

# Response of Africanized and European Honey Bees to Lactose in Sucrose Syrup

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## Introduction

Application of biotechnology to control Africanized honey bees may be possible by genetically engineering domestic honey bee stocks to be resistant to some agent which is toxic or debilitating to Africanized bees. The agent could then be made available to foragers in an area, and susceptible wild colonies, including Africanized honey bees, would be controlled. Africanized honey bees are usually considered to be undesirable due to their increased propensity to defend their nests with greater numbers of bees stinging over a wider radius from the colony, reduced honey production, increased swarming and absconding, reduced effectiveness in pollination management systems and increased costs to beekeepers (Collins *et al.* 1982, Rinderer *et al.* 1985, Danka *et al.* 1987).

The toxic agent chosen to control Africanized bees should have minimal effects on the environment and non-target species. One such chemical is lactose, a natural sugar which is toxic to honey bees (Barker 1977, Sylvester 1979, Peng 1981). One control procedure would be to place feeders of sugar syrup containing lactose in locations where wild bees need to be eliminated. Bees visiting the feeder would imbibe the syrup and carry it back to their colonies. Only unprotected bees would be killed. The procedure's success depends on reliable acceptance of the syrup by the bees and acceptable protection from the toxicant of the desirable bees. Using present technology, desirable bees could be protected by moving them out of the area or covering them so they could not visit the feeder.

Another way to protect desirable bees would be to genetically engineer them to be resistant to the toxicant. The DNA sequences that code for bacterial enzymes which metabolize lactose and the accompanying end product, galactose, are available for transfer into honey bee genomes using recombinant DNA technologies. However, gene transfer procedures have not yet been developed for bees.

A stock of bees which had been genetically engineered to include an identifiable marker gene would also have

other uses. The presence of the marker could be used as a criterion for certified stock. The presence of the marker could also be followed in other studies, such as mating control and population dynamics.

This study was conducted to evaluate the suitability of lactose as an agent to control Africanized bees.

## Materials and Methods

**Honey bee stocks:** This research was conducted at an ARS field laboratory near Acarigua, Venezuela in 1987. European bees were obtained from colonies headed by European queens (commercially available stocks) brought from the United States. Africanized bees were obtained from colonies produced by swarms caught in an area known to have a high concentration of Africanized bees, with confirmation based on field evaluation of behavior. No morphometric analyses were conducted. The queens were marked and colonies were examined periodically to ensure that the original queen was present. Five colonies of each type were chosen for testing.

**Establishment of cages:** Combs containing emerging adult worker bees were removed from each of these colonies and placed in emergence cages in an incubator (35°C, 50% RH). Newly emerged worker bees, ages 0-24 h, were placed in groups of 30 in hoarding cages (16 cages per colony) similar to those of Kulinčević and Rothenbühler (1973). Each cage was fitted with a 20 ml water vial and a 20 ml syrup vial, but no pollen substitute was provided. The cages were then placed in a large, walk-in incubator (35°C, 50% RH).

**Lactose treatments:** Syrup treatments were randomly assigned within each replicate group of cages. Sucrose syrup (50% wt/vol) was used as the control solution and as the base for the three lactose solutions. Lactose (Sigma Chemical Co. L-3625) was added to aliquots of 50% sucrose syrup at 5, 10, and 15% (wt/vol) rates. Each source colony was represented in each of the 4 treatment groups by 4 replicate cages, for a total of 40 cages/sugar treatment (160 cages total).

## Abstract

Africanized and European honey bees (*Apis mellifera*) were placed in hoarding cages and their hoarding and mortality rates were measured for four treatments: 50% sucrose syrup, 50% sucrose + 5% lactose, 50% sucrose + 10% lactose, and 50% sucrose + 15% lactose. Africanized bees responded similarly to European bees at all treatment levels. Lactose caused significantly increased mortality and significantly decreased hoarding at each increase of dosage. The mortality levels might be suitable for a toxicant-based control system but the decreased levels of hoarding strongly suggest that bees in the field would not collect enough lactose-containing syrup for the system to be effective. Thus, a toxicant system using lactose does not appear to be feasible.

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**Experimental conditions:** The weight of syrup removed from each vial was recorded on alternate days. Based on our previous research (unpublished observations), bees have the highest hoarding rates and the highest correlation of hoarding rate with field colony weight gain 3 to 6 days

after being caged. The hoarding rates were therefore calculated and analyzed for these days. Bees that died in each cage were removed when the syrup was weighed and counted. Because of the much longer survival of bees in the control cages, these cages were continued only until mortality reached 50% or more. Days to median mortality ( $LT_{50}$ ) were calculated as the number of complete test days (in 2-day units) until at least half of the bees in the cage had died.

**Analysis:** An analysis of variance with the following model was used to analyze syrup hoarding and  $LT_{50}$  data.

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \Gamma_{k(i)} + \beta\Gamma_{jk(i)} + \epsilon_{ijkl}$$

where

$\mu$  = overall mean

$\alpha_i$  = the effect due to the  $i^{\text{th}}$  bee type

$\beta_j$  = the effect due to the  $j^{\text{th}}$  treatment

$\alpha\beta_{ij}$  = interaction of bee type and treatment

$\beta\Gamma_{jk(i)}$  = interaction of treatment and colony for  $i^{\text{th}}$  bee type

$\Gamma_{k(i)}$  = random effect due to the  $k^{\text{th}}$  colony of the  $i^{\text{th}}$  bee type;  $N(0, \sigma^2)$

$\epsilon_{(ijkl)}$  = error term for the  $l^{\text{th}}$  cage for  $i^{\text{th}}$  bee type,  $j^{\text{th}}$  treatment and  $k^{\text{th}}$  colony,  $N(0, \sigma^2)$

The Waller-Duncan K-ratio test was used for mean separations. A one-way analysis of variance was conducted on days to median mortality for the two bee types for each treatment and for the amount of syrup hoarded by the two bee types for each treatment (SAS 1987).

## Results

The difference between the races for days to median mortality ( $LT_{50}$ ) over all treatments is not significant (Table 1). The mean  $LT_{50}$  for races within treatments was significantly lower for Africanized bees for the 5% and 10% lactose levels. The mean  $LT_{50}$  was significantly different among all treatments. The mortality curves for each treatment and race are shown in Figures 1 and 2.

The hoarding trends generally parallel mortality trends. The difference between the races for hoarding rate over all treatments is not significant ( $P > 0.1$ ). However, European bees hoarded significantly more than Africanized bees in the control and sucrose + 10% lactose treatments (Table 2). The treatment means for hoarding by both races combined were significantly different among all treatments ( $P > 0.0001$ , Table 2).

## Discussion

Africanized and European honey bees responded similarly to the toxic effects of lactose, and thus would be equally susceptible to its use as a control agent.

Hoarding rates for both bee types decreased with lactose concentration, which suggests that bees detect lactose in syrup and find it unattractive. This effect would be even more pronounced in the field where competing food sources often would be available. Thus, consumption in the field would likely be reduced and not enough lactose would be consumed for the treatment to be effective for the

control of feral bees.

Any application of genetic engineering is expensive. In bees, the technology for transferring genes is not yet available. Therefore, any preparatory work directed toward genetic engineering should be applied to procedures and characters which are particularly valuable and likely to be effective. The use of lactose as a selective toxicant to control Africanized bees does not appear to be effective.

The significantly higher hoarding rate of European bees compared to Africanized bees in the control cages using sucrose confirms the report by Rinderer *et al.* (1982) that European bees have higher hoarding rates in cages. The significantly shorter life span of Africanized bees in the 5% and 10% treatment cages may be due to a higher metabolic rate in Africanized bees and therefore a higher susceptibility of individual bees to toxins.

Peng (1981) reported that significantly higher mortality occurred in bees fed 10% and 15% lactose compared to controls but not in bees fed 5% lactose. However, her bees were in small hives kept in screen flight cages and they were fed a pollen supplement. They were also adult bees of unmeasured age shaken from colonies, rather than newly emerged adult bees. Therefore, those bees may have been under less stress and may have been better able to resist the effects of lactose. However, a more likely explanation is

that bees which can take cleansing flights can excrete undigested lactose and unabsorbed galactose (Peng 1981) and thereby minimize their effects. The 5% lactose treatment may be very near a threshold, so that inability to excrete feces containing lactose may lead to significantly more mortality than in the control treatment.

### Conclusions

Africanized and European honey bees are both adversely affected by lactose. In the field, honey bees probably would not consume enough lactose for it to be an effective toxicant for control of Africanized bees.

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Table 1

*Effects of lactose in sucrose syrup on mortality of European (E) and Africanized (A) honey bees caged with empty comb. Results are presented as mean ± SE. Standard errors for treatment by race means apply only to race comparisons. Acarigua, Venezuela, 1987.*

Treatment	European	Days to median mortality (LT <sub>50</sub> )		
		Africanized	P>F	(E+A)*
Sucrose 50% wt/vol	18.5±0.7	19.7±0.7	0.2	19.1±0.4
Sucrose + 5% lactose wt/vol	13.4±0.4	11.2±0.4	0.0002	12.3±0.4
Sucrose + 10% lactose wt/vol	12.0±0.3	9.8±0.3	0.0001	10.9±0.4
Sucrose + 15% lactose wt/vol	9.1±0.4	8.8±0.4	0.6	9.0±0.4
All treatments	13.3±0.3	12.4±0.3	0.1	

\*Means in this column are all significantly different (P<0.05) by the Waller-Duncan K-ratio T test.

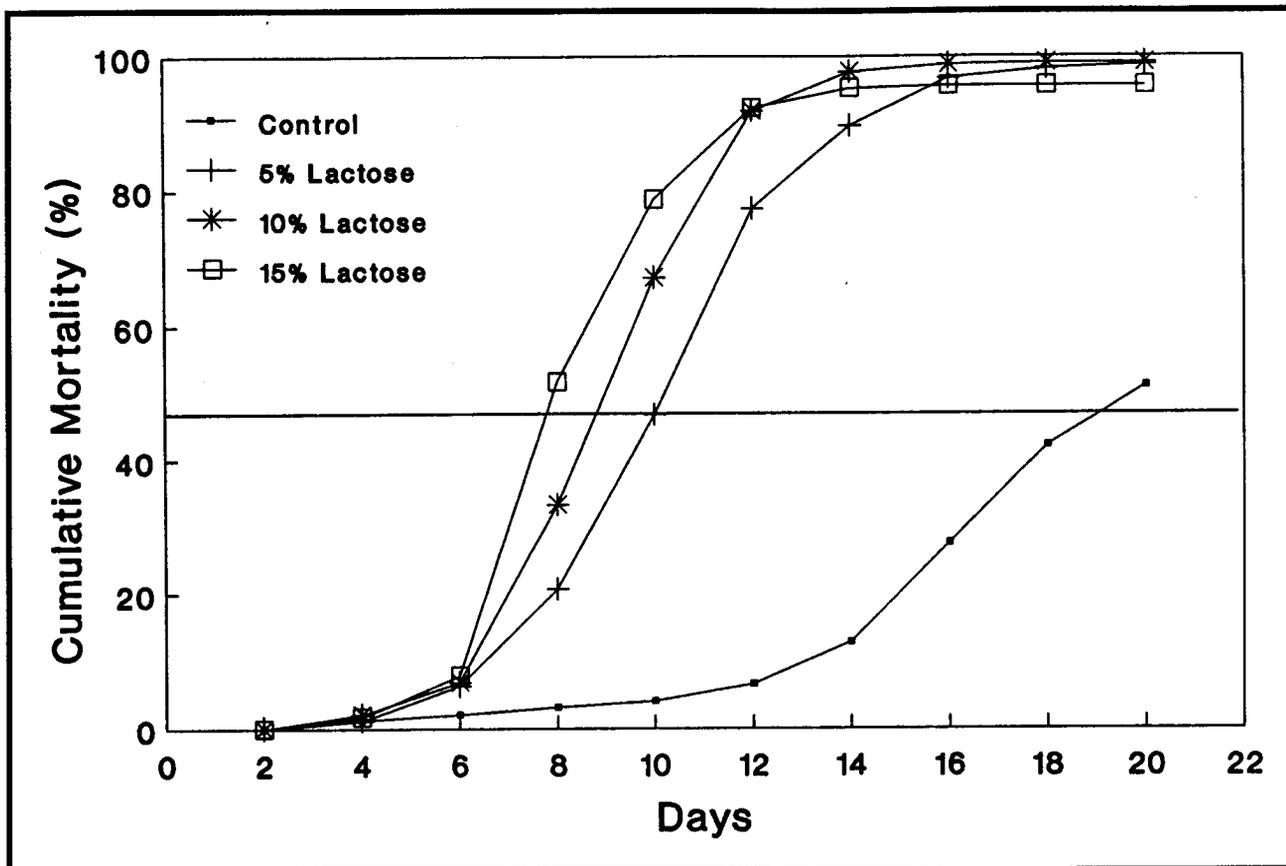


Figure 1. Effects of lactose in sucrose syrup on mortality of Africanized honey bees.

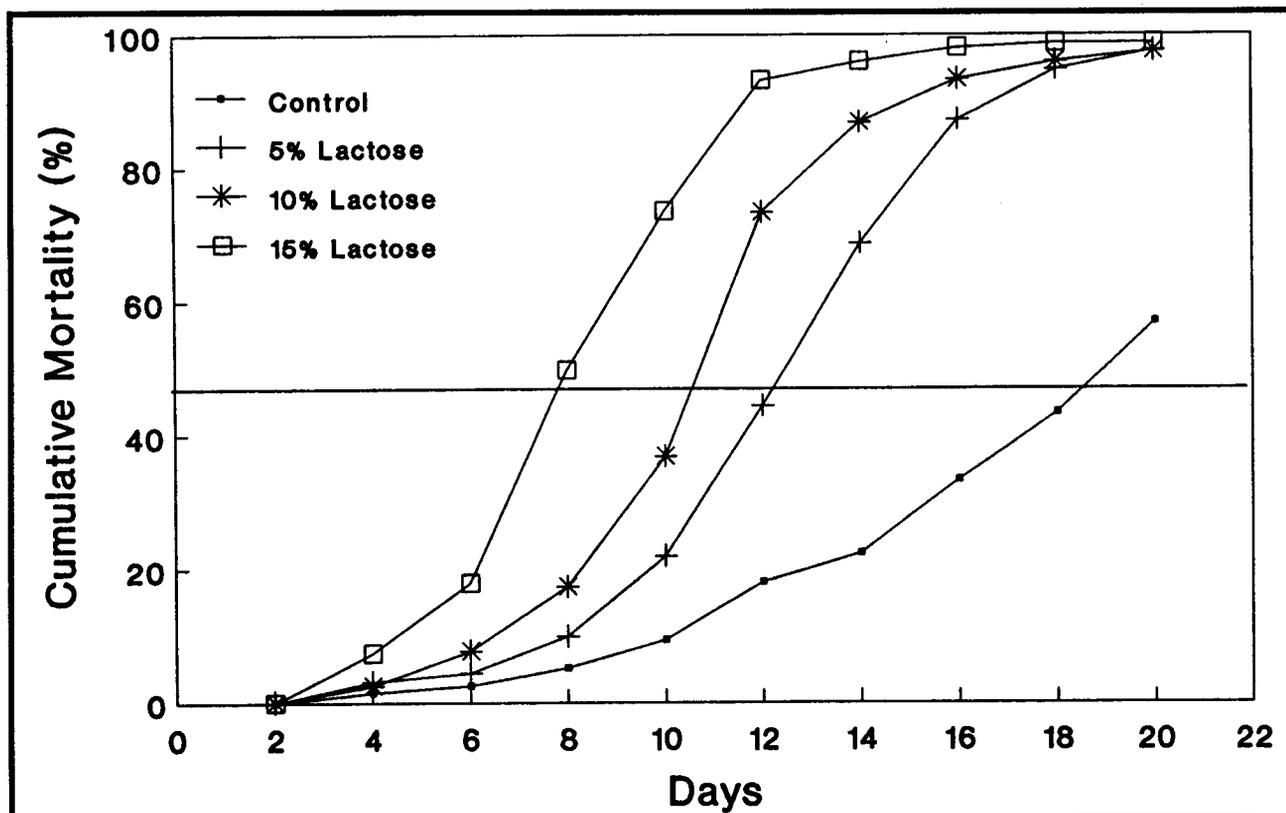


Figure 2. Effects of lactose in sucrose syrup on mortality of European honey bees.

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**Table 2**

*Effects of lactose in sucrose syrup on hoarding rates of European (E) and Africanized (A) honey bees caged with empty comb. Results are presented as mean ± SE. Standard errors for treatment by race means apply only to race comparisons. Acarigua, Venezuela, 1987.*

Treatment	Grams syrup taken/cage on days 3+4+5+6			
	European	Africanized	P>F	(E+A)*
Sucrose 50% wt/vol	7.1±0.7	5.0±0.7	0.05	6.1±0.4
Sucrose + 5% lactose wt/vol	5.7±0.8	3.9±0.8	0.1	4.8±0.4
Sucrose + 10% lactose wt/vol	4.0±0.4	2.4±0.4	0.01	3.2±0.4
Sucrose + 15% lactose wt/vol	1.6±0.1	1.7±0.1	0.5	1.6±0.4
All treatments	4.6±0.6	3.2±0.6	0.1	

\*Means in this column are all significantly different (P<0.05) by the Waller-Duncan K-ratio T test.