6-1996

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Abstract

Some ecotypes of the honey bee, Apis mellifera L., show excessive levels of colony defense that have occasionally resulted in human and animal deaths. In cases where death has occurred, the victim, animal or human, has often been confined or panicked into an area from which it cannot escape. Our study was done to evaluate the use of repellents to reduce the severity of the stinging during accidental disturbances of excessively defensive colonies. Three mosquito repellents (diethyl-meta-toluamide, 2-ethyl-1,3-hexandiol, and dimethyl phthlate) and 2 odiferous compounds known to be repellent to honey bees (benzaldehyde and menthol) were tested in European (Texas) and Africanized (Mexico) apiaries by victims in protective clothing. When sprayed as an aerosol at the defending worker bees, all the compounds significantly reduced the number of bees around the victim and the number of stings in a patch of suede exposed during the test. D EET was consistently the most effective repellent. A number of materials could be developed as repellents for emergency use by individuals that are at high risk of encountering wild honey bee colonies in the course of their daily activity.

Keywords

Apis melliferam, repellents, colony defense, diethyl-meta-tolumide, menthol, stinging

Disciplines

Entomology | Systems Biology | Toxicology | Weed Science

Comments

This article is from *Journal of Economic Entomology* 89 (1996): 608-613.

Use of Insect Repellents for Dispersing Defending Honey Bees (Hymenoptera: Apidae)

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J. Econ. Entomol. 89(3): 608–613 (1996)

ABSTRACT Some ecotypes of the honey bee, *Apis mellifera* L., show excessive levels of colony defense that have occasionally resulted in human and animal deaths. In cases where death has occurred, the victim, animal or human, has often been confined or panicked into an area from which it cannot escape. Our study was done to evaluate the use of repellents to reduce the severity of the stinging during accidental disturbances of excessively defensive colonies. Three mosquito repellents (diethyl-meta-toluamide, 2-ethyl-1,3-hexandiol, and dimethyl phthlate) and 2 odiferous compounds known to be repellent to honey bees (benzaldehyde and menthol) were tested in European (Texas) and Africanized (Mexico) apiaries by victims in protective clothing. When sprayed as an aerosol at the defending worker bees, all the compounds significantly reduced the number of bees around the victim and the number of stings in a patch of suede exposed during the test. DEET was consistently the most effective repellent. A number of materials could be developed as repellents for emergency use by individuals that are at high risk of encountering wild honey bee colonies in the course of their daily activity.

KEY WORDS *Apis mellifera*, repellents, colony defense, diethyl-meta-tolumide, menthol, stinging

THE HONEY BEE, *Apis mellifera* L., has a well-developed social response for the defense of its nest. During this response, worker bees may leave the nest, approach an intruder, and engage in defensive behavior (Collins et al. 1980). Sometimes a threat display of loud buzzing and flying at the face of an intruder may be sufficient to protect the colony. A more vigorous defense involves stinging, which is painful and marks the victim for further attack (Maschwitz 1964, Collins and Blum 1982). Additional stimuli from an intruder (for example, dark colors, strong odors, or movement) will increase the severity of the stinging response. If a victim receives sufficient stings, severe illness or death may result. Schumacher et al. (1990) suggested that venom from 1,160 stings is the average toxic dosage for humans of average weight and good health.

As the Africanized honey bee, (*Apis mellifera scutellata,* crossed with various European subspecies previously imported to the Western hemisphere) has expanded its range through much of South America, Central America, and Mexico, and into the United States, severe stinging incidents, some of which have resulted in death (Taylor and Williamson 1975, Hellmich and Rinderer 1991), continue to occur. Therefore, we began research to identify materials that would reduce the number of stings inflicted during colony defense. Collins and Hellmich (1988) described a preliminary study in Venezuela in 1988, when a number of compounds were evaluated for further testing. We included known insect repellents, several compounds that were used by beekeepers to drive bees from honey stores for harvesting (Tew 1992), and others that had been reported as repellent to bees (Woodrow et al. 1965). Testing included topical application to persons working with colonies and spray applications to bees defending disturbed colonies. Because topical applications gave no indications of successful repellency, we used only materials sprayed directly at the attacking workers in later tests. Several of the materials tested proved to be unsuitable because they were severely irritating to humans or they were not effective in keeping bees away. Here we report results of 2 experiments done to evaluate compounds for repellency to honey bee workers during colony defense.

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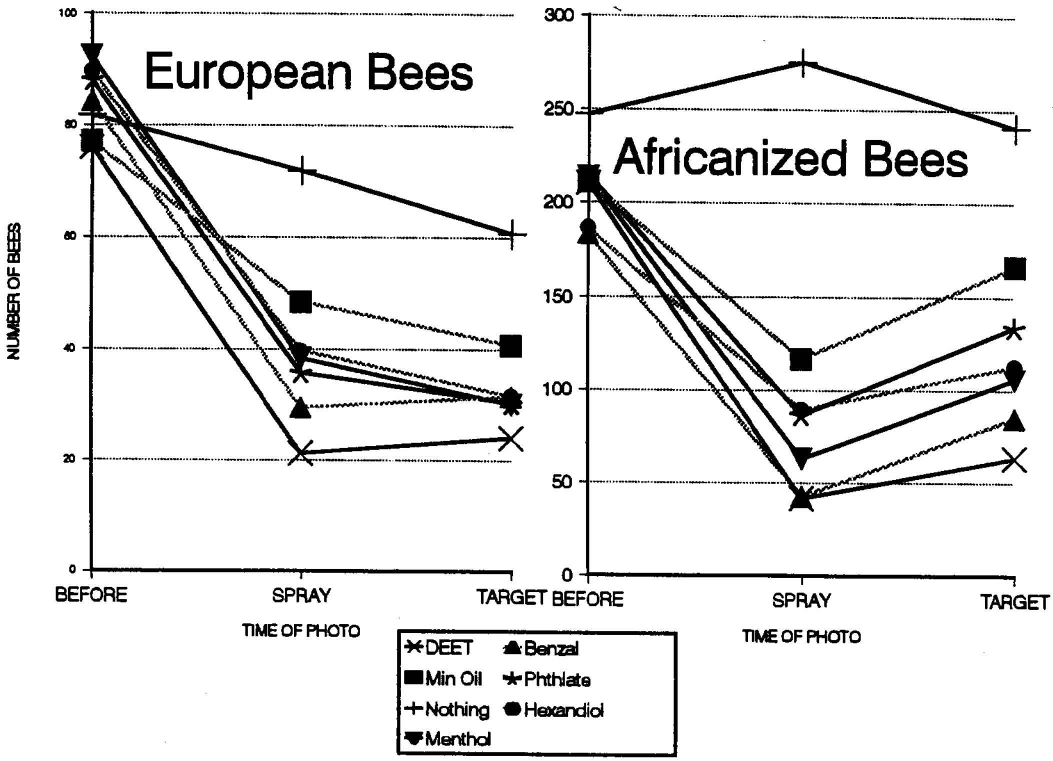

Fig. 1. Number of defending honey bees in the air during a test of repellent aerosol sprays. BEFORE, before treatment; SPRAY, during spraying of the repellent; and TARGET, after spraying when a suede target was waved. Treatments were significantly different from the control. DEET, diethyl-meta-toluamide; benzal, benzaldehyde; min oil, mineral oil; phthlate, dimethyl phthlate; hexanediol, 2-ethyl-1,3-hexanediol. Each point represents the mean of 36[48] observations (2 photographs per trial, 3 trials, 2 replicates per trial, on 3[4] d).

Materials and Methods

Five products were chosen, including 3 commercially available mosquito repellents—diethyl-meta-toluamide (DEET, McCabe et al. 1954), 2-ethyl-1,3-hexanediol (Granett and Haynes 1945, King 1954), and dimethyl phthlate (King 1954)—and 2 beekeeping products—benzaldehyde (oil of bitter almond, Townsend 1963) and menthol (Wilson and Collins 1989). We purchased all materials except 1 from Aldrich (Milwaukee, WI). Menthol crystals were obtained from Mann Lake Bee Supply (Hackensack, MN). All of the repellents were diluted to 15% by volume (by weight for menthol) in mineral oil. Controls of mineral oil alone and no spray were included in the experimental design. The liquids were dispersed with compressed-air aerosol cans (Sure Shot Atomizer Sprayer, Milwaukee Sprayer, Milwaukee, WI).

In experiment 1, we used only European honey bee colonies of several commercial stocks in Weslaco, TX; experiment 2 was done in Tapachula, Chiapas, Mexico. Africanized honey bees had been present in that area for 5 yr before the experiment. Samples of worker bees were collected from each colony in the Tapachula apiary and identified by morphometric analysis (Daly and Balling 1978, Rinderer et al. 1993). Eleven colonies were Afri-

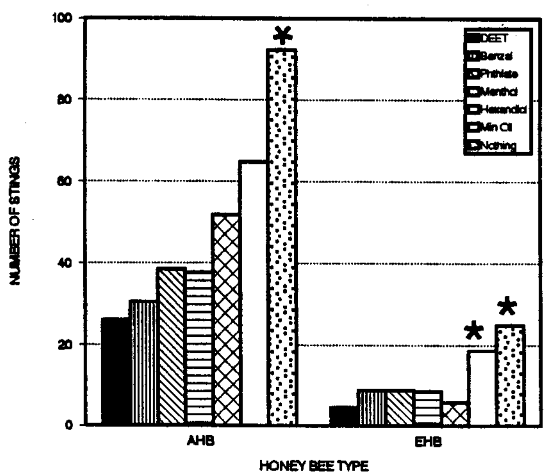

Fig. 2. Number of stings in suede targets exposed to defending bees still present after the repellent spray treatment. *, controls were significantly different from the treatments ($P < 0.05$; least squares means [SAS Institute 1988]).

Fig. 3. Photographic series taken during a trial sequence with Africanized honey bees. Number of bees in air around the victim is representative of a mean response. (a) before treatment, (b) while repellent was sprayed, (c) while a suede target is waved, column A, no spray; B, DEET.

canized, 2 were Africanized with evidence of introgression of European genes, 2 were European with evidence of introgression of African genes, and 1 was European. All colonies in the apiary where testing was done were disturbed by vigorous pounding on hives, opening covers, and brushing bees from the entrance.

For each replicate of the experiment, 3 people wearing white, hooded coveralls with facial screening (standard beekeeper protective equipment) walked (1 at a time) among the disturbed colonies and attracted numerous defending bees. They then stood in front of a white backdrop (183 by 198 cm). Two black-and-white photographs were taken of the experimenter's back and the flying bees. The experimenter then sprayed the test compound around his/her upper body for 10 s. An average of 46.3 ml of oil–repellent mixture was used during the 10 s. At 5 and 10 s after the beginning of spraying, photographs were taken. After the spray, a

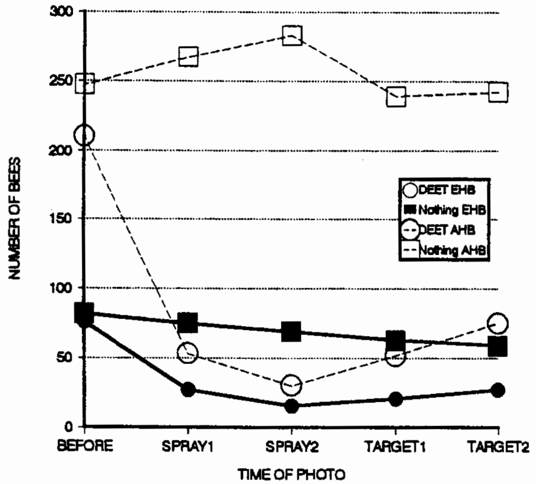

Fig. 4. Mean number of bees in the air from each of the timed photographs taken during the trial sequence. EHB, European honey bees; AHB, Africanized honey bees; DEET, diethyl-meta-toluamide.

dark suede patch (100 cm^2) was waved slowly in front of the experimenter for 15 s at head height. Photographs were taken at 10 and 15 s after the waving began. The number of flying bees in the area defined by the backdrop were counted from the negatives. The stings left in the suede patch were also counted.

For a complete replicate, each of the 3 experimenters did the test sequence with each of the 5 chemicals and 2 controls. All experimenter–chemical combinations were done in different random order for each replicate. Two replicates were completed per day. Experiment 1 (Texas) was done on 4 d (19, 20, 21 September and 10 October 1989). Experiment 2 (Mexico) was done on 3 d (9, 10, 11 September 1991). Day 4 of experiment 2 was deleted because of drastically reduced numbers of bees with stings, because of their vigorous stinging on days 1–3. Thus, each compound was tested 24 times in experiment 1 and 18 times in experiment 2. In experiment 1, the coveralls were washed each evening to remove any residues from the day's tests. No facilities were available for washing the coveralls during experiment 2.

Because of missing data, results were analyzed with a general linear models procedure for unbalanced analysis of variance (ANOVA) (SAS Institute 1988). The variables were day, replicate nested within day, chemical, chemical by day, coverall, and chemical by coverall. The final analysis for each response variable was repeated without the nonsignificant effects included in the model. Type III partial sums of squares were used. Pairwise comparisons of the least squares means were made by t-test.

Results and Discussion

Effect of Chemical. The levels of activity of defending workers before the test materials were sprayed were not significantly different by chemical (European honey bee $F = 0.27$, df = 6, $P > 0.952$; Africanized honey bees $F = 1.06$, df = 6, $P > 0.394$) (Fig. 1), that is, the testing began with similar numbers of bees in the air around the experimenter for all treatments. The numbers of bees in the air around the experimenter during spraying (European honey bee $F = 5.28$, df = 6, $P < 0.0001$; Africanized honey bees $F = 38.94$, df = 6, $P < 0.0001$) and presentation of the suede patch (European honey bee $F = 5.37$, df = 6, $P < 0.0001$; Africanized honey bees $F = 18.41$, df = 6, $P < 0.0001$), and the numbers of stings in the patch (European bees $F = 8.45$, df = 6, $P < 0.0001$; Africanized bees $F = 13.94$, df = 6, $P < 0.0001$) were all reduced by the use of an aerosol

Table 1. Intensity of defense by pursuing *A. mellifera* workers when aerial repellent sprays were used

Stage of test	Mean no. bees in the air[a]							
	Day 1		Day 2		Day 3		Day 4	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
			European bees					
Before treatment	24.8a	45.5a	106.0b	90.2b	119.8b	76.6a	130.3b	80.8a
During treatment	10.0c	15.4c	45.3de	32.6d	61.8ef	33.0d	79.3f	48.0d
	(40)	(34)	(43)	(36)	(52)	(43)	(77)	(59)
After treatment, moving target	7.9g	17.3g	37.4h	29.1h	63.2i	22.7h	68.3i	38.7h
	(32)	(38)	(35)	(32)	(53)	(30)	(52)	(48)
			Africanized bees					
Before treatment	170.3j	231.0k	244.5k	207.6k	202.5k	201.5k	—	—
During treatment	105.6m	141.7n	107.0m	86.9m	83.1m	83.3m	—	—
	(62)	(61)	(44)	(42)	(41)	(44)	—	—
After treatment, moving target	126.7n	186.5o	133.9n	122.1n	102.6n	105.9n	—	—
	(74)	(81)	(55)	(59)	(51)	(53)	—	—

Means in the same row followed by the same letters are not significantly different ($P > 0.01$; least squares means [SAS Institute 1988]). Rep, replicate.

[a] Number in parentheses (%) is the fraction of bees remaining during treatment or target presentation (after treatment), expressed as a percentage of the numbers before treatment.

Table 2. Rates of stinging by defending *A. mellifera* after repellent sprays were used

Bee ecotype	Mean no. stings in suede target							
	Day 1		Day 2		Day 3		Day 4	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
European	16.3a	9.2a	2.5b	6.3b	15.6a	9.0a	16.6a	13.6a
Africanized	87.8c	64.8d	56.2e	38.3f	25.5f	19.9f	—	—

Means in the same row followed by the same letters are not significantly different ($P > 0.01$; least squares means [SAS Institute 1988]).

spray (Figs. 1 and 2). The control in which nothing was sprayed at the defending workers resulted in significantly more bees and more stings than did any of the spray treatments. Spraying mineral oil alone had some effect in reducing the number of bees in the air and number of stings, however, this treatment was numerically less effective than the repellents. DEET was consistently the most effective material used. Representative photographs from a control (nothing) and a repellent (DEET) test are shown in Fig. 3 (experiment 2).

Effect of Bee Type. The Africanized colonies in Chiapas had a more intensive response to the disturbances by the experimenters than did the European colonies in Texas (Fig. 4; see also y-axis scales in Fig. 1, and Fig. 3). In both cases, the repellents (especially DEET) reduced the number of bees in the air to <50 after 10 s. Even with the reduced stinging when repellents were used, the Africanized honey bees stung the suede much more often than did European honey bees.

Other differences in the pattern of defensive response by the 2 bee types where apparent when the time frame of the experiments was examined. The Africanized apiary always had a large number of bees responding to the disturbance (Table 1); however, less stinging occurred as the test progressed, both within a day (2 replicates) ($F = 4.44$, df = 3, $P < 0.0055$) and across the 3 d of the experiment ($F = 43.36$, df = 2, $P < 0.0001$) (Table 2). A greater proportion of bees were driven off by the spray during later tests and fewer returned to sting the patches when they were presented. Day 4 of the test with these bees was canceled because of the lack of sufficient defending workers that were still stinging.

In contrast, bees from the European apiary responded to disturbance of their colonies with more bees flying on test days 2, 3, and 4 and the 2nd replicate of day 1. However, the numbers of defenders had decreased by the 2nd replicate on days 2, 3, and 4 (Table 1). The percentage of bees present during spray and target waving was also somewhat greater on days 3 and 4. The level of defensive behavior by Africanized honey bees generally decreased from day to day with continued disturbance; with European bees, defensive behavior increased, at least for the 1st event of a day.

In experiment 2 with Africanized honey bees, we noted a significant interaction ($F = 1.97$, df = 12, $P < 0.036$) of chemical-by-day for the number of bees in the air during spraying. With Africanized honey bees, DEET and benzaldehyde were always effective at repelling bees. The other compounds were less effective when bees were most defensive (that is, day 1, Table 3).

Effect of Person. In both of the experiments, 1 person consistently brought more defending bees from the apiary (experiment 1, $F = 9.52$, df = 2, $P < 0.0002$; experiment 2, $F = 9.94$, df = 2, $P < 0.0001$; Table 4.). This was not the same person for experiment 1 as for experiment 2. The differences may be attributable to odors or cleanliness of the coverall, or to differences in the behavior of the person; that is, some of the experimenters were

Table 3. Interaction of repellent and test day on the intensity of defense by Africanized honey bees during spraying of test material

Repellent	Mean no. bees in the air		
	Day 1	Day 2	Day 3
DEET	48.5ab	35.7a	41.0a
Benzaldehyde	57.5ab	35.6a	35.4a
Menthol	105.7bc	35.9a	47.0ab
Dimethyl phthlate	125.5c	77.2ac	56.6a
Ethyl hexandiol	145.8c	70.3ad	52.2a
Mineral oil	155.2c	117.6cd	77.3ad
Nothing	227.3e	306.4f	290.4f

Means within column or row followed by the same letter are not significantly different ($P > 0.05$; least squares means [SAS Institute 1988]). $F = 1.97$, df = 12, $P < 0.036$.

Table 4. Effect of individual protective gear and behavior on intensity of defense by Africanized honey bees during test of repellents (experiment 2 only)

	Bee suit-experimenter		
	R	C	W
	Mean no. bees in air		
Before treatment	256.0a ± 13.2	197.0b ± 13.2	175.7b ± 13.2
During treatment	130.6c ± 8.4	90.2d ± 8.4	85.5d ± 8.4
After treatment, target	150.3e ± 8.7	131.5e ± 8.7	107.1f ± 8.7
	Mean no. stings		
Stings in target	15.8g ± 1.7	10.1h ± 1.7	7.4h ± 1.7

Means in the same row followed by the same letter are not significantly different ($P > 0.05$; least squares means [SAS Institute 1988]).

more aggressive in stimulating colony defense for each test.

The results of our study indicate that several compounds will disperse honey bees defending their colony when sprayed at the flying workers. The most effective of the materials tested was DEET. All of the repellents were effective against both Africanized and European honey bee workers. Within 5 s after spraying began, the bees started leaving the area around the victim. As long as the material was being sprayed, the bees continued to move away and stay away. When spraying stopped, however, some of the bees returned (Fig. 4).

These compounds were not tested immediately adjacent to the colonies harboring the defending bees. The experimenters walked at least 5 m from the colonies to do the test. We do not claim that spraying the repellents on a colony will abort the entire defensive event, as some critics (e.g., Schmidt and Spangler 1991) have implied. However, our results indicate that some materials, with proper development and labeling, could be made available as personal safety tools for persons at risk of encountering defensive honey bee colonies in the course of their daily activity.

Acknowledgments

Sincere thanks for fine technical assistance go to Noe Buenrostro, Roy Medrano, Kristina Williams, and Raul Rivera (Honey Bee Research Unit, SARL, USDA-ARS, Weslaco, TX); Gustavo Lopez Bautista (Centro de Investigacciones Ecologicas del Sureste, Tapachula, Mexico); and Lorraine Davis Beaman and Dan Winfrey (Honey Bee Breeding, Genetics and Physiology Laboratory, USDA-ARS, Baton Rouge, LA). Initial testing was done in cooperation with Miel Primavera (Acarigua, Portuguesa, Venezuela). We thank the Screwworm Research Laboratory, USDA-ARS, Tuxtla Guitierrez, Mexico, for the vehicle used during the Tapachula study.

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Received for publication 10 May 1994; accepted 25 January 1996.