Behavioral Response of Two Species of Stingless Bees and the Honey Bee (Hymenoptera: Apidae) to GF-120

ENOC GÓMEZ-ESCOBAR,[1,2] PABLO LIEDO,[1] PABLO MONTOYA,[2] RÉMY VANDAME,[1] AND DANIEL SÁNCHEZ[1,3]

ABSTRACT We present the results of evaluating the response of three species of bees, *Trigona fulviventris* (Guérin), *Scaptotrigona mexicana* (Guérin-Meneville), and *Apis mellifera* (L.), to food sources baited with the toxic bait GF-120 (NF Naturalyte), a spinosad-based bait exclusively used to manage fruit flies. Groups of foragers were trained to collect honey and water from a feeder located 50 m from the colonies. Once a sufficient number of foragers were observed at the experimental location, the training feeder was changed to two or three feeders that offered either honey and water, GF-120, Captor (hydrolyzed protein), GF-120 and honey (4:6), or Captor and honey (1:19). *T. fulviventris* and *S. mexicana* rarely visited GF-120, Captor, or their mixtures with honey, while ≈28.5 and 1.5% of *A. mellifera* foragers visited the GF-120 and honey and Captor and honey mixtures, respectively. Our results show that GF-120 clearly repels *T. fulviventris* and *S. mexicana*, whereas for *A. mellifera*, repellence is not as marked when GF-120 is combined with highly nutritious substances like honey.

KEY WORDS *Trigona fulviventris*, *Scaptotrigona mexicana*, *Apis mellifera*, foraging, ecotoxicology

GF-120 (NF Naturalyte, Dow Agrosciences LLC, Indianapolis, IN) is a toxic bait used in the management of fruit flies (Diptera: Tephritidae). It is composed of hydrolyzed protein, ammonium acetate, fructose, vegetable oils, surfactants, and the macrolide spinosad as the insecticide (Dow Agrosciences 2006). Because GF-120 is applied at 80 ppm spinosad in up to 5 mm-diameter drops at a rate of 80 drops per square meter, it appears to have minimal effects on insect pollinators such as social bees (Burns et al. 2001, Miles 2003). However, in laboratory tests, this formulation was classified as highly toxic (Edwards et al. 2003), and because there is no way to completely keep GF-120 drops from reaching food sources, such as pollen and nectar, it represents a potential threat to bees. Mangan and Moreno (2009) studied the attractiveness of GF-120 and its components and found that *Apis mellifera* (L.) was slightly attracted to feeders offering honey or invertose mixed with different constituents of GF-120. They concluded that the formulation is repellent for this species, and thus, any pollen or nectar mixed with GF-120 would be avoided by bees. However, although it was also noted that a number of workers continued

to consume the honey and ammonium acetate or invertose and Solulys (corn steep liquor composed mainly by proteins and lactic acid, by Roquette Inc., Gurnee, IL), the authors never tested the complete GF-120 formulation, which represents an underlying risk if it has a similar effect as the tested mixtures. Sánchez et al. (2012) showed that the stingless bee *Plebeia moureana* (Ayala) collects GF-120 mixed with 1.0 M sucrose continuously; foragers do not stop collecting this mixture potentially because spinosad acts slowly in the digestive and nervous systems of insects, further allowing this substance to accumulate in the colony. To date, tests to specifically determine whether *A. mellifera* foragers reject GF-120 when mixed with foods with high caloric content have not been carried out.

Although *A. mellifera* has been traditionally considered an adequate model species to assess the toxicity of insecticides, recent studies show that this is not necessarily true (Hardstone and Scott 2010, Cresswell et al. 2012). Other social bees, particularly the tribe Meliponini (Hymenoptera: Apidae), have been commonly absent from these assessments even though they may be more sensitive than *A. mellifera,* as shown by Valdovinos-Núñez et al. (2009). The aim of this study was therefore to evaluate the response (attraction or repellence) of the highly social bees *A. mellifera*, *Scaptotrigona mexicana* (Guérin-Meneville), and *Trigona fulviventris* (Guérin) to GF-120 alone or mixed with honey. Unlike *A. mellifera*, the stingless bees *S. mexicana* and *T. fulviventris* live in small colonies with up to a dozen thousand individuals, in tree

Table 1.　Mean (± SE) number of unmarked bees attracted to each feeder in the seven experiments

Experiment	Feeders	*S. mexicana*	*T. fulviventris*	*A. mellifera*
1	Honey	4.1 ± 0.5a	9.5 ± 0.7a	22.1 ± 1.2a
	Honey	6.6 ± 1.1a	8.8 ± 0.8a	22.5 ± 1.8a
2	Honey	8.5 ± 1.2a	15.8 ± 1.2a	23.9 ± 1.6a
	GF-120/water	0.0b	0.0b	0.4 ± 0.1b
3	Honey	10.47 ± 1.2a	15.2 ± 0.9a	24.5 ± 2.3a
	GF-120/honey	0.0b	0.0b	9.8 ± 1.7b
4	GF-120/honey	0.0	0.0	5.4 ± 0.9a
	GF-120/honey	0.0	0.0	4.4 ± 0.7a
5	Honey	10.8 ± 1.4a	7.4 ± 0.6a	29.1 ± 2.3a
	GF120/honey	0.0b	0.0b	8.1 ± 0.8b
	GF120/water	0.0b	0.0b	0.2 ± 0.04c
6	Honey	8.6 ± 0.8a	12.5 ± 0.9a	30.8 ± 2.0a
	Captor/water	0.0b	0.2 ± 0.1b	0.2 ± 0.1b
7	Honey	13 ± 1.2a	14.3 ± 0.9a	26.2 ± 1.9a
	Captor/honey	0.1 ± 0.04b	0.0b	1.5 ± 0.6b

Data were pooled across colonies, replicates, and 10-min intervals. Means followed by the same letters in each column are not significantly different (*P* > 0.05).

cavities and in the ground, respectively; they lack a sting, relying upon their mandibles for defense (Michener 2013). They can recruit nestmates to rich food sources as pollen and nectar, which makes them good competitors for *A. mellifera* (Sánchez and Vandame 2013). More importantly, they are pollinators of both cultivated and wild plants, so their contribution to ecosystems is at least as important as that of *A. mellifera.*

Materials and Methods

This work was carried out in El Colegio de la Frontera Sur (ECOSUR) in Tapachula, Chiapas, Mexico, from August to November 2012 with colonies of *A. mellifera* (*N* = 2) and *T. fulviventris* (*N* = 1), and from August to September 2013 with *S. mexicana* (*N* = 4). For all species, groups of 5–10 bees were trained to collect a honey:water (1:1) solution from a feeder located 50 m from the colonies following the procedure used by Sánchez et al. (2011). Honey (moisture of 17.8%) used in these experiments was obtained from the apiaries of ECOSUR and came from a single harvest to prevent differences in attractiveness across experiments. These bees were distinctively marked on the thorax to prevent trapping them along with unmarked newcomers during experiments; if marked foragers are trapped recruitment of nestmates ceases, resulting in low number of newcomers and reducing the probability of detecting any potential effect in our treatments. At the experimental location, the training feeder was removed and replaced with feeders offering a variety of test solutions (Table 1). The distance between feeders was 30 cm. The following solutions were offered in the test feeders: 1) GF-120 prepared according to the manufacturer's instructions (40% GF-120 and 60% water), 2) a mixture of GF-120 and honey (40% GF-120, 50% honey, and 10% water), 3) Captor 300 (hydrolyzed protein from vegetable hydrolysis used as an attractant for fruit flies; Química Lucava,

Mexico) prepared according to manufacturer's instructions (5% Captor and 95% water), 4) a mixture of Captor and honey (5% Captor, 50% honey, and 45% water), and 5) honey solution (50% honey and 50% water, as in training). Captor 300 was used instead of Solbait bait because it is the attractant commonly used in the region where the study was conducted. Seven experiments were carried out with six replicates each. In six experiments, foragers were offered a choice between two solutions; in one experiment, three solutions were offered. A replicate consisted of 1 h of observation, divided in six 10-min intervals (time intervals). During 2 min at the end of each interval, all unmarked foragers were trapped and registered, leaving 8 min for marked bees to recruit more nestmates. In 1 d, two replicates were carried out, with 2–3 h between replicates. Bees were kept in nylon mesh cages (30 by 30 by 30 cm) and released at the end of all the experiments. To avoid pseudoreplication (counting a forager more than once, which would inflate our results and bias our statistical analysis) and to prevent a severe reduction in colonies' foraging force (which would derive in a reduction in the number of bees visiting the feeders, thus obscuring differences in choice frequency among feeders), experiments 4–7 were conducted 4 wk after experiments 1–3. To prevent site learning by marked bees, which could bias the decision of unmarked bees through social facilitation (Slaa et al. 2003), we changed the relative positions of the feeders every 10 min. Data sets consisting of six replicates per colony, each replicate with six time intervals, could be analyzed using linear mixed models using SPSS v19 software (SPSS 2010), which consider the effect of temporal autocorrelation on the data (number of unmarked bees counted every time interval) when analyzing possible differences in the attractiveness of feeders within experiments.

Results and Discussion

Our data revealed no significant difference among colonies of the same species in all experiments when analyzed by linear mixed models (*P* > 0.05). A mean per experiment per species (sum of unmarked bees trapped in one experiment divided by N colonies times six replicates times six time intervals) was calculated as shown in Table 1. We did not find differences in the number of foragers trapped among the six time intervals (*P* > 0.05), except in experiment 4 for *S. mexicana* and *T. fulviventris,* as no bee was captured: foraging flow was kept constant during experiments. We found that *T. fulviventris* and *S. mexicana* never visited the GF-120, GF-120 and honey, or Captor feeders and rarely visited the Captor and honey feeder. However, the response of *A. mellifera* was different. In experiment three, 71.5% of foragers visited the feeder with honey, while the remaining 28.5% visited the GF-120 and honey feeder (Table 1); it is clear that GF-120 is not as dissuading as previously suggested by Mangan and Moreno (2009). However, it is important to note that these authors did not evaluate the GF-120 and honey mixture. This methodological difference sug-

gests that under actual field applications, bees might not collect drops of GF-120 alone but they could collect resources such as nectar, and perhaps pollen mixed with GF-120, but this has to be proven (Mangan et al. 2006, Sánchez et al. 2012); moreover, attractiveness of resources mixed with GF-120 may vary according to the requirements of the colony. Another difference was that they used non Africanized bees, whereas in our region all bees are Africanized. Why some foragers collect GF-120 while others do not is unclear. One possible answer may be found in the studies of *A. mellifera* by Laloi and Pham-Delegue (2010) and Scheiner and Arnold (2010), who showed that there is variability in the threshold of gustative behavior and learning among individual bees; therefore, some of our foragers could have lower rejection thresholds for the GF-120 and honey solution than others. More studies are required to further understand this phenomenon.

Finally, our results showed that the response of different species of bees to the toxic bait GF-120 varies; it is not possible to generalize its safety and repellency using a single species as representative of all bees or pollinators. Studies aimed to evaluate the safety of chemicals on nontarget species must address these differences and consider the areas where such chemicals are to be used.

Acknowledgments

References Cited

Burns, R. E., D. L. Harris, D. S. Moreno, and J. E. Eger. 2001. Efficacy of spinosad bait sprays to control mediterranean and caribbean fruit flies (Diptera: Tephritidae) in commercial citrus in Florida. Fla. Entomol. 84: 672–678.

Cresswell, J. E., C. J. Page, M. B. Uygun, M. Holmbergh, Y. Li, J. G. Wheeler, I. Laycock, C. J. Pook, N. H. de Ibarra, N. Smirnoff, et al. 2012. Differential sensitivity of honey bees and bumble bees to a dietary insecticide (imidacloprid). Zoology 115: 365–371.

Dow Agrosciences. 2006. Naturalyte fruit fly bait. Techn. Bull. Dow Cl: 0326.

Edwards, C.R.E., C.K.G. Erber, and G.J.H. Unt. 2003. A laboratory study to evaluate the toxicity of the Mediterranean fruit fly, *Ceratitis capitata*, bait, Success 0.02 CB, to the honey bee, *Apis mellifera*. Apidologie 34: 171–180.

Hardstone, M. C., and J. G. Scott. 2010. Is *Apis mellifera* more sensitive to insecticides than other insects? Pest Manage. Sci. 66: 1171–1180.

Laloi, D., and M. H. Pham-Delegue. 2010. Patriline-level variability in olfactory learning in the honey bee. Apidologie 41: 436–442.

Mangan, R. L., and A. T. Moreno. 2009. Honey bee foraging preferences, effects of sugars, and fruit fly toxic bait components. J. Econ. Entomol. 102: 1472–1481.

Mangan, R. L., D. S. Moreno, and G. D. Thompson. 2006. Bait dilution, spinosad concentration, and efficacy of GF-120 based fruit fly sprays. Crop Prot. 25: 125–133.

Michener, C. D. 2013. The Meliponini, pp. 3–17. *In* P. Vit, S.R.M. Pedro and D. Roubik (eds.), Pot-Honey. Springer New York, NY.

Miles, M. 2003. The effects of spinosad, a naturally derived insect control agent, to the honeybee. Bull. Insectol. 56: 611–624.

Sánchez, D., and R. Vandame. 2013. Stingless bee food location communication: from the flowers to the honey pots, pp. 187–199. *In* P. Vit, S.R.M. Pedro and D. Roubik (eds.), Pot-Honey. Springer, New York, NY.

Sánchez, D., J. C. Nieh, and R. Vandame. 2011. Visual and chemical cues provide redundant information in the multimodal recruitment system of the stingless bee *Scaptotrigona mexicana* (Apidae, Meliponini). Insect Soc. 58: 575–579.

Sánchez, D., E. de J. Solórzano, P. Liedo, and R. Vandame. 2012. Effect of the natural pesticide spinosad (GF-120 formulation) on the foraging behavior of *Plebeia moureana* (Hymenoptera: Apidae). J. Econ. Entomol. 105: 1234–1237.

Scheiner, R., and G. Arnold. 2010. Effects of patriline on gustatory responsiveness and olfactory learning in honey bees. Apidologie 41: 29–37.

Slaa, E. J., J. Wassemberg, and J. C. Biesmeijer. 2003. The use of field-based social information in eusocial foragers: local enhancement among nestmates and heterospecifics in stingless bees. Ecol. Entomol. 28: 369–379.

SPSS. 2010. SPSS statistics V19. SPSS, Chicago, IL.

Valdovinos-Núñez, G. R., J.J.G. Quezada-Euán, P. Ancona-Xiu, H. Moo-Valle, A. Carmona, and E. R. Sánchez. 2009. Comparative toxicity of pesticides to stingless bees (Hymenoptera: Apidae: Meliponini) J. Econ. Entomol. 102: 1737–1742.