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Research

Ecotoxicology

Effect of GF-120 (Spinosad) Aerial Sprays on Colonies of the Stingless Bee *Scaptotrigona mexicana* (Hymenoptera: Apidae) and the Honey Bee (Hymenoptera: Apidae)

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Abstract

Despite their relevant contribution to the conservation of tropical ecosystems and crop productivity through pollination, the stingless bees (Apidae: Meliponini) can be considered a group of neglected species in the assessment of pesticides upon nontarget organisms. In this article, we evaluated the effect of aerial sprays of the spinosad-based fruit fly toxic bait GF-120 upon colonies of the stingless bee *Scaptotrigona mexicana* Guérin (Hymenoptera: Apidae), an economically important and abundant species in some landscapes of Mexico, located in mango orchards. Colonies of the honey bee *Apis mellifera* L. (Hymenoptera: Apidae) were used for comparison. Eight colonies (four of *A. mellifera* and four of *S. mexicana*) were moved into each of two mango orchards, one was used as a control, with no insecticide application, and other received five weekly aerial sprays of GF-120. Foraging activity and strength of colonies of both species were measured nine times over the fruiting season, previous, during and after insecticide application. We did not find a significant difference in foraging activity and strength between exposed and control colonies of *A. mellifera* during the observation period. However, colonies of *S. mexicana* seemed to be affected by the exposure, as revealed by a reduction in colony strength. However, 1 yr later, with no insecticide applications, the colonies of both species were evaluated and found to be in good conditions. Our results showed that weekly aerial sprays of GF-120 are unlikely to generate acute poisoning in both species, even if in acute toxicity tests this product has been found to be highly active.

Key words: Ecotoxicology, spinosyn, pollinator, tephritid fruit fly, native bee

The discovery and development of synthetic compounds used to control agricultural pests have made agriculture an activity that depends extensively on pesticides. If used judiciously, pesticides have several advantages, like rapid control of outbreaks and increased crop production; however, there are several problems associated with pesticide use, like environmental pollution, emergence of public health issues, resistance, and reduction of biodiversity (Horrigan et al. 2002, Aktar et al. 2009). Therefore, new substances are developed to minimize these harmful effects to achieve environmentally friendly agriculture.

Spinosad, a blend of the macrolides spinosyn A and spinosyn D, which are produced by fermentation of the soil actinomycete *Saccharopolyspora spinosa* (Actinomycetales: Pseudonocardiaceae) Mertz & Yao is considered a natural pesticide nontoxic for mammals and birds, somewhat toxic to fish and very active against several lepidopteran, coleopteran and dipteran pests (Sparks et al. 2001, Mayes et al. 2003, Thompson et al. 2006). It is widely used as a green pesticide and organic farming has found no issues incorporating this substance within its pest management strategies (Zehnder et al. 2006, Arthurs and Dara 2018). Many studies have shown that some risk is involved, though, particularly if natural enemies or pollinators get in contact with wet residues (Cisneros et al. 2002, Williams et al. 2003, Desneux et al. 2007, Biondi et al. 2012). Yet spinosad is acknowledged as a better alternative to synthetic pesticides, so new spinosad-based formulations have been developed, with even friendlier environmental profiles.

GF-120 (NF Naturalyte, Dow AgroSciences LLC, Indianapolis, IN) is a bait formulation developed for controlling fruit flies (Diptera: Tephritidae) that incorporates spinosad as the active ingredient. It also contains ammonium-releasing compounds to attract fruit flies, which also repel bees, such as *Apis mellifera* L. (Hymenoptera: Apidae) (Mangan and Moreno 2009). The reconstituted formulation contains 80 mg/liter of active ingredient and 7.5 liters/ha of the bait are aerially applied at ultra-low volume with large droplets (≥5 mm in diameter), which implies a further substantial reduction in the exposure risk to nontarget organisms. Several studies have shown that GF-120 sprays do not pose an important mortality factor to honey bees colonies (Kirkland 1999, Spencer et al. 2003). Our research has shown that, even though GF-120 is not completely repellent to honey bees (Gómez-Escobar et al. 2014) as suggested by Mangan and Moreno (2009), these pollinators can tune their foraging activity to avoid GF-120 contaminated food sources (Cabrera-Marín et al. 2015). However, it is unknown the amount of GF-120 that reaches the colonies of this social insect, so sublethal effects resulting of a potential chronic exposure to GF-120 cannot be discarded, particularly given that honey bee foragers are not dissuaded from walking on GF-120 treated surfaces (D. Sánchez, unpublished data).

Studies on the effect of GF-120 upon stingless bees (Apidae: Meliponini), the sister taxa of honey bees (Apidae: Apini) and bumble bees (Apidae: Bombini), is scarce. This is rather paradoxical, since stingless bees are considered essential for the maintenance of food webs in neotropical ecosystems (Michener 2013). Sánchez et al. (2012) found that some stingless bee species are reluctant to collect GF-120 treated food sources, but Gómez-Escobar et al. (2014) found that others are not. Surprisingly GF-120 does not seem to deter stingless bees from walking on surfaces treated with it (D. Sánchez, unpublished data). More importantly, yet, no studies on the effect of aerial applications of GF-120 upon stingless bees have been carried out, though it is known that activity of pesticides might be higher in stingless bees than in the honey bees given the short range of their flights (Valdovinos-Nunez et al. 2009). These gaps in our knowledge have led local stingless beekeepers to assume that GF-120 aerial sprays are detrimental to their colonies. The aim of this study was to gather evidence 1) on the effect of GF-120 aerial sprays on colony strength and foraging activity and 2) on the activity of fresh and dry residues of this insecticide bait upon the stingless bee *Scaptotrigona mexicana* Guérin (Hymenoptera: Apidae). In this study, we included *A. mellifera* to compare the well-known response of this species with that of our focal species.

Materials and Methods

Study Site and Bee Species

The study was carried out from February to April 2013 in the mango orchards 'El Zapotal' (100 ha; N 14°47′21.22″, W 92°26′27.80″) and 'Santa Elena' (180 ha; N 14°49′52.11″, W 92°23′55.57″), both at 25 m.a.s.l. in the municipality of Mazatan, Chiapas, Mexico. At this time of year bee foragers collect food from plants outside the orchard, small shrubs inside, and from exposed, ripe fruit pulp, either hanging or over the ground, since flowering in mango trees is almost over. This is the time when fruit flies infest mangoes and GF-120 is applied over the orchards. We wanted to investigate if GF-120 was detrimental to bee colonies under this condition.

Four colonies of *A. mellifera* and four colonies of *S. mexicana* were moved into the center of each orchard and positioned rowwise, with 5 m of distance between neighboring colonies. GF-120 was prepared and aerially applied as suggested by the manufacturer: GF-120 mixed with water at a 6:4 proportion and sprayed at a density of eighty 5-mm diameter droplets per square meter.

Measuring Colony Strength and Foraging Activity

Measurements of foraging activity and colony strength were carried out between 8:00 a.m. and 12:00 p.m. since in this period foraging activity is at its peak. To measure foraging activity, we video recorded the entrance of each colony during 15 min, which allowed us to register the foraging activity of all eight colonies from one orchard in one day. We measured the strength of *A. mellifera* colonies by opening the hives, taking photographs of both sides of all combs and counting the workers in each picture. Since combs are built horizontally in the case of *S. mexicana*, we removed all combs carefully, separated each comb and took a picture of both sides. Next, all combs were placed back in their hives. We used a Nikon D3100 camera (Nikon Corp., Tokyo, Japan) for taking videos and photographs. Video files were analyzed frame by frame using the iMovie v9.0.4 software in a MacBook Pro running under Mac OS X Lion (Apple Inc.), which allowed us to have an exact count of the number of foragers entering and exiting during that period. Photographs were analyzed using the Preview v5.5.3 software in the same MacBook pro computer by manually counting the workers in each photograph.

GF-120 Aerial Application

GF-120 was aerially sprayed over 'El Zapotal' in 1, 8, 15, 22 and 29 March, starting at 6 am, when colonies initiate foraging. No application was carried out over 'Santa Elena'. To assure that GF-120 was applied as recommended by the manufacturer we placed ten 30 × 40 cm rectangular pieces of cardboard over the ground in the orchard while the aircraft (flying at an altitude of 150 m and speed of 150 km/h) was spraying the pesticide (at 1.4 kg/cm^2 output pressure from two nozzles with 20 mm diameter orifices) and the number of 5-mm droplets were counted and divided by the area of the pieces of cardboard. One week before the first application of GF-120, colony strength and foraging activity were measured only once in all colonies to have a basal condition to compare with. In order to evaluate the effect of applications upon bee colonies, we took a single measurement of colony strength and foraging activity of all colonies one day post-application. Colony strength and foraging activity were also measured in the control treatment in the same dates as in the GF-120 treatment. Once a week, during the next 3 wk after the last application of GF-120, we also measured colony strength and foraging activity in both treatments. So we had three separated situations: 1) pre-application (one measurement), 2) during applications (five measurements) and, 3) post-application (three measurements). Finally, the status of the colonies was qualitatively evaluated 1 yr later only by visual inspection by two well-trained beekeepers, one specialized on stingless bees and the other on honey bees, who evaluated colony strength, foraging force, brood amount, defensiveness, and food (pollen and honey) reserves.

Residual Exposure to Dry and Wet GF-120

Contact between foragers and GF-120 could occur during application (while flying or harvesting resources) or post application (collecting contaminated resources or walking on treated surfaces). In a previous study, we found that *S. mexicana* is reluctant to collect rich food sources treated with GF-120 (Gómez-Escobar et al. 2014), so this possibility was not tested here. Thus, we focused on determining the effect of bees walking on treated (wet and dry) surfaces. On a piece of filter paper (30 cm length, 32 cm width) we placed GF-120 according to the following treatments: 1) twenty droplets of 10 µl of GF-120, simulating a uniform aerial spray of the recommended application rate of 80 drops/m². 2) Fifty milliliters of GF-120 to have the filter paper completely saturated with the formulation (thus representing a continuous exposure), which was used immediately (fresh GF-120 treatment). 3) Same as the previous treatment but left drying for 2 h at room temperature (dry GF-120 treatment) before use. The control treatment consisted of a filter paper, in which 20 water droplets of 10 µl were placed uniformly. Each filter paper was introduced into a 30 cm height, 10 cm diameter PVC container covered with mesh caps, also treated with GF-120 or water according to the treatment. In each container groups of 15–20 bees collected directly from the colony were confined. Bees were exposed for 15 min in all treatments. After exposure, all bees were transferred to cages (wooden frame, mesh walls, 30 × 30 × 30 cm) and fed with 50% sucrose solution. Mortality was recorded at 24 h by counting the number of dead bees (insects that did not respond to a stimulus with a toothpick).

Statistics

Since in the GF-120 aerial sprays experiments we collected repeated measures on the same subjects (i.e., colonies) over several weeks, we carried out the statistical analyses using a mixed linear model approach (Sánchez et al. 2011) using the R software (R Development Core Team 2012) and the lme4 package (Bates et al. 2015). We tested the assumptions of homogeneity of variances and normality of residuals by visually analyzing Q-Q plots. Proportion of mortality in the residual experiments was analyzed with the Exact Wilcoxon rank sum test using the package exactRankTests (Hothorn and Hornik 2015) run within the R software. We set the significance level at 0.05.

Results

Aerial Spray—Foraging Activity

For each colony, the number of foragers exiting and entering were added, divided by two, rounded down and used as the 'foraging flow activity' variable for analysis. For *A. mellifera*, we found that

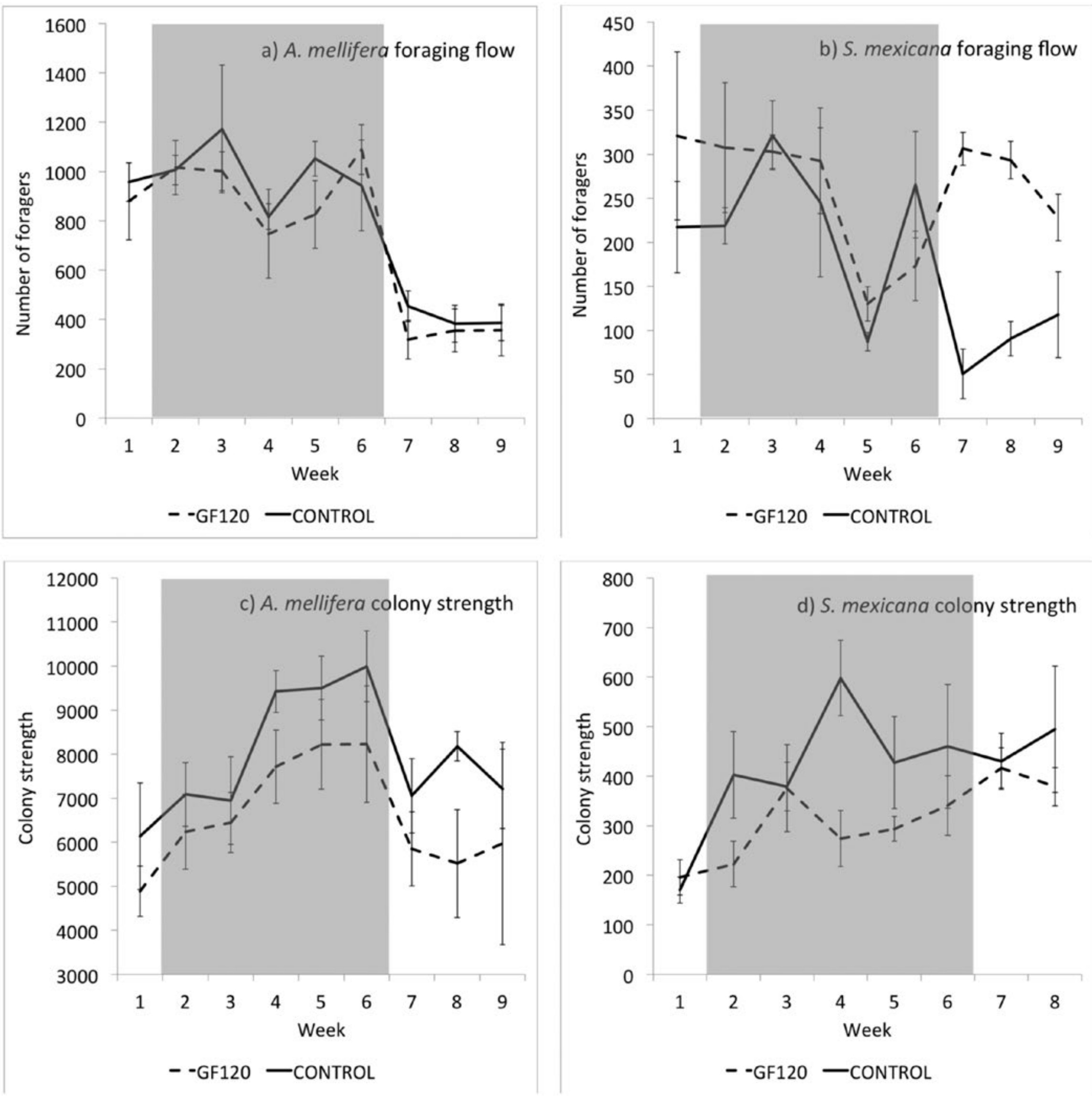

Fig. 1. Foraging activity (a and b) and colony strength (c and d) (mean ± standard error) over the study period. The shaded area represents the period in which the five aerial sprays of GF-120 took place.

foraging flow was significantly different over weeks ($\chi^2 = 95.63$, df = 8, $P < 0.001$), but no significant difference was observed between the GF-120 and the control treatments ($\chi^2 = 0.36$, df = 1, $P = 0.55$; Fig. 1a). In the case of *S. mexicana*, foraging activity was statistically significant over weeks ($\chi^2 = 23.94$, df = 8, $P < 0.01$) and treatment ($\chi^2 = 6.25$, df = 1, $P = 0.01$; Fig. 1b).

Aerial Spray—Colony Strength

In *A. mellifera*, colony strength behaved similarly to foraging activity: it was not significantly different between control and exposed colonies ($\chi^2 = 1.65$, df = 1, $P = 0.20$), but changed significantly over the 9 wk ($\chi^2 = 53.59$, df = 8, $P < 0.001$, Fig. 1c). The control and exposed colonies of *S. mexicana* showed significant differences ($\chi^2 = 5.65$, df = 1, $P = 0.020$) and foraging varied as weeks passed as well ($\chi^2 = 19.37$, df = 7, $P < 0.001$, Fig. 1d). Surprisingly, foraging activity was higher in the exposed colonies post-exposure.

The inspection of all colonies of both species 1 yr later revealed that all of them were in good conditions, with healthy queens, sufficient number of workers and brood and enough food reserves.

Residual Exposure to Dry and Wet GF-120

Mortality in the recommended application rate treatment was not significantly different from the non-treated control either in *A. mellifera* ($W = 28$, $P = 0.12$), or in *S. mexicana* ($W = 12.5$, $P = 0.42$). The complete coverage with dried GF-120 caused mortality higher than in the control treatment in *S. mexicana* ($W = 5$, $P < 0.01$) but not in *A. mellifera* ($W = 59$, $P = 0.51$). When exposed to complete coverage of fresh GF-120 mortality was significantly higher in both species (*S. mexicana*: $W = 0$, $P < 0.001$; *A. mellifera*: $W = 3$, $P < 0.001$) than in the non-treated control. In all cases, *S. mexicana* seemed to be more susceptible to GF-120 than *A. mellifera* (Fig. 2a and b).

Discussion

In this article, we explored the effects of the widely used spinosad-based bait GF-120 on colonies and workers of the stingless bee *S. mexicana* and of the honey bee *A. mellifera*. We found that *S. mexicana* seems to be more sensitive to GF-120; however, 1 yr after the experiments, all colonies of this species seemed to be in acceptable conditions. Overall, it seems that GF-120, if applied judiciously, is not detrimental to the species included in this study.

Foraging flow in *A. mellifera* followed a steady pattern quite similar in both exposed and unexposed colonies (Fig. 1a) even though strength was slightly lower (but statistically indistinguishable) in exposed colonies (Fig. 1c). Similar findings were reported by Burns et al. (2001) and Spencer et al. (2003). Surprisingly foraging flow increased in the colonies of *S. mexicana* after exposure to GF-120 (Fig. 1b), which might be a natural response to a slight reduction in strength (Fig. 1d). However, it may also be possible that other factors were responsible for increasing the foraging flow as pointed out by Biesmeijer et al. (1999), who found that colonies of the stingless bee *Melipona beecheeii* Bennett (Hymenoptera: Apidae) did not increase their foraging force when mortality of workers reached up to 50%, but they did in pollen shortage conditions.

Colonies of *S. mexicana* seemed to be more sensitive than *A. mellifera* colonies. This difference may be related to the lower number of workers that constitute their colonies (healthy wild colonies of up to 7,000 workers; Arzaluz-Gutiérrez et al. 2002), compared with that of the honey bee (dozens of thousands). That is, the more populated honey bee colonies can afford the stress of the pesticide since a moderate reduction in the number of workers may be rapidly compensated, but the smaller stingless bees cannot. Moreover, the shorter flight ranges of the small *S. mexicana* foragers of less than 1 km given its 5.2–5.4-mm body size (Wille 1983, Ayala 1999) might increase the likelihood of encountering a GF-120 droplet, since their foraging area could be restricted to the mango orchard; on the

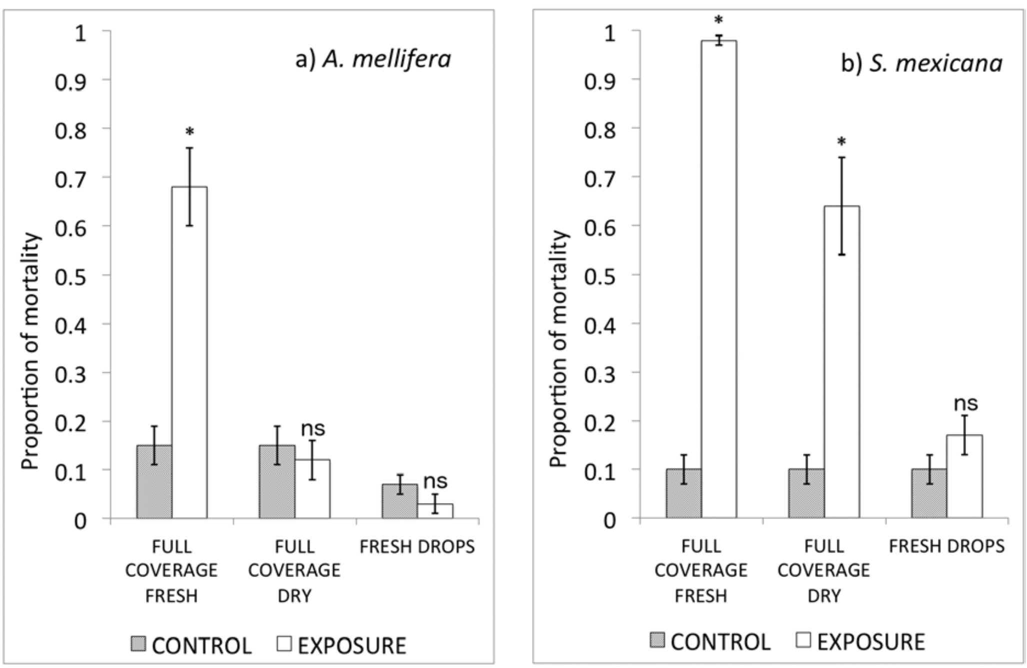

Fig. 2. Proportion of mortality (mean ± standard error) at 24 h post-exposure to GF-120 in any of three forms of exposure: Full coverage fresh (a filter paper saturated with freshly prepared, liquid GF-120), full coverage dry (same as full coverage fresh, but the paper was left to dry for 2 h before exposure), and fresh drops (a filter paper with drops of GF-120 at a density of 80 drops per square meter). *Statistically different from the control; ns: no significant difference.

other hand, the honey bee can fly up to several kilometers (Ribbands 1951), far from the boundaries of the orchards we carried out the experiments in.

We were especially interested in the reduction of the strength in the *S. mexicana* colonies potentially caused by GF-120. In a previous work, Cabrera-Marin et al. (2016) found that falling droplets of GF-120 hardly reach immobile honey bees, and that though they can collect GF-120 treated food sources (Gómez-Escobar et al. 2014), they preferred to switch to GF-120 free food sources (Cabrera-Marín et al. 2015). Thus, being *S. mexicana* smaller insects than honey bees, the probability of a GF-120 drop reaching a forager is lower. In a previous work, Gómez-Escobar et al. (2014) found that *S. mexicana* workers actively avoided GF-120 treated sugar solution. Other potential source of contact is trough tarsi. Our results show that tarsal exposure to GF-120 wet residues are more detrimental than to dry residues in both species, though mortality was higher in *S. mexicana*. However, the recommended coverage treatment (80 drops/m^2) did not cause mortality higher than control in both species. Thus, it is unlikely that the reduction in strength observed in *S. mexicana* colonies can be attributed to tarsal contact of foragers in the field experiment, since the main threat was to entering in contact with fresh residues of the insecticide.

We can conclude that, under the conditions of our experiments, aerial spraying of GF-120 at recommended rate cause minimal impact on the two social bees. Though GF-120 is more toxic when liquid than when dry, the application rate significantly reduces the probability of contact. Since mango trees were not flowering, collecting pollen contaminated from these trees was unlikely, though insecticide drift cannot be completely ruled out. A minor reduction in colony strength was observed, but colonies did not collapse even 1 yr after the experiments. Despite GF-120 was applied five times on a weekly basis, our data do not support the hypothesis that this formulation is highly toxic to social bees under field conditions.

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References Cited

Aktar, M. W., D. Sengupta, and A. Chowdhury. 2009. Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip. Toxicol. 2: 1–12.

Arthurs, S., and S. K. Dara. 2018. Microbial biopesticides for invertebrate pests and their markets in the United States. J. Invertebr. Pathol. doi:10.1016/j.jip.2018.1001.1008

Arzaluz-Gutiérrez, A., F. Obregón-Hernández, and R. Jones. 2002. Optimum brood size for artificial propagation of the stingless bee, *Scaptotrigona mexicana*. J. Apic. Res. 41: 62–63.

Ayala, R. 1999. Revisión de las abejas sin aguijón de México (Hymenoptera: Apidae: Meliponini). Folia Entomol. Mex. 106: 1–123.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67: 1–48.

Biesmeijer, J., M. Born, S. Lukács, and M. J. Sommeijer. 1999. The response of the stingless bee *Melipona beecheii* to experimental pollen stress, worker loss and different levels of information input. J. Apic. Res. 38: 33–42.

Biondi, A., V. Mommaerts, G. Smagghe, E. Viñuela, L. Zappalà, and N. Desneux. 2012. The non-target impact of spinosyns on beneficial arthropods. Pest. Manag. Sci. 68: 1523–1536.

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.

Cabrera-Marín, N. V., P. Liedo, R. Vandame, and D. Sánchez-Guillén. 2015. Foraging allocation in the honey bee, *Apis mellifera* L. (Hymenoptera, Apidae), tuned by the presence of the spinosad-based pesticide GF-120. Neotrop. Entomol. 44: 166–172.

Cabrera-Marín, N. V., P. Liedo, and D. Sánchez. 2016. The effect of application rate of GF-120 (Spinosad) and Malathion on the mortality of *Apis mellifera* (Hymenoptera: Apidae) foragers. J. Econ. Entomol. 109: 515–519.

Cisneros, J., D. Goulson, L. Derwent, D. Penagos, O. Hernandez, and T. Williams. 2002. Toxic effects of spinosad on predatory insects. Biol. Control. 23: 156–163.

Desneux, N., A. Decourtye, and J. M. Delpuech. 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52: 81–106.

Gómez-Escobar, E., P. Liedo, P. Montoya, R. Vandame, and D. Sánchez. 2014. Behavioral response of two species of stingless bees and the honey bee (Hymenoptera, Apidae) to GF-120. J. Econ. Entomol. 107: 1447–1449.

Horrigan, L., R. S. Lawrence, and P. Walker. 2002. How sustainable agriculture can address the environmental and human health harms of industrial agriculture. Environ. Health Perspect. 110: 445–456.

Hothorn, T., and K. Hornik. 2015. exactRankTests: exact distributions for rank and permutation tests. R package version 0.8–28. http://CRAN.R-project.org/package=exactRankTests.

Kirkland, R. 1999. Evaluation of Success 2SC on honey bees. Report 219–99. Dow AgroSciences, Indianapolis, IN.

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.

Mayes, M. A., G. D. Thompson, B. Husband, and M. M. Miles. 2003. Spinosad toxicity to pollinators and associated risk. Rev. Environ. Contam. Toxicol. 179: 37–71.

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.

R Development Core Team. 2012. R: a language and environment for statistical computing computer program. R Development Core Team, Vienna, Austria.

Ribbands, C. R. 1951. The flight range of the honey-bee. J. Anim. Ecol. 20: 220–226.

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). Insec. Soc. 58: 575–579.

Sánchez, D., E. Solórzano-Gordillo, 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.

Sparks, T., G. Crouse, and G. Durst. 2001. Natural products as insecticides: the biology, biochemistry and quantitative structure-activity relationships of spinosyns and spinosoids. Pest. Manag. Sci. 57: 896–905.

Spencer, J., J. Ibarra, and P. Rendón. 2003. Effect of Spinosad on honey bees (Hymenoptera: Apidae) in Guatemala. Southwest. Entomol. 28: 211–216.

Thompson, G. D., S. H. Hutchins, and T. C. Sparks. 2006. Development of spinosad and attributes of a new class of insect control products. *In* E. Radcliffe, W. Hutchison and R. Cancelado (eds.), Radcliffe's IPM World Textbook. University of Minnesota, St. Paul, MN.

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

Wille, A. 1983. Biology of the stingless bees. Annu. Rev. Entomol. 28: 41–64.

Williams, T., J. Valle, and E. Viñuela. 2003. Is the naturally derived insecticide Spinosad® compatible with insect natural enemies? Biocontrol. Sci. Techn. 13: 459–475.

Zehnder, G., G. M. Gurr, S. Kühne, M. R. Wade, S. D. Wratten, and E. Wyss. 2006. Arthropod pest management in organic crops. Annu. Rev. Entomol. 52: 57–80.