

# Comparative Toxicities of Four Topically Applied Insecticides to Africanized and European Honey Bees (Hymenoptera: Apidae)

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**ABSTRACT** Contact toxicities were established for acetone formulations of azinphosmethyl, carbaryl, methyl parathion, and permethrin applied to workers of Africanized and European honey bee (*Apis mellifera* L.) types. For each insecticide, 95% fiducial limits at the LC<sub>50</sub> levels for the two bee types did not overlap. Africanized bees showed greater tolerance to all the chemicals except carbaryl; differences in tolerance to each of the four chemicals were all about 2-fold. The order of toxicity of the compounds on the Africanized bees was permethrin > carbaryl > azinphosmethyl > methyl parathion; on the European honey bees, the order of toxicity was permethrin > azinphosmethyl > carbaryl > methyl parathion. Significant differences between the bee types were noted in the slopes of the probit regressions estimated for three of the compounds. The Africanized bees responded more homogeneously than the European bees to azinphosmethyl; European bees reacted more homogeneously than Africanized bees to carbaryl and permethrin.

HONEY BEES (*Apis mellifera* L.) provide the majority of crop pollination services in the United States. The value of bee-mediated pollination is estimated at nearly \$20 billion annually (Levin 1983). Africanized honey bees (*A. m. scutellata* Lepeletier, crossed with a variety of honey bee subspecies of European origin) are predicted to continue their northward encroachment and to become established in the southern and coastal areas of the United States (Taylor and Spivak 1984). Should this occur, they will become an intrinsic part of the complex agricultural production systems in those areas. Recent discoveries of Africanized bee nests in California underscore the possibility of such a scenario.

A major problem currently plaguing crop pollination is pollinator losses through contact with pesticides. Much research effort has been directed at resolving this situation, resulting in increasingly precise predictors of pesticidal hazards to honey bees and recommendations for avoiding deleterious chemical applications (e.g., Atkins et al. 1981). Central to understanding the bee-pesticide interaction is the establishment of specific dose/response relationships of bees to the various compounds used for pest management. Many of these relationships have been documented for typical North American honey bee stocks (Atkins et al. 1973, 1975). The possibility of widespread Africanization of bees in the United States adds a new dimension to the problem. Hence, toxicity determinations may be important not only relative to pollination disruption and associated bee losses to beekeepers, but also in potential Africanized bee

abatement strategies (Gary 1971, Stibick 1984). With these considerations, we compared responses of Africanized and European bees to a group of insecticides commonly encountered by bees foraging in agroecosystems.

## Materials and Methods

The effects of technical grade azinphosmethyl (Chem Service), carbaryl (Chem Service), methyl parathion (Chem Service), and permethrin (*cis-trans*-, 40:60) (FMC) were investigated. These materials were chosen because they represent three classes of widely used insecticides (organophosphates, carbamates, and pyrethroids). All compounds were dissolved in acetone at a rate of 0.5 g/liter; test concentrations were formulated as appropriate serial dilutions of the stock solutions. We initially screened four concentrations of each chemical, then added a fifth, mid-mortality-range concentration, based on initial responses.

Tests were performed in Sarare, Venezuela (09°44'N, 69°08'W), during March and April 1984, on honey bees from Africanized colonies started from local feral swarms and European colonies derived from several commercial "Italian" stocks imported from the United States. Sealed brood combs were brought into the laboratory and placed in an incubator (maintained at 35°C, 50% RH), where worker bees were allowed to eclose. Unanesthetized adults, <24 h old, were treated individually on the nota with a 2- $\mu$ l aliquot of insecticide-acetone solution applied with an electric microapplicator. After treatment, bees were held individually

Table 1. Responses of Africanized (Afr) and European (Eur) honey bees to topically applied insecticides<sup>a</sup>

Compound	Bee type	n <sup>b</sup>	LC <sub>50</sub> (95% FL), µg per bee	LC <sub>95</sub> (95% FL), µg per bee	Slope (SEM) <sup>c</sup>
Azinphosmethyl	Afr	844	0.105 (0.095-0.118)	0.243 (0.207-0.299)	3.51 (0.25)a
	Eur	947	0.055 (0.045-0.070)	0.248 (0.243-0.740)	1.95 (0.17)b
Carbaryl	Afr	1,000	0.053 (0.035-0.068)	0.458 (0.381-0.950)	1.37 (0.08)b
	Eur	700	0.122 (0.105-0.141)	0.549 (0.457-0.684)	1.96 (0.12)a
Methyl parathion	Afr	1,000	0.324 (0.284-0.370)	0.919 (0.780-1.112)	2.83 (0.16)a
	Eur	799	0.165 (0.146-0.191)	0.402 (0.322-0.550)	3.32 (0.32)a
Permethrin ( <i>cis</i> -: <i>trans</i> -, 40:60)	Afr	891	0.028 (0.023-0.032)	0.195 (0.154-0.260)	1.51 (0.10)b
	Eur	949	0.015 (0.013-0.017)	0.061 (0.049-0.079)	2.06 (0.13)a

<sup>a</sup> Based on 24-h mortality; bees held individually at 35°C, 50% RH after treatment.

<sup>b</sup> Total number of insects treated to estimate concentration/mortality regressions.

<sup>c</sup> Slopes for the two bee types within chemicals followed by the same letter are not significantly different based on the criterion of nonoverlap of twice the SEM.

under 22.2-ml paper cups inverted over a waxed-paper-covered board. A 5-mm<sup>3</sup> cellulose sponge soaked in 50% sucrose solution (vol/vol) was placed with each bee for feeding. The boards, holding 50 cup-cages each, were stacked in a separate incubator (35°C, 50% RH). Mortality was assessed after 24 h; lack of movement in response to prodding was used as the criterion for mortality. In each replication, new paper cups, waxed paper, and feeder sponges were used.

Each concentration of all the compounds was tested on 50 bees from each of a randomly selected group of five Africanized and five European stock colonies. A set of acetone-treated bees was used as a control for each colony and chemical to adjust for baseline mortality (Abbott 1925). We experienced some problems with contamination in the holding incubator and chose to discard test groups associated with control mortalities higher than 18%. Concentration/mortality regressions for each bee type and insecticide combination (based on four or five concentrations with 50-250 bees per point) were estimated by probit analysis (Finney 1971). Equality of slopes within each chemical was judged using the criterion of nonoverlap of twice the SEM.

### Results

For each of the chemicals, 95% FL of the LC<sub>50</sub>'s for the honey bee types did not overlap (Table 1). The 95% FL of the LC<sub>95</sub>'s for the bee types did not overlap for methyl parathion or permethrin. European bees were relatively more susceptible than Africanized bees to three of the four insecticides examined; only to carbaryl were they more tolerant. The difference in tolerance at the LC<sub>50</sub> was 2.3-fold for carbaryl, while for the other compounds it was 1.9- to 2.0-fold. At the LC<sub>95</sub>, the tolerances were 2.3- and 3.2-fold for methyl parathion and permethrin, respectively. The order of toxicity of the compounds (LC<sub>50</sub>) on the Africanized bees was permethrin > carbaryl > azinphosmethyl > methyl parathion; on the European bees, the order of toxicity was permethrin > azinphosmethyl > carbaryl > methyl parathion.

The Africanized population reacted in a more

homogeneous fashion to azinphosmethyl, as revealed by a significantly steeper slope for the probit regression for Africanized bees compared with that for European bees (Table 1). European bees were significantly more homogeneous in their responses to carbaryl and permethrin. Slopes were similar for the two bee types treated with methyl parathion.

### Discussion

Africanized and European honey bees exhibited different susceptibility responses to the four insecticides bioassayed: the Africanized population was more tolerant to three of the test chemicals. Africanized worker honey bees are ca. 10% smaller than their European counterparts (Heinrich 1979, Otis 1982, Rinderer et al. 1982); they therefore might be expected to be more susceptible to the insecticide. Yet, using our procedure, they generally were not. One important implication of the tolerance differences manifested is that Africanized bees may be selectively favored compared with European bees when both types are exposed to insecticides, as in agricultural production systems in the United States. In areas of indiscriminate insecticide use, this may promote faster and more widespread dominance of the Africanized type. If Africanized bees become prevalent and are used as crop pollinators, bee poisoning problems may be less severe than are presently experienced with European bees. Still, the fact that the susceptibility differences found between the two bee types were relatively small indicates that the observed differences may be less important than careful use of pesticides when trying to protect pollinators.

The observed selectivity may be predicated on a host of physiological, biochemical, and behavioral differences between the bee types. Penetration rates of chemicals may have been affected by quantitative differences in the ecotypes' cuticular composition (unpublished data). Also, sclerotization rates of the newly eclosed bees we treated may have influenced penetration. Sclerotization is a process directly affecting chemical penetration

of the integument and one affecting activity levels in these young adult insects. During the course of the work we noticed that the Africanized bees were more advanced ontogenetically than their European counterparts. The Africanized stock exhibited a very high activity level, which frequently included flying and stinging, although they were <24 h old; European bees were comparatively lethargic. Winston and Katz (1982) also found accelerated ontogeny in Africanized bees. Several workers (Jay 1963, Kerr et al. 1972, Fletcher 1978, Winston 1979, Harbo et al. 1981) have observed differences in development times between the bee ecotypes. These behavioral and developmental patterns are manifestations of physiological processes, indicating some degree of variance in physiology of the bee types.

Biochemical differences between Africanized and European honey bees have been detected (Mestriner and Contel 1972, Sylvester 1976, Contel et al. 1977, Martins et al. 1977, Nunamaker and Wilson 1981, Carlson and Bolten 1984). These differences, together with physiological differences, indicate the possibility of metabolic dissimilarities affecting pharmacodynamics. No data exist to implicate other components of insecticide selectivity (storage, excretion, and penetration to, attack of, and consequences of attack of the active site [O'Brien 1967]) as bases for the observed tolerance differences.

The extent of pesticide use in the tropics is difficult to assess. Use seems to be relatively light compared with that in the United States (Crane and Walker 1983), although more geographically concentrated. It is unlikely that much resistance has been developed by the very large population of Africanized bees in South America. In North America, Tucker (1980) concluded that vigor tolerance was exhibited by worker bees after a nine-generation selection for tolerance to carbaryl. Anderson and Atkins (1968) found no change in tolerance to carbamate or organophosphorus chemicals during 8 years of tests.

Generally, the  $LC_{50}$ 's obtained for the North American bees in this study were lower than those reported elsewhere (azinphosmethyl, 0.063–0.425  $\mu\text{g}$  per bee; carbaryl, 0.08–1.54  $\mu\text{g}$  per bee; methyl parathion, 0.061–0.428  $\mu\text{g}$  per bee; and permethrin, 0.11–0.16  $\mu\text{g}$  per bee [Georghiou and Atkins 1964, Graves and Mackensen 1965, Stevenson 1968, Atkins et al. 1973, 1975, Tucker 1980]). Batista et al. (1974) determined the toxicities of insecticides to Africanized honey bees in Brazil. For carbaryl, the  $LC_{50}$  was 0.124  $\mu\text{g}$  per bee (slope = 3.336); for methyl parathion, the  $LC_{50}$  was 0.061  $\mu\text{g}$  per bee (slope = 7.509). Much of the variability between these results and our findings may reflect differences in materials and methods employed. For example, all the studies except that of Tucker (1980) used bees of mixed ages, effectively increasing mean age. Age has been reported to regulate some toxicological responses in honey bees: bees <24 h

old are more sensitive to carbaryl, while sensitivity to methyl parathion is increased in older bees (Mayland and Burkhardt 1970).

Posttreatment holding temperature has a demonstrable effect on sensitivity of insects to some insecticides. Activity of organophosphorus compounds increases with higher temperatures; pyrethroids typically show a negative temperature coefficient, though interspecific differences have been demonstrated (Sparks et al. 1983). Carbaryl shows a negative temperature coefficient when tested against honey bees (Georghiou and Atkins 1964). Our tests were done at temperatures approximating those found within honey bee nests, whereas most other researchers have tested insecticides at lower temperatures.

Atkins et al. (1973, 1975) used a dusting technique (Atkins et al. 1954) in establishing many insecticide toxicities to honey bees but the other referenced results are topical-contact toxicities; in the majority of method comparisons,  $LC_{50}$ 's obtained by topical application are relatively lower. Group size can affect toxicity responses in honey bees (Graves and Mackensen 1965); this factor also varied in the tests reported.

From a regulatory aspect, these results suggest that an insecticide which selectively removes Africanized bees from mixed groups of the two bee types could be found. Carbaryl is the only insecticide we examined to which European bees appear to be somewhat more tolerant. However, although carbaryl was 2.3-fold as toxic to Africanized bees as to Europeans at the  $LC_{50}$ , the responses of bee types converged at the  $LC_{95}$ . Classically,  $LC$  values representing high mortality levels are used when making choices of insecticides to control a pestiferous target (e.g., Africanized bees) in the presence of a desirable species (i.e., European bees).

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