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The Effect of an Organic Pesticide on Mortality and Learning in Africanized Honey Bees (*Apis mellifera* L.) in Brasil

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Abstract: Seven experiments were conducted. First, the influence of the consumption of different concentrations of the organic pesticide Bioganic® on mortality was assessed at 11 different time intervals in Africanized honey bees (*Apis mellifera* L.) as was direct application of the pesticide to the abdomen. Results indicated that the pesticide was not lethal to bees regardless of concentration at any intervals tested whether consumed directly or applied to the abdomen. Second, the effects of different concentrations of the pesticide on Pavlovian conditioning and complex learning were examined in harnessed foragers. Results suggest that the pesticide affected learning; however, this conclusion may be erroneous because the bees would not feed on the pesticide, thus making it impossible to properly assess Pavlovian conditioning and complex learning. Consequently, the effect of the agrochemical on complex learning was examined in free flying bees trained to land on targets. The results of free flying experiments indicated that bees did not avoid a target associated with the smell of the pesticide but did avoid the target if they had to drink the pesticide.

Key words: Organic pesticide, honey bee, behavior, learning

INTRODUCTION

These experiments investigated the effects of Bioganic® Lawn and Garden Spray Multi-Insect Killer (Bioganic Safety Brands, USA), an organic pesticide, on learning and mortality in Africanized honey bees (*Apis mellifera* L., Hymenoptera: Apidae) in the northeastern state of Paraiba, Brasil. This organic pesticide is unique because it is composed almost entirely of essential oils. To examine the effect of the pesticide on mortality, bees in one group consumed pesticide and in another group the pesticide was applied directly to their abdomens. Effects on learning were assessed using proboscis conditioning in harnessed foragers and in free-flying bees trained to visit the laboratory. Olfactory conditioning of proboscis extension is a popular technique utilized to estimate the influence of lethal and sublethal inorganic agrochemicals on honey bee behavior, but there is no literature to date on the effect of organic insecticides on learning[1-3]. The free-flying procedure was also utilized to investigate the ability of the organic insecticide to serve as a discriminative stimulus and as a reward. To our knowledge, this is the first published account of using free-flying bees to investigate the effect of an agrochemical on learning.

In Experiment 1 we investigated the effects of consumption of various concentrations of the organic pesticide as well as application of 100% pesticide to the abdomen on mortality in a sample of Africanized honey bees. In Experiment 2 we investigated whether an unconditioned stimulus (US) of 1.56% or 6.25% organic pesticide solution influenced acquisition or extinction of a simple Pavlovian association. In Experiment 3 we investigated whether consumption of 1.56% or 6.25% organic pesticide solution influenced complex learning as represented by the ability to discriminate between two conditioned stimuli (CSs) – one paired with a US and the other not paired with a US. In Experiment 4 we investigated whether the odor of 6.25% pesticide solution could function as a CS. In Experiment 5 we investigated whether the odor of 6.25% pesticide solution could be used in a discrimination experiment. We selected these organic pesticide values in anticipation of future work on the use of essential oils to control aphids. In Experiment 6 we used the free-flying procedure to determine whether under natural conditions honey bees could discriminate the odor of the organic pesticide from a control odor. In Experiment 7 we used the free-flying procedure to determine whether the organic pesticide could function as a reward in the same way sucrose does.

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MATERIALS AND METHODS

The seven experiments were conducted at the Federal University of Paraiba: Campus III located in the city of Bananeiras. All experiments were conducted during the months of June, July, and August of 2005. These months constitute the winter or “rainy season” in northeast Brasil. To control for calendar variables and fluctuating hive conditions, animals from all experiments were run simultaneously. All learning experiments employed unpaired control groups or the use of discriminative stimuli to control for non-associative effects, and bees were selected from multiple laboratory colonies. The organic pesticide utilized in all experiments was Bioganic® Lawn and Garden Spray Multi-Insect Killer. The pesticide is composed entirely of plant and tree oils (4% each of thyme, clove, sesame, with the remaining 88% consisting of unspecified combinations of water, soybean oil, wintergreen oil and lecithin).

Insects: The proboscis and free-flying training methods are identical to those utilized in our previous work in Brasil[4]. For the proboscis conditioning experiments, foraging honey bees (*Apis mellifera* L.) were captured in glass vials from laboratory hives, placed in an ice water bath, and while unconscious harnessed in metal tubes. Upon regaining consciousness, they were fed 1.8 M sucrose solution until satiated and set aside for use approximately 24 h later. Only those animals that vigorously extended their proboscis to sucrose stimulation during a pretest were used in experiments.

Apparatus and stimuli: The conditioned stimuli (CS) in experiments 2 - 7 consisted of the odors of: 6.25% organic pesticide diluted in water, cinnamon (Gilbertie’s, Easton, CT), or citronella (*Cymbopogon winterianus*). The citronella was steam distilled in the laboratory. The unconditioned stimuli (US) in these experiments consisted of a 1 µl droplet of either 1.8 M sucrose, 1.56% organic pesticide, or 6.25% organic pesticide applied with a Hamilton microsyringe. The latter corresponds to the labeled rate and both concentrations were made palatable to the honey bees by diluting them in 1.8 M sucrose. We have used the 1 µl droplet of an agrochemical in previous research and believe this to be a good approximation of what a honey bee would encounter in the field.

The CS odors were applied neat (approximately 3 µl) each day on a 1-cm^2 piece of filter paper (Whatman no. 4) attached to a 20 ml plastic syringe to create an odor cartridge. To apply the odor, the plunger of the syringe is pulled back to the 20 ml mark and quickly depressed. This method, although unautomated, is highly effective and inexpensive. Research designed to directly compare automated and unautomated proboscis conditioning techniques revealed no differences in conditioning[5]. It should be noted that in rural areas of Brasil automated apparati is often difficult to obtain and not practical.

Proboscis conditioning experiments: For all proboscis conditioning experiments, the CS duration was 2 s, US duration approximately 1 s (the time needed to consume a 1 µl droplet) and the intertrial interval (ITI) 10 min in paired animals and 5 min in unpaired and animals receiving discrimination training.

A conditioning trial began by picking up a bee and placing it in front of a ventilation fan for several seconds, after which the appropriate stimuli were introduced. After application of the stimuli, the animal was returned to a holding area and a second animal was run. A trace conditioning procedure was used where the CS was presented first followed by the US. The CS and US presentations did not overlap. If the animal extended its proboscis during the CS but before the US a ‘1’ was recorded. If the proboscis did not extend to the CS ‘0’ was recorded. Responses were recorded visually.

Free-flying experiments: The free-flying technique was used by first establishing a feeder containing an 8% sucrose solution. Foraging bees were attracted to the feeder and bees were captured individually in a matchbox, placed on a gray target constructed from a 5.5 cm diameter disposable petri dish, and marked with nail polish while feeding on a .6 ml droplet of 1.8 M sucrose. If the bee did not return to the target on its own, it was recaptured and returned to the gray target.

When the bee returned to the gray target twice of its own accord, the gray target was replaced with the two odor targets used in training. The odor targets were also gray, but have .5-cm holes equally spaced around the circumference of the dish. A cotton ball with 20µl of an odor served as discriminative stimuli. In these experiments, the odors of 6.25% organic pesticide and erva-doce (*Foeniculum vulgare* Apiaceae, 3.12%, diluted with 1% neutral detergent, Minuano, Friboi Ltda, Luiania, GO) served as the discriminative stimuli and 1.8 M sucrose and 6.25% organic pesticide in 1.8 M sucrose served as rewards (.6 ml droplet). In preparation for these experiments, we conducted a feeding test using Minuano detergent and it had no effect on learning or mortality in a test sample of 30 bees (for details on the free-flying procedure and how it is used with Africanized honey bees, see references 4 and 10).

Experiment 1-mortality: In Experiment 1 we investigated whether consuming various concentrations of the organic pesticide led to death. Two hundred forty bees were divided randomly into 8 groups of 30 for feeding tests; a ninth group of 62 bees received 100% organic pesticide applied directly to their abdomens. In the feeding tests, each group differed in pesticide concentration (0% - water only, 1.56%, 3.12%, 6.25%, 12.5%, 25%, 50%, and 100%). Except for the 0% and 100% groups, the pesticide was diluted in 1.8 M sucrose. Antennae of bees in the 0% and 100% groups were stimulated with sucrose, thus causing proboscis extension. Bees in the 0 % (water only) group and in the 100% pesticide group were then allowed to feed on the pesticide solution. To determine whether topical application of the pesticide to honey bees would give results that differed from feeding tests, the abdomens of a ninth group of 62 bees were exposed to a 1 µl droplet of 100% organic pesticide. After either consumption or application of organic pesticide, all animals were observed over the course of 11 time intervals totaling 4 h (5, 10, 15, 20, 25, 30, 45, 60, 120, 180, 240 min).

Experiment 2-acquisition and extinction of Pavlovian association, harnessed: In Experiment 2 we investigated whether a US of 1.56% or 6.25% organic pesticide influenced acquisition or extinction of a simple Pavlovian association. One hundred twenty bees were randomly divided into 6 groups of 20 subjects each. Three groups received a CS of citronella odor paired with either a US of: 1) sucrose only, 2) 1.56% pesticide, or 3) 6.25% pesticide. To control for nonassociative effects, each of the 3 paired groups were linked to 3 groups receiving unpaired pseudorandom CS/US presentations. Paired animals received 12 acquisition trials followed by 12 extinction trials in which the US was omitted. Extinction trials were included to determine whether the pesticide influenced persistence of a learned response. Animals in the unpaired groups received 24 trials, 12 each of the CS and US respectively. To equate the ITI for both paired and unpaired animals, all unpaired animals received an ITI of 5 min – half that used for paired animals.

Experiment 3-complex learning, harnessed: In Experiment 3 we investigated whether consumption of 1.56% pesticide solution or 6.25% pesticide solution influenced complex learning as represented by the ability of honey bees to discriminate between two conditioned stimuli – one paired with a US and the other not. Sixty bees were randomly divided into 3 groups of 20. One group received a CS+ of an odor paired with sucrose and the other two a CS+ of an odor paired with either 1.56% or 6.25% organic pesticide, respectively. The CS- consisted of odor alone – no US was presented. The CS odors were citronella and cinnamon. For half the animals in each of the three groups, the CS+ was citronella odor and the CS- cinnamon odor. For the remaining 10 animals in each group, the CS+ was cinnamon odor and the CS- citronella odor. The order of CS+ and CS- was pseudorandom and the ITI was 5 min.

Experiment 4-pesticide as a conditioned stimulus, harnessed: In Experiment 4 we investigated whether the odor of 6.25% pesticide solution could function as a CS. This experiment was similar to Experiment 2 with the exception that the CS was the odor of pesticide and the US was 1.8 M sucrose solution. Forty animals were randomly divided into two groups of 20. In one group, animals received a paired CS-US presentation. In the second group animals received unpaired CS/US presentations.

Experiment 5-pesticide as a discriminative stimulus, harnessed: In Experiment 5 we investigated whether the odor of 6.25% pesticide solution could function as a discriminative stimulus. The experiment was similar to Experiment 3, but the odor of 6.25% pesticide solution was used as a cue rather than as a US. Forty animals were randomly divided into 2 groups. Group 1 received a CS+ of the odor of 6.25% pesticide solution and a CS- of citronella odor. Subjects in Group 2 received a CS+ of citronella and a CS- of the odor of 6.25% pesticide solution. The US was 1.8 M sucrose. Each animal received 24 training trials with 12 being CS+ and 12 CS-. The order of CS+ and CS- was pseudorandom.

Experiment 6-pesticide as a discriminative stimulus, free-flying: In Experiment 6 the free-flying procedure was utilized to determine whether under natural conditions honey bees would discriminate the odor of the organic pesticide from a control odor. Twelve animals were randomly divided into two groups of 6. The training odors were 6.25% pesticide and citronella, respectively. Each animal received 24 training trials in which it was confronted with two targets differing in odor (known in the conditioning literature as a simultaneous discrimination). One odor target always contained a drop of sucrose (S+) and the other always contained a drop of water (S-). For 6 of the animals the S+ was 6.25% pesticide and the S- was citronella. In the remaining animals the S+ was citronella and the S- was pesticide. The targets were positioned approximately 30 cm apart (center to center) and all animals received 24 training trials, with one trial per visit. Following a trial, the targets were removed and thoroughly washed. The position of S+ and S- was pseudorandom from trial to trial. Landing on the S+ target was considered a correct choice.

Following the 24 training trials, each animal received a 10 min extinction test. Both targets contained water and the number of landings on each target was counted over the course of twenty 30 s intervals. Persistence during the extinction test is one method to estimate the strength of any learned association formed during the previous 24 training trials. Following extinction, the animal was captured and not permitted to return to the hive.

Experiment 7-pesticide as reward, free-flying: In Experiment 7 we used the free-flying procedure to determine whether the organic pesticide could function as a reward as sucrose does. This experiment was equivalent to the harnessed discrimination training described in Experiment 3. The method used was similar to that in the previous experiment. Each animal received 24 training trials using a simultaneous discrimination task. Following training, extinction began in which both targets contained water. The major difference between the two experiments is that both training targets contained sucrose reward, but 6.25% pesticide was imbedded within one of them. The training odors were erva-doce and citronella. Twelve animals were used, with 6 receiving an S+ of erva-doce and sucrose and an S- of citronella with sucrose/pesticide. The remaining animals received an S+ of citronella and sucrose and an S- of erva-doce with sucrose/pesticide. Landing on the target that contained the sucrose/pesticide reward was considered an error. Following the 24 training trials all animals received 10 min of extinction in which both targets contained a drop of water.

Data analysis: Analyses for all experiments were performed in SPSS utilizing the General Linear Model Multivariate Analysis of Variance[6]. Raw data were transformed into mean number of responses across trials for all experiments except the mortality experiment, in which each trial interval was tested separately. In addition, α was set at .05 for all experiments, unless heterogeneity of variances was present, in which case α was set at .01.

RESULTS AND DISCUSSION

Mortality: We began this research by asking whether or not the organic insecticide Bioganic® was safe for Africanized honey bees. Based on the results of this research, the answer is yes. The results of our consumption and abdomen tests revealed no significant differences in mortality at any of the 11 intervals tested ($F_{10,2260} = .48$; P = n.s., partial eta squared = .015) nor was there an effect of concentration ($F_{1,7} = .48$; P = n.s., partial eta squared = .01). There were no differences between the abdomen and feeding tests when 100% Bioganic® was applied ($F_{1,1} = .08$; P = n.s., partial eta squared <.01, (Table 1 for mortality rates).

Simple Pavlovian and Pavlovian discrimination experiments: There were no differences in CS or US responses between any of the paired and unpaired groups (Ps >.05), therefore subsequent analyses were collapsed across groups. Figure 1 shows the results of the simple Pavlovian conditioning. At first glance, it appears that pesticide severely effected learning. The Pavlovian association proceeded normally in animals that received a US of sucrose only.

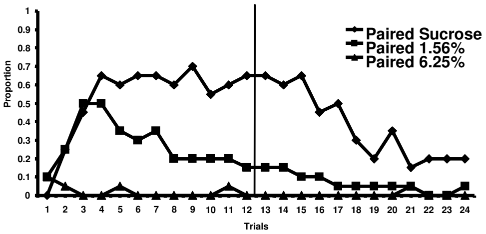

Fig. 1: Acquisition and extinction of simple Pavlovian conditioning in harnessed bees. The CS is citronella and the US sucrose, 1.56%, or 6.25% pesticide. Trial 13 is the transition from acquisition to extinction

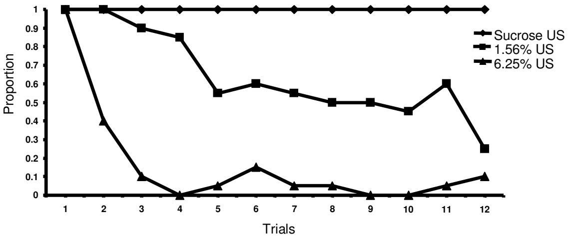

Fig. 2: Unconditioned responses to sucrose, 1.56% or 6.25% pesticide over 12 acquisition trials in Experiment 2

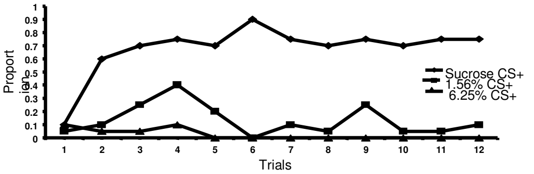

Fig. 3: Acquisition of complex learning in the honey bee as represented by discrimination learning. The odors of citronella or cinnamon function as the CS+ (and CS-). The US is sucrose, 1.56%, or 6.25% pesticide. For clarity, only the CS+ responses are shown

The association however, was unstable in animals that received 1.56% pesticide and non-existent in animals that received 6.25% pesticide, although mean responses to the CS and to the US were not statistically significantly different from each other. Statistical analysis revealed significant differences in mean CS responses between groups ($F_{2,117} = 28.05$; P = .001, partial eta squared = .32, Table 2 for means and standard deviations). Many animals stopped feeding on the pesticide even though it was diluted with 1.8 M sucrose. This result, illustrated in Fig. 2, posed a problem because a proper assessment of Pavlovian conditioning

Table 1: Mortality rates for bees in each group at each interval in minutes

Group	5	10	15	20	25	30	45	60	120	180	240
0	0	0	0	0	0	0	0	0	2	3	0
1.56	0	0	0	0	0	0	0	0	0	1	0
3.125	0	0	0	0	0	0	0	0	1	1	0
6.25	0	0	0	1	0	0	0	0	0	2	0
12.5	0	0	0	0	0	0	0	0	1	1	1
25	0	0	0	0	0	0	0	0	1	1	1
50	0	0	0	0	0	0	0	0	0	2	1
100	0	0	0	0	0	0	0	0	0	3	0
Abdomen	0	0	0	0	0	0	0	0	2	3	0

[a] *n* = 30 for all groups except Abdomen group; *n* = 62 for this group.

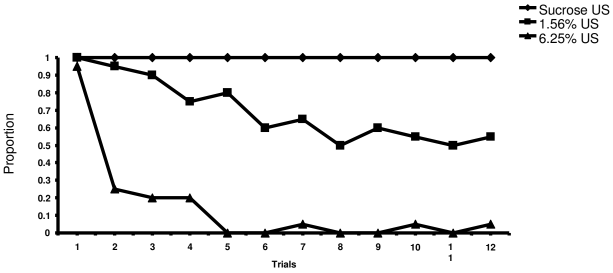

Fig. 4: Unconditioned responses to sucrose, 1.56%, or 6.25% pesticide during the 12 CS+ trials in Experiment 2

in harnessed bees requires that the bees feed on the US. Statistical analysis revealed significant differences in mean US responses ($F_{2,117} = 19.13$; $P = .001$, partial eta squared = .25). Follow-up Tukey HSD tests indicated the control group responded significantly more often to the CS and to the US than the 1.56% or the 6.25% pesticide groups.

A similar pattern of results emerges when the discrimination experiments are considered. Figure 3 shows the results of the harnessed discrimination experiment in which 1.56% or 6.25% served as the US. For clarity, only the CS+ curves are presented. Once again, the data suggests a learning deficiency produced by exposure to the pesticide. Analyses showed statistically significant differences in mean CS+ responses ($F_{2,57} = 60.48$; $P = .001$, partial eta squared = .68 Table 3). As in the simple Pavlovian experiment, the deficiency is directly related to the lack of feeding on the pesticide. Responses to the US are presented in Fig. 4. Analysis of US responses revealed group mean differences in consumption of the pesticide ($F_{2,57} = 113.84$; $P = .001$, partial eta squared = .80). Follow-up Tukey HSD tests indicated the control group responded significantly more often to the CS and than either the 1.56% or the 6.25% pesticide groups, however, there were no significant differences in mean response between the pesticide groups. In addition, the control group responsed significantly more often to the US than the pesticide groups, and the 1.56% pesticide group responded to the US more frequently than did the 6.25% group. A subsequent analysis was performed on the US responses between experiments 2 and 3 and revealed a group effect but no significant differences between the two experiments when feeding on the pesticide ($F_{5,174} = 202.90$; $P = .001$, partial eta squared = .85). Follow-up Tukey HSD tests indicated the control group responded significantly more often to the US than the 1.56% or the 6.25% pesticide groups. In addition, mean responses to the US was higher than in the 6.25% group.

The results of the simple Pavlovian and Pavlovian discrimination experiments with pesticide as a US were inconclusive because harnessed bees stopped feeding on the pesticide. Figure 5 shows the results when 6.25% pesticide was used as a CS and is quite surprising. In a research program spanning almost 10 years we have never seen such high levels of conditioning in Africanized honey bees. However, animals in the unpaired control group also showed considerable levels of responding. Statistical analysis revealed no differences between animals receiving paired or unpaired training ($F_{1,38} = 3.44$; $P = .07$, partial eta squared = .08). The mean and standard deviation for the paired group were 0.93 and 0.04 and for the unpaired group they were 0.87 and 0.14, *n* = 20 subjects per group. We do not know why the odor of the pesticide was so attractive to our sample. It is known that soybean flowers are attractive to foraging bees and may be a primary nectar source and the Brazilian research institute responsible for honey bee research recommends including soybean flour in artificial diets for honey bees[7,8]. The organic pesticide contains an unspecified amount of soybean oil. These results, while not supporting learning, illustrate the importance of employing unpaired control groups when assessing agrochemicals.

The attractiveness of 6.25% pesticide was supported in the discrimination experiment. When animals were required to discriminate pesticide from citronella they did so readily. Preliminary analyses indicated no significant differences in mean CS+ or CS- responses between groups (all *P*s > .05), therefore the groups were combined in subsequent analyses. The results shown in Fig. 6 reveal that harnessed bees learned to respond to a CS+ of 6.25% pesticide if it signaled food and to withhold responding when it did not (CS-). Statistical analysis revealed significant differences in mean CS+ and CS-responses, ($F_{1,78} = 86.98$; $P = .001$, partial eta squared = .527). For CS+ responses, the mean and standard deviation were 0.69

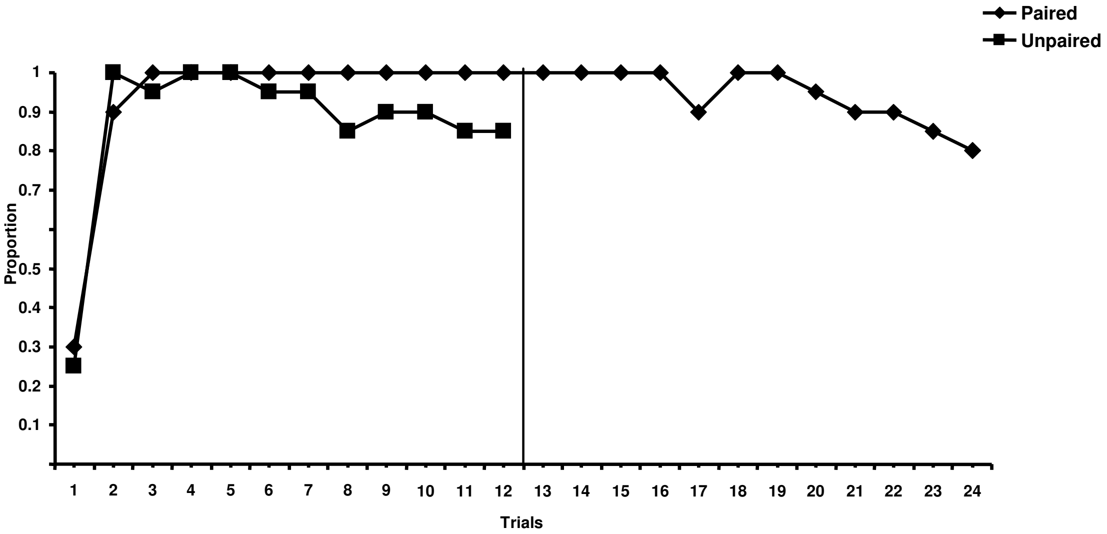

Fig. 5: Acquisition and extinction of simple Pavlovian conditioning with a CS odor of 6.25% pesticide and a US of sucrose. Unpaired control animals received a pseudorandom sequence of CS and US. Trial 13 is the transition from acquisition to extinction

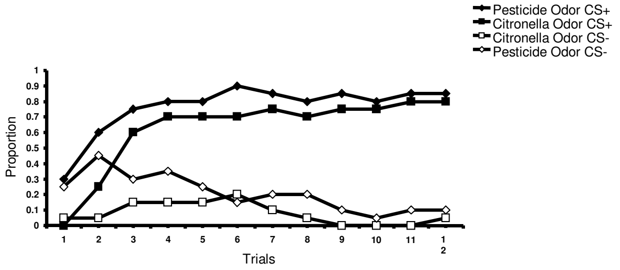

Fig. 6: Discrimination learning in harnessed honey bees with 6.25% pesticide and citronella serving as CS+ (and CS-). Each animal received 24 training trials, 12 each with the CS+ and CS-

and 0.31, and for CS- responses the mean and standard deviation were 0.14 and 0.38, n = 20 subjects per group.

Free – flying experiments: The results of harnessed experiments revealed no significant effect on mortality when pesticide was consumed or applied directly to the abdomens of bees, refusal to feed on a pesticide US even when diluted with high molarity sucrose, and that the odor of pesticide was highly attractive and discriminable, which suggested that results of the free-flying experiments would provide a better assessment of the organic pesticide.

Figure 7 shows the acquisition results of the use of the pesticide as a discriminative stimulus. Free-flying bees readily associated the odor of pesticide with a sucrose reward and learned to discriminate pesticide from citronella. Preliminary analyses showed no significant differences in mean S+ or S- responding between the two groups (Ps > .05) , therefore the groups were combined in subsequent analyses (n = 12). Analyses showed significant differences between mean number of S+ and S- responses during training trials ($F_{1,22}$ = 251.10; P = .001, partial eta squared = .92). For S+ responses the mean and standard deviation were 0.73 and 0.12 and for S- they were 0.11 and 0.05. The acquisition results are supported by the extinction results, illustrated in Fig. 8, in which both targets now contained water. There are clear differences between the two curves. As the duration of extinction progresses over 10 min, bees landed more frequently on the target previously associated with reward. Analyses showed significant differences in mean number of S+ and S- responses during extinction trials ($F_{1,22}$ = 44.53; P = .001, partial eta squared = .70).

Table 2: Mean responses in harnessed bees, Pavlovian association

Group	Response type	Mean	S.D.
Control	CS	0.40	0.30
1.56%		0.08	0.217
6.25%		0.06	0.13
Control	US	0.83	0.23
1.56%		0.58	0.43
6.25%		0.38	0.27

[a] n = 120, forty subjects per group.

Table 3: Mean responses in harnessed bees, complex learning experiment

Group	Response type	Mean	S.D.
Control	CS+	0.68	0.29
1.56%		0.13	0.18
6.25%		0.03	0.05
Control	US	1.00	0.00
1.56%		0.70	0.30
6.25%		0.15	0.10

[a] n = 60, twenty subjects per group.

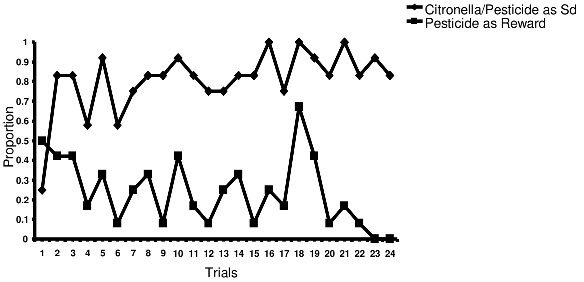

Fig. 7: Acquisition results of the free-flying discrimination Experiments 6 and 7

The mean and standard deviation for S+ responses during extinction intervals were 1.09 and 0.40 and for S- responses were 0.30 and 0.11.

Figure 7 also shows the results when the animal chose between sucrose only and pesticide/sucrose as a reward and clearly illustrates that honey bees selected the sucrose only reward over the pesticide/sucrose target. Again, preliminary analyses showed no significant differences in mean S+ or S- responding between the two groups (*P*s > .05), therefore they were combined in subsequent analyses ($n = 12$). Analyses revealed significant differences in mean S+ and S- responses during training trials ($F_{1,22} = 75.18$; $P = .001$, partial eta squared = .77). The mean and standard deviation for S+ responses were 0.84 and 0.14, and for S- responses the mean and standard deviation were 0.32 and 0.16. This supports the view that animals were refusing to consume the pesticide when harnessed. Given free choice, bees chose the target odor associated with sucrose. The acquisition results are supported by the extinction results. Figure 8 shows when both targets now contained water, bees landed more frequently on the target that formerly contained sucrose only. Analyses showed significant differences in mean S+ and S- responses ($F_{1,22} = 49.00$; $P = .001$, partial eta squared = .69). The mean and standard deviation for S+ responses during extinction intervals were 0.26 and 0.13 and for S- responses they were 0.93 and 0.31.

These results show the importance of using both harnessed and free-flying bees, the importance of employing unpaired control groups, reporting US response data, and reveal a limitation of the harnessed bee procedure. If bees do not consume the agrochemical and mortality studies indicate that bees are not killed by consuming the agrochemical, the only alternative is to use the free-flying method. Unfortunately, this method is time consuming and it is difficult to control all the relevant training variables.

These experiment also represents what we believe to be only the second agrochemical study on learning in Africanized honey bees conducted on a northeastern Brasil sample and, indeed, possibly in Brasil. It is critically important to conduct agrochemical assessments within a country especially when such data is used to make policy decisions on the application of agrochemicals within that country.

Fig. 8: Extinction results for the free-flying Experiments 6 and 7

Relying on data generated in the United States or in European laboratories to make public policy decisions in Brasil is a mistake; more empirical data is needed. Consider, for example, a recent report showing that exposure to endosulfan had no effect on a European honey bee sample[2]. However, a previous experiment showed that there was an effect[9]. When discussing the results of this experiment, the authors of the report failed to mention that an Africanized honey bee sample was used in Brasil, and that learning was affected gradually. The initial learning curves were similar to control animals and the effect of endosulfan was revealed only as training progressed. The literature suggests at least 26 behavioral differences between European and Africanized honey bees with learning capacity and pesticide tolerance among them[4,9-11]. Given the presence of so many behavioral differences it is possible that differential sensitivity to agrochemicals also exists.

The assessment of agrochemicals on honey bees and other animals in Brasil must be made with great care. Brasil is the fifth largest country in the world, covers half of South America, spans four time zones and contains three major climatic regions. The state of Paraiba, for example, contains 12 micro-regions and is part of the Northeast "sertão" which encompasses about 10% of Brasil and is subject to long periods of drought[12]. Such diversity must be taken into account when applying data generated from foreign laboratories. The social science literature is littered with examples of misapplications of the comparative method[13]. What is needed is more comparative data generated under Brazilian conditions in Brasilian laboratories.

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REFERENCES

1. Abramson, C.I., J. Squire, A. Sheridan and P.G. Mulder Jr., 2004. The effect of insecticides considered harmless to honey bees (*Apis mellifera* L.): Proboscis conditioning studies using the insect growth regulators Confirm®2F (Tebufenozide) and Dimilin®2L (Diflubenzuron). Environ. Entomol., 33: 378-388.
2. Decourtye, A., J. Devillers, E., Genecque, K., Le Menach, H., Budzinski, S., Cluzeau and M.H. Pham-Delègue, 2005. Comparative sublethal toxicity of nine pesticides on olfactory learning performance of the honeybee *Apis mellifera*. Arch. Environ. Contam. Toxicol., 48: 242-250.
3. Decourtye, A. and M.H. Pham-Delègue, 2002. The Proboscis Extension Response: Assessing the Sublethal Effects of Insecticides on the Honey Bee. In: Honey Bees: Estimating the Environmental Impact of Chemicals. (Eds. J. Devillers and M.H. Pham-Delègue), pp: 67-84. Taylor & Francis, Ltd., London.
4. Abramson, C.I. and I.S. Aquino, 2002. Behavioral studies of learning in the Africanized honey bee (*Apis mellifera* L.). Brain Behav. and Evol., 59: 68-86.
5. Abramson, C.I. and B.J. Boyd, 2001. An automated apparatus for conditioning proboscis extension in honey bees (*Apis mellifera* L.). J. Entomol. Sci., 36: 78-92.
6. SPSS, 2002. SPSS11.5 for Windows, SPSS Incorporated, Chicago, IL.
7. Ellis, M.D., 1998. Bee Tidings, May, 10.
8. Embrapa Meio-Norte, 2003. Produção de mel. Sistema de Produção, 3, Versão Eletrônica, July.
9. Abramson, C.I., I.S. Aquino, F.S. Ramalho and J.M. Price, 1999. The effect of insecticides on learning in the Africanized honey bee (*Apis mellifera* L.). Arch. Environ. Contam. Toxicol., 37: 529-535.
10. Abramson, C.I., I.S. Aquino, M.C. Silva and J.M. Price, 1997. Learning in the Africanized honey bee: *Apis mellifera* L. Physiol. Behav., 62: 657-674.
11. Winston, K.L., 1992. Killer Bees: The Africanized honey bee in the Americas. Harvard University Press, Cambridge, MA.
12. Weil, T., 1975. Area handbook for Brazil. American University, Washington, D.C.
13. Gould, S.J., 1996. The Mismeasure of Man. revised and expanded. W. W. Norton, New York.