculture of México and Latin America.

Acknowledgments

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