

Foraging Allocation in the Honey Bee, *Apis mellifera* L. (Hymenoptera, Apidae), Tuned by the Presence of the Spinosad-Based Pesticide GF-120

NV CABRERA-MARÍN, P LIEDO, R VANDAME, D SÁNCHEZ

El Colegio de la Frontera Sur, Tapachula, Chiapas, Mexico

Keywords

Ecotoxicology, macrolide, recruitment, tephritidae

Correspondence

D Sánchez, El Colegio de la Frontera Sur, Carretera Antiguo Aeropuerto Km 2.5, Tapachula, Chiapas, Mexico CP 30700; dsanchez@ecosur.mx

Edited by Fernando B Noll – UNESP

Received 2 September 2014 and accepted 12 December 2014

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Abstract

Agroecosystem management commonly involves the use of pesticides. As a result, a heterogeneous landscape is created, in which suitable and unsuitable spaces are defined by the absence/presence of pesticides. In this study, we explored how foragers of the honey bee, *Apis mellifera* L., adapt to such context. We specifically evaluated the effect of GF-120, a spinosad-based fruit fly toxic bait, on the allocation of foragers between food sources under the hypothesis that foragers will move from food sources with GF-120 to food sources without it. We thus carried out three experiments: in experiment 1, a group of foragers was trained to collect honey solution from a feeder; next, this feeder offered a GF-120/honey solution. A minority of foragers continued collecting the GF-120/honey solution. In experiment 2, we trained two groups of foragers from a colony to two food sources equally rewarding. Next, GF-120 was added to one of the food sources. We found that the majority of foragers moved from the GF-120-treated feeder to the feeder without GF-120 and that the minority that continued visiting the GF-120-treated feeder did not collect the GF-120/honey solution. In a third experiment, we wanted to know if foragers in an experimental setup as in experiment 1 would perform waggle dances: none of the foragers that collected GF-120/honey were observed dancing. Our results emphasize the importance of “food refuges” for non-target species, since they minimize the impact of agrochemicals upon them.

Introduction

Current rate of biodiversity loss, partially originated by agricultural practices, emphasize the importance of developing pest control approaches that embrace preventive measures and interventions with reduced risks to non-target species (Tilman *et al* 2002). In this sense, pesticides should be carefully controlled and their use considered only after agroecological techniques have shown to be insufficient to control pest populations (Horrigan *et al* 2002). Moreover, pesticides should be used as a complement in pest management, not as the primary tactic, so that pest populations become effectively managed and beneficial organisms be marginally affected (Carvalho 2006). Such chemicals should also be environmentally friendly, so the impact upon non-target

organisms is further decreased (Leng & Reddy 2012). Pesticides of natural origin seemingly satisfy these requirements. Insect pollinators are a good model to evaluate the effect of these eco-friendly substances upon non-target species for several reasons: (1) net revenues by insect pollination are significant; (2) ecological benefits are immense, since they are necessary for maintaining food webs in nature; and (3) they are usual visitors of crops, sharing space, time, and risks with pests (Mayes *et al* 2003, Miles 2003, Bailey *et al* 2005, Morandin *et al* 2005, Klein *et al* 2007, Besard *et al* 2011, Garibaldi *et al* 2013).

Both managed and wild bees are known to increase the productivity of many crops; the honey bee, *Apis mellifera* L., and the bumble bees, *Bombus* spp., are the managed species responsible for pollinating a variety of important crops

(Velthuis & Doorn 2006, Klein *et al* 2007, Aizen & Harder 2009). Recent studies have evaluated the proficiency of the other highly social tribe of Apidae, the Meliponini or stingless bees, as pollinators of many other crops with promising results (Heard 1999, Torres-Ruiz *et al* 2013). Pollinators are heavily affected by agricultural practices, particularly pesticide use, which is a paradoxical situation given their contribution to crop production. Use of safer alternatives to synthetic pesticides has derived in the discovery of less harmful substances like spinosad, a mixture of microbial macrolides with insecticidal properties (Cisneros *et al* 2002, Miles 2003, Williams *et al* 2003). Spinosad has proven to be safe to honey bees in many settings; in experiments in which spinosad was applied at 175 g/ha on alfalfa fields, no significant difference was observed in honey bee worker mortality in treated and untreated plots (Mayer 1999). Further field studies demonstrated the low impact of spinosad on the honey bee, revealing its convenience as a low-impact pesticide on non-target species (Mayes *et al* 2003). A special formulation of spinosad was developed to control fruit flies, a pest that costs millions USD/year worldwide, which improves its specificity and reduces the amount of active ingredient in it, turning it less active to pollinators and natural enemies (Miles 2003, Mangan & Moreno 2009). The GF-120 formulation provides specificity against fruit flies by the addition of substances that release NH_3 , which putatively attracts fruit flies but repels bees. Many studies have proven the value of this formulation on fruit fly control (Prokopy *et al* 2003, Yee & Chapman 2005, Flores *et al* 2011), its efficacy, similar to that of conventional pesticides (Vargas *et al* 2003, Urbaneja *et al* 2009), and its lack of attractiveness to honeybees (Mangan & Moreno 2009).

A recent study revealed that the stingless bee *Plebeia moureana* Ayala and *A. mellifera* are not completely reluctant to collect a mixture of GF-120 and honey (Sánchez *et al* 2012, Gómez-Escobar *et al* 2014); this is a very important finding since in oral and topical toxicity tests, spinosad and GF-120 were found to be very active in honey bees (Edwards *et al* 2003, Miles 2003). In fact, even when few foragers would collect small quantities of GF-120, sublethal effects due to chronic ingestion of spinosad could have detrimental effects or act synergistically with other substances in the long term, as shown in the honeybee with other pesticides (Vandame *et al* 1995, Vandame & Belzunces 1998, Desneux *et al* 2007, Aliouane *et al* 2009, Biondi *et al* 2012). In addition to ingestion, bee foragers can contact GF-120 during application. Fortunately, the probability that they enter in contact with GF-120 drops (at 80 ppm of spinosad) during application seems to be minimal, since it is applied as 3–5 mm Ø droplets, spaced by 10–15 cm (Spencer *et al* 2003). Much of the concern comes from foragers gathering contaminated resources. Actually, GF-120 is applied in plantations or in areas where alternate hosts of fruit flies may be found. In either case, blooming plants are commonly available, and thus,

there is some probability that foragers enter in contact with the pesticide. On the other hand, there could be patches free of GF-120. These spaces can become more attractive than contaminated ones, and foragers then could move from contaminated resources to uncontaminated ones. We actually do not know if this happens in any species of social bees.

A special behavior described in many species of social bees could potentially increase the probability of contact between foragers and resources reached by GF-120; this is referred to as food recruitment and occurs when scouts send nestmates to profitable resources by means of communication mechanisms (Sánchez & Vandame 2013). The worst scenario is that GF-120 reaches very attractive, widely available food sources, in which the content of sugar is high; such attractive but contaminated resources may be accepted by foragers, as shown by Sánchez *et al* (2012) and Gómez-Escobar *et al* (2014), and be profitable enough to communicate its location to nestmates. This possibility greatly increases the exposure of the colony: if foragers succeed at recruiting nestmates, the amount of GF-120 inside the colony could build up rapidly. Several studies have shown that the decision of foragers to communicate nestmates the location of rich food sources largely depends upon their individual perception of profitability (Scheiner *et al* 2004), so foragers with a high rejection threshold therefore could initiate waggle dances to inform nestmates about the location of GF-120-treated food sources. This possibility remains to be explored.

There is a large gap in our understanding about how bees modify their behavior when facing contaminated environments. Thus, we carried out a series of experiments to know the effects of the pesticide GF-120 on *A. mellifera* foraging behavior. This is of particular interest to us since GF-120 is planned to gradually substitute the organophosphate Malathion in the coming years in Southern Mexico. We aimed to answer the following questions: (1) do *A. mellifera* foragers actually collect the putatively repellent GF-120 in semifield conditions; (2) if foragers are visiting a food source without GF-120, will nestmates visiting a food source with GF-120 move to the non-toxic alternative; and (3) will successful foragers attempt to recruit nestmates to food sources treated with GF-120?

Material and Methods

Experiments were carried out in Tapachula, Chiapas, Mexico, from August to November 2012, in the middle of the rainy season, when there are few natural food sources available and thus foragers can be trained to our feeders. Queenright *A. mellifera* colonies in good conditions were used. Since there were feral colonies of this species in our experimental field, we paint-marked all foragers from our test colonies using a device in the entrance of the test colonies (Mikery-Pacheco *et al* 2013); it basically consists of a 25×

10×8-cm wooden box that holds a piece of sponge imbibed in water-based paint, which paints the thorax of incoming and outgoing foragers (Fig 1). Any unmarked forager in the feeders (which could potentially come from a colony other than ours) was trapped using suction tubes and kept in plastic jars until completion of the experiments. Since experience could bias the choice behavior in our foragers (Sánchez *et al* 2008), all thorax-marked foragers that were observed in the feeders were manually painted in the abdomen with a different color. Therefore, foragers that visited the feeder had two color dots. In subsequent replicates, all unmarked foragers and foragers painted in both the abdomen and the thorax were trapped; we only allowed inexperienced, thorax-marked foragers in each replicate. With this procedure, we also avoided pseudoreplication and could consider all replicates as independent among them.

Experiment 1—do honey bees collect a GF-120/honey mixture?

In this experiment, the objective was to determine whether foragers would continue visiting a food source treated with GF-120 (NF Naturalyte, Dow Agrosiences, Indianapolis, IN) when no other choice was available. Ten to 20 foragers were trained to collect a honey solution (60% honey, 40% water) from a feeder located 50 m south (south feeder (SF)) from the test colony (Fig 2a), following Sánchez *et al* (2011) training procedures. All these foragers were already marked on the thorax by the Mikery-Pacheco's device, so we had to mark them yellow on the dorsal side of their abdomens to distinguish them from newcomers. This was the *training phase*, and ended once 100–150 newcomers were observed at the feeder and their abdomens were painted. In the *test phase*, the honey solution was removed and a GF-120 solution was placed, prepared as recommended by the manufacturer, but using honey instead of water (60% honey, 40% GF-120 concentrate, final concentration of spinosad 80 ppm), to have a solution with identical honey content as the solution used



Fig 1 Device used to mark *Apis mellifera* foragers in our experiments. In this particular case, foragers are being marked red in the thorax and blue in the abdomen. For more details, consult Mikery-Pacheco *et al* (2013).

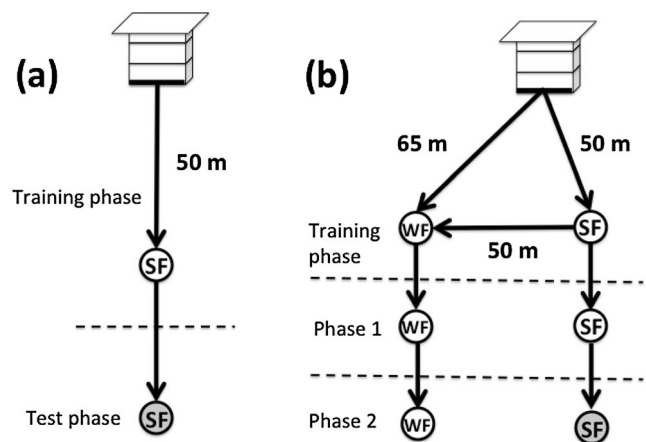


Fig 2 a Setup of experiment 1 and b experiment 2. WF west feeder, SF south feeder. The feeder is offering GF-120/honey (shaded circles). The feeder offers honey solution (white circles).

during training. We allowed foragers to adapt to the new situation for 15 min and then started registering the number of foragers collecting the GF-120/honey mixture every minute for 15 min. Overall, nine replicates were carried out: three replicates per each of three colonies.

Experiment 2—do honey bees exposed to GF-120-treated food sources move to GF-120-untreated food sources?

The objective of this experiment was to know whether foragers could move from GF-120-treated food sources to untreated ones. A group of 40–50 foragers was trained to collect honey solution from the SF. Next, approximately half of these foragers were trained to collect the honey solution in another feeder located 50 m west from the SF and 65 m from the colony (west feeder (WF); Fig 2b). Thus, we had two groups of foragers from the same colony foraging simultaneously in two different locations. Each group of foragers was distinctively marked in the abdomen (red for SF foragers, blue for WF foragers) to know the feeder they were trained to. Thus, three colors could be noticed during the experiments: white on the thorax of all foragers, painted by the Mikery-Pacheco's device, red in the abdomen of SF foragers and blue in the abdomen of WF foragers. After training was completed, we recorded the numbers of foragers moving between feeders every minute for 20 min; we called this period of time *phase 1*, a measure of location fidelity in the experimental conditions; in *phase 2*, the honey solution in the SF was removed and the GF-120/honey mixture was offered for 40 min; the WF always offered honey solution. We could distinguish foragers that moved between feeders by observing the color in their abdomens; such foragers were trapped and counted during both phases and feeders every minute. Three replicates per each of two colonies were carried out.

Experiment 3—do foragers that collect GF-120 perform waggle dances?

In experiment 1, we observed some foragers collecting the GF-120/honey mixture. In experiment 3, we aimed to investigate whether such foragers attempted to recruit nestmates through waggle dance. Two queenright colonies housed in glass hives placed inside a laboratory were connected outdoors through a plastic tube, allowing foragers to collect honey solution from a feeder located 50 m south of the laboratory. Since these colonies' foragers were marked with the Mikery-Pacheco's device and some were visiting other food sources in different places, we marked the foragers visiting our feeder on their abdomens. Once 100–150 foragers were observed in the feeder, phase 1 started and we video-recorded dancers in the colony using a Nikon D3100 camera (Nikon Corp., Tokyo, Japan) for 30 min. In phase 2, the honey solution was exchanged for a GF-120/honey solution and 30 more minutes were recorded. Since in phase 2 the majority of the foragers stop visiting this feeder, and potentially could visit other resources, we marked the foragers that kept visiting the feeder with orange over the yellow marks. Finally, in phase 3, we put back the honey solution and 30 more minutes were recorded; again, foragers that visited the feeder were painted blue over any mark on the abdomen to recognize them in the videos. A total of three replicates per each of two colonies were conducted.

Data analysis

Data from experiment 1 (number of foragers in training and test phases) and experiment 3 (number of foragers performing the waggle dance in the three phases) were analyzed by a repeated measures ANOVA approach, which is able to handle autocorrelated (time) data. Data from phase 2 of experiment 2 was further divided into two phases of 20 min each before applying a repeated measures analysis, so we end up with three phases (Fig 4). Every replicate in all experiments was considered an independent subject in our analysis. Data were analyzed in R v3.0.2. (R Development Core Team 2012) using the ezANOVA function in the ez package v4.2-2. (Lawrence 2012).

Results

Experiment 1—do honey bees collect GF-120/honey mixture?

In all nine replicates, only 3.8 ± 0.83 (mean \pm SD) foragers continued collecting the GF-120/honey mixture in the test phase, which was significantly lower than 121.1 ± 19.33 foragers observed collecting the honey solution in the training phase

(Fig 3; $F=337.15$; $df=1, 8$; $p<0.001$). Foragers were clearly discouraged from collecting the treated resource. Interestingly, we observed several foragers overflying and examining the GF-120/honey solution, but none of these collected it; these individuals were not counted for the analysis.

Experiment 2—do honey bees visiting food sources with GF-120 move to GF-120-untreated food sources?

In phase 1, the number of foragers that moved from the SF to the WF and vice versa was insignificant: 8 and 11, respectively. In this phase, the feeders offered the same resource and were located at a distance that reduced the flow of foragers between them by chance, which may help to explain these numbers. Similarly, only five foragers moved from WF to SF in phase 2. We think that such location fidelity is the consequence of offering a rich resource. Moreover, visitation by the foragers trained to the WF did not significantly change over the 60 min of observation (Fig 4; $F=3.25$, $df=2, 10$; $p=0.08$; Mauchly's sphericity test, $p=0.07$). However, when the SF offered the GF-120/honey solution in phase 2, a significant number of foragers started migrating to the WF (Fig 4; $F=16.02$, $df=2, 10$; $p<0.001$; Mauchly's sphericity test, $p=0.25$). Only a few foragers remained visiting the SF but did not collect the mixture. WF foragers were never observed collecting the GF-120/honey solution in the SF after phase 1. Since all foragers that moved from the SF to the WF were trapped as they were arriving, a decline in the number of these foragers was observed as the experiment progressed, as shown in Fig 4. We did so to avoid hundreds of foragers visiting the WF feeder so counting would become difficult. These results clearly indicate that SF foragers redirected their efforts toward safer, insecticide-free resources.

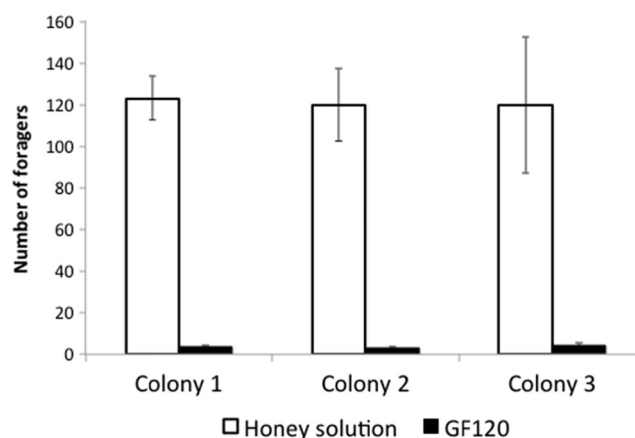


Fig 3 Number of *Apis mellifera* foragers observed during training (empty columns) and test (solid columns) phases of experiment 1.

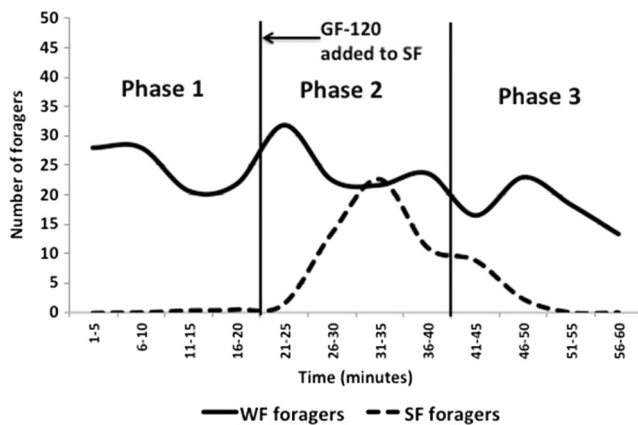


Fig 4 Average number of *Apis mellifera* foragers observed in the west feeder; foragers observed in the south feeder are not shown (see “Results”). WF foragers: bees trained to collect food from west feeder; SF foragers: bees trained to collect food from south feeder. The WF offered sugar solution in phases 1 and 2. The SF feeder offered sugar solution in phase 1 and GF-120 solution in phase 2, which explains why SF foragers were observed moving from their south feeder to the west feeder from the beginning of phase 2. To avoid excessive recruitment, SF foragers were systematically captured as soon as they appeared in

Experiment 3—do GF-120 collecting foragers waggle dance?

On average (\pm SE) 16.3 ± 1.08 , 0.0 ± 0.00 , and 5.8 ± 0.48 foragers were observed performing the waggle dance in phases 1, 2, and 3, respectively; these averages are significantly different ($F=31.14$, $df=2, 10$; $p<0.001$). Further pairwise tests revealed that the absence of foragers dancing in the second phase caused the statistical difference (pairwise paired t test: phase 1–2: $p<0.001$; phase 1–3: $p=0.635$; phase 2–3: $p<0.001$). Thus, we can affirm that foragers that collected GF-120/honey did not attempt to recruit nestmates.

Discussion

To keep the negative impact of agricultural practices upon beneficial organisms as low as possible, a deep understanding of animals’ behavioral response to agrochemicals is essential. In this study, we show how honey bee foragers adjusted their foraging efforts according to the presence of the pesticide GF-120. We found that even though some foragers were still willing to collect GF-120 mixed with honey in experiment 1, we did not observe in experiment 2 any foragers collecting this mixture perhaps because an alternate food source without GF-120 was available. Moreover, we did not observe any of the foragers that collected GF-120/honey performing waggle dances in experiment 3. Honey bees thus seem to modify their foraging efforts according to the presence or absence of pesticides.

In experiments 1 and 3, some foragers continued collecting GF-120/honey mixture, despite it had repellent and toxic substances (Mangan & Moreno 2009). Given the substantial content of non-sugar compounds in the GF-120/honey mixture, we wondered why such minority of foragers collected it. Pankiw & Page (1999) showed that the probability, or threshold, for a worker to carry out certain tasks is genetically related; other studies have shown that behaviors like sucrose responsiveness and olfactory learning are also genetically determined (Laloi & Pham-Delegue 2010, Scheiner & Arnold 2010). Therefore, it is possible that some genes are involved in the threshold for GF-120 acceptance and that the foragers that continued collecting GF-120/honey had the highest rejection threshold in the tested colonies, i.e., the genetic variability within the colony could allow some foragers to do hazardous tasks like collecting GF-120/honey.

Another explanation might be associated with the presence of alternative food sources. In experiments 1 and 3, one feeder was offered, which forced some foragers to keep collecting from this single food source, even though it had GF-120. However, two feeders were available in experiment 2. In phase 1 of experiment 2, a typical location fidelity pattern could be observed, since very few foragers moved between the two feeders. In phase 2, however, foragers that were visiting the SF suddenly found GF-120 in the food. Such foragers readily moved to the untreated WF, so no foragers were observed collecting GF-120/honey in the SF in the rest of the experiment. Wray *et al* (2011) demonstrated that private, internal information is overridden by social information when it is less profitable to follow own experience. However, it is difficult to determine which communication mechanisms (waggle dance, sounds, social facilitation, or pheromone deposition) could be involved in the movement of foragers from the SF to the WF. This remains for future research.

Foragers that collected GF-120/honey did not attempt to recruit nestmates through waggle dance. This result is in agreement with the findings of Abbott and Dukas (2009) who observed foragers performing fewer waggle dances than nestmates coming from equally profitable, but less dangerous patches. Moreover, it has been shown that food profitability affects both duration and rate of waggle-run production in the honey bee (Seeley *et al* 2000) and also the intensity of the round dance (Waddington & Kirchner 1992). Similar findings have been reported with sound production in stingless bees (Aguilar & Briceño 2002, Nieh *et al* 2003) and food alerting behavior in *Bombus* spp. (Dornhaus & Chittka 2001, Dornhaus & Cameron 2003). The GF-120 content in the honey solution was clearly discouraging foragers from communicating its presence, but it was good enough to be collected by themselves.

Our results stress the importance of leaving spaces without pesticides, i.e., patches that may be considered “refuges.” We used a highly concentrated honey solution (60%

honey solution, roughly 48% of sugar content), which represents a highly attractive resource. Such sugar concentrations are not unusual in nature (up to 60% w/v; Gottsberger *et al* 1984), so it is not improbable that honey bees collect contaminated resources if no other option is available, particularly in dearth conditions. Same reasoning applies to other pesticides since many studies have found them in high concentrations in pollen and honey, which reveals that honey bees do not reject food sources with agrochemicals (Chauzat *et al* 2006, Mullin *et al* 2010). Therefore, the next step is to implement our findings in field tests, by leaving untreated patches, placing rich food sources (feeders) or both, observing colony strength and quantifying chemicals in bee products as pesticides are applied.

Acknowledgments We are very grateful to Agustín Méndez and Miguel Cigarroa for helping with *A. mellifera* colonies. Also, we appreciate the assistance of Erik Solórzano and Leonardo Godínez during experiments. This research was carried out thanks to support by the CONACYT-SAGARPA Project no. 163413 and SEP-CONACYT Project no. 106043.

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