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Effect of the Natural Pesticide Spinosad (GF-120 Formulation) on the Foraging Behavior of *Plebeia moureana* (Hymenoptera: Apidae)

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ABSTRACT In this study we evaluated the effects of the biorational pesticide, Spinosad (GF-120 formulation), on foraging behavior in the stingless bee *Plebeia moureana* (Ayala). Several foragers were individually trained to collect an unscented 1.0 M sucrose solution (31% sucrose wt:wt) from a blue plate in one arm of a Y-tube maze. The other arm offered plain water on a yellow plate. After 20–30 visits to the setup, the sucrose solution was exchanged for a sucrose solution mixed with one of five concentrations of GF-120 and 30 consecutive choices of each bee were recorded. Interestingly, the foragers collected the sucrose solution with GF-120 at all concentrations. Our results show that: 1) the GF-120 formulation, when applied at the recommended concentration and mixed with food, does not discourage engaged foragers and, 2) foraging behavior over time is not significantly impaired by the continuous collection of GF-120.

KEY WORDS meliponini, biorational, pesticide, social, *Saccharopolyspora spinosa*

With the advent of biological control approaches to regulate populations of insect pests, natural substances that may be more environmentally friendly have increasingly been used as alternatives to synthetic insecticides. Spinosad is a prime example; it is a mixture of the macrolide molecules, spinosyn A and spinosyn D, produced by the actinomycete bacteria, *Saccharopolyspora spinosa* (Mertz & Yao), which is currently used against many crop pests around the globe with remarkable success (Thompson et al. 2000). Given such worldwide usage, several studies have focused on the potential side effects of Spinosad on nontarget species, particularly an important agricultural pollinator, the honey bee *Apis mellifera* (L.) (Thompson and Sparks 2002, Mayes et al. 2003). In oral exposure tests, Spinosad is considered to be very harmful to honey bee foragers (24 h $LC_{50} = 0.053\ \mu g$ active ingredient [AI]/bee); however, no significant adverse colony level effect has been found in field tests with honey bees foraging on Spinosad-treated flowering crops (Miles 2003). Nonetheless, steps have been taken to reduce honey bee intake of Spinosad through an altered pesticide formulation. For example, The GF-120 NF Naturalyte formulation (Dow Agrosciences, Indianapolis, IN) targets fruit flies (Diptera, Tephritidae) by adding ammonia (NH_3), which is attractive to fruit flies and putatively repels honey bees (Mangan and Moreno 2009). Such specificity allows for the use of Spinosad at much lower concentrations than other formulations without compromising its effectiveness for at least 10 d under field conditions (Flores et al. 2011). For example, ≈0.5 g AI/ha of the GF-120 formulation of Spinosad at a concentration of 80–96 mg AI/liter can be used against fruit flies as compared with concentrated suspensions used for other arthropods (25–150 g AI/ha, at a concentration 45–3,600 mg AI/liter; Mayes et al. 2003). Because of these characteristics, the GF-120 formulation may have negligible side effects on beneficial insects. In fact, Spinosad is considered to be one of the least detrimental bioinsecticides to nontarget organisms like natural enemies (Williams et al. 2003). However, when there is a shortage of floral food, such as occurs in the rainy season, even low concentrations of Spinosad, and the dissuasive ammonia, may not be sufficient to discourage bee foragers from gathering nectar and pollen containing GF-120.

Surprisingly, there are currently no published studies on the effects of Spinosad on stingless bees (Apidae: Meliponini), although they are considered as key providers of pollination services for agricultural and natural systems in the tropics (Roubik 1989). Therefore, we evaluated whether free flying foragers of the stingless bee *Plebeia moureana* (Ayala), an abundant local pollinator (Ayala 1999) would collect sucrose solution containing different concentrations of GF-120 under seasonal conditions of food dearth. In this study, our goal was to answer two questions. 1) Will *P. moureana* foragers reject the GF-120 formulation mixed with sucrose when no other profitable food source is available? 2) Is foraging behavior impaired when these free-flying foraging are orally exposed to sucrose solution treated with GF-120?

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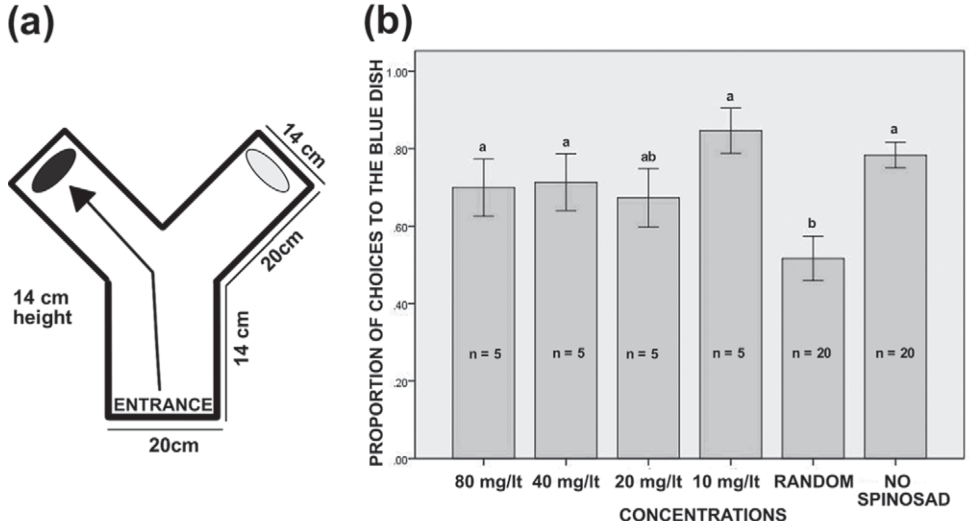

Fig. 1. (a) Apparatus used in this study, arrow indicates the route followed by foragers to reach the reward on the blue plate (b) mean proportion (±95% SE) of *P. moureana* foragers that chose the blue plate in the different concentrations of active ingredient. Same letters indicate no significant difference ($P > 0.05$). Number (n) of tested bees in each concentration is shown.

Materials and Methods

The current study was conducted in the campus of El Colegio de la Frontera Sur, Tapachula, Chiapas, Mexico, from August to September 2011, in the middle of the rainy season, when there are very few natural food sources available. Forty free flying *P. moureana* foragers from one wild colony were individually trained, following Sánchez et al. (2011) procedures, to collect 1.0 M (31% wt:wt) sucrose solution from a 0.2 ml tube (with a hole in its lid), placed in the center of a vertical blue plate in one of the arms of a Y-tube choice apparatus made entirely of transparent Plexiglas. The opposite arm offered plain water in a tube on a yellow plate (Fig. 1a). This Y-tube apparatus was located 10 m south from the colony so foragers could freely fly back and forth between them. Plates were randomly moved from one arm to the other after each visit to reduce potential site learning bias. This training phase lasted, on average, 23 ± 11 min (mean ± SD), that is, 20–30 training visits.

In the test phase that immediately followed, we kept the Y-tube maze at the same location and recorded 30 consecutive choices from each of the trained bees, but in this phase the sucrose solution was exchanged for one of five concentrations of GF-120 solution mixed with sucrose solution (final concentrations of 0, 10, 20, 40, and 80 mg/liter of Spinosad, all at 1.0 M sucrose, Fig. 1b). The yellow dish always offered only water. The test phase lasted, on average, 35 ± 15 min, and thus foragers were exposed to Spinosad and their responses were tested over this average time interval. Such concentrations range from near the field application dose (80 mg/liter, worst scenario) to concentrations that may still useful to control fruit flies (Flores et al. 2011). To avoid odor marking bias, both plates were exchanged for clean ones every five visits. The Y-apparatus was throughout cleaned with 95% ethanol before testing with each bee.

Data were analyzed using Generalized Estimating equations, assuming a binomial distribution and log-linking the data to the mathematical model. To account for the time-correlated (repeated measures) nature of the data, an autoregressive working correlation matrix structure was also included in the model (Ballinger 2004). To test whether the frequency of choices departed from a random distribution, we compared this data with a randomly generated data set. All analyzes were done with IBM SPSS statistics release 19.0.0. (SPSS Institute, Cary, NC).

Results and Discussion

Interestingly, foragers did not reject the GF-120 and sucrose mixture at any of the tested concentrations. Foragers choose sucrose solutions contaminated with GF-120 as often as the control (sucrose-only) feeder (sequential Sidak significance, $P > 0.5$, df = 1 in all pairwise comparisons; Fig. 1b). Moreover, foragers continued to visit the sucrose solutions at significantly higher proportions than would be expected based upon random choices ($P < 0.05$ in all cases except in the 20 mg/liter concentration; Fig. 1b). Only one forager (40 mg/liter), stopped coming to the setup after 20 visits, so data from this nonvisiting forager was not used. Bees did learn to associate feeder color with food during their training, because they chose the blue plate significantly more often than the yellow plate for all 30 visits in all concentrations ($P < 0.05$; Fig. 2); the proportion of visits to the blue plate is also significantly higher than the proportion obtained with the random data set ($P < 0.05$; Fig. 2). Even though these results were obtained from a single *P. moureana* colony, they

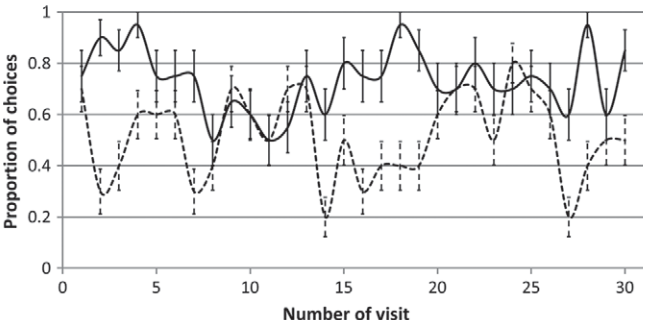

Fig. 2. Average proportion (±95% SE) of choices to the blue plate by *P. moureana* foragers over thirty visits. Solid line: data of concentrations 10–80 mg/liter of Spinosad pooled (n = 20); dashed line: computer randomly generated data set.

clearly show that the GF-120 formulation might not be as dissuasive for bees as initially thought. Future studies should be conducted with additional colonies and species to gain a better understanding of the Spinosad's effects on stingless bees.

In conclusion, the GF-120 formulation is not rejected by the stingless bee *P. moureana* at manufacturer-recommended field concentrations when mixed with sucrose solution and in a season of dearth when natural food sources are highly limited. Moreover, foragers continued to visit the sucrose solution at all of the tested levels of contamination with GF-120 and did not significantly decrease their rate of visitation over time, the 30 consecutive visits tested in our experiment (Fig. 2). In contrast, honey bees will decrease their visitation of sucrose solution contaminated with certain levels of the pesticide imidacloprid (Decourtye and Devillers 2010).

These findings have interesting implications for our understanding of how nontargeted insects are exposed to and, over the short term, respond to the Spinosad GF-120 formulation. First, *P. moureana* foragers will not be discouraged from collecting GF-120 treated nectar. We tested this in a condition of natural food dearth, but future studies should examine whether this is also true when natural food sources are abundant. However, our results are consistent with honey bee studies in green houses and in extensive monocultures (both cases in which there are relatively few other food options) in which single applications of Spinosad (not the GF-120 formulation) did not stop foragers from collecting food (Mayes et al. 2003). More recently Mangan and Moreno (2009) investigated the effects of the individual components of the GF-120 formulation on honey bee choice behavior. They showed that ammonium acetate, the putatively repellent constituent in the GF-120 formulation, does not discourage all the foragers feeding on an invertose sugar solution. We found similar results using the Spinosad GF-120 formulation mixed with sucrose: stingless bees did not abandon the contaminated food.

Given that stingless bee foragers were not discouraged from collecting GF-120 treated food, and did not lose their ability to visually orient toward this food, the pesticide would reach other members of the colony through food deposited in honey pots and sharing directly with nestmates. One *P. moureana* forager can transport on average ≈1 μl of nectar per trip (unpublished data), thus a foraging force of 60–100 individuals would deliver 72–120 μg of Spinosad-contaminated nectar (assuming they are collecting a 40 mg/liter solution) to the colony in 30 trips, a quantity sufficient to kill multiple bees. Foragers are less affected than larvae in such situation, because foragers empty their stomach after each trip, while larvae are enclosed for several days with contaminated food.

The absence of any immediate effect on our foragers could be because of the fact that Spinosad acts more slowly than conventional insecticides (Bret et al. 1997). Thus, the average 35 ± 15 min observation period of this study was not sufficient to observe any adverse effect of Spinosad. In the bumblebee *Bombus impatiens* (Cresson), individuals that were exposed to Spinosad at a concentration of 0.8 mg/kg during the larval stage developed into slower foragers than those exposed to lower or no Spinosad concentrations (Morandin et al. 2005). Thus, the effects may become more apparent in the next generation if larvae develop into slower and thus poorer foragers. Over the long-term, this should reduce colony fitness. Overall, our findings show that it will be important to carry out more studies on the effects of Spinosad on stingless bees, particularly on the doses that foragers and larvae are cumulatively exposed to and on potential long-term behavioral effects. Stingless bees have, to date, not been a focus of such pesticide studies, but are an important tropical pollinator. Our understanding of the unintended consequences of pesticide use would therefore be enhanced by more studies using stingless bee models.

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