

## Responses of Three Genetically Different Stocks of the Honeybee to a Virus from Bees with Hairless-Black Syndrome

THOMAS E. RINDERER, WALTER C. ROTHENBUHLER,  
AND JOVAN M. KULINČEVIĆ

*Department of Entomology,  
The Ohio State University,  
Columbus, Ohio 43210*

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Test samples, each of 50 honeybees (*Apis mellifera*), from three different stocks (hairless-black resistant, hairless-black susceptible, and commercial) were fed over a 3-day period a total dose per bee of  $4 \times 10^9$ ,  $4 \times 10^7$ ,  $4 \times 10^6$ ,  $4 \times 10^4$ , or 0 virus particles in sucrose syrup. Mortality differences due to dose and stock variables were observed. By the last day of the experiment, mortality in control groups of all three stocks averaged less than 4%, whereas samples of susceptible, commercial, and resistant stocks receiving  $4 \times 10^9$  virus particles per bee averaged 94, 60, and 40%, respectively. The responses to other doses reflected the same trends.

### INTRODUCTION

The occurrence, severity, and characteristics of hairless-black syndrome in the honeybee, *Apis mellifera*, have been discussed elsewhere (Kulinčević et al., 1969; Rothenbuhler et al., 1969). Horvath and Rothenbuhler (1972) have described the histopathology of the malady.

A two-way selection experiment over four generations has yielded two lines of the honeybee, one resistant and one susceptible, to an inoculum made by simply macerating in water bees displaying hairless-black syndrome and by clarifying via centrifugation at 3000g for 1 hr (Kulinčević and Rothenbuhler, 1975). Although there were indications that the pathogen was viral in nature, no virus was isolated or identified in the inoculum. At the time that tests of the fourth generation were finished, Rinderer (1974) isolated a nonoccluded virus from the heads of sick bees exhibiting the syndrome, and demonstrated that this virus, in the absence of ribonuclease, produced high mortality in test populations of a commercial stock of honeybee. Whether or not the selected lines were resistant and susceptible to the isolated virus became an obvious question. This investigation was designed to

answer that question and to explore dose-mortality relationships.

### MATERIALS AND METHODS

Tests of mortality were conducted on samples of 50 bees each, contained in cages identical to those described by Kulinčević, Rothenbuhler, and Stairs (1973), and kept in an incubator at approximately 35°C and 50% relative humidity.

The bees involved in these tests came from nine colonies each of the resistant and susceptible stocks, and five colonies of commercial stock. In order to test stocks of bees rather than individual colonies, each 50-bee sample tested was composed of five groups of ten bees emerged from five combs, each from a different colony. In resistant and susceptible stocks, a specific group of five colonies provided bees for each test of an entire dilution series of virus. Replications of this test involved other groupings of colonies. Each test of commercial stock involved the same five colonies.

Tests with resistant and commercial stocks were replicated five times; tests with the susceptible line were replicated four times. This group of tests was divided into two experiments. The first experiment,

started on September 16, 1973, was composed of three tests of a virus dilution series with both resistant and commercial stocks and two tests with the susceptible stock. The second experiment, started on September 20, consisted of two tests with all three stocks. Inasmuch as a fresh virus suspension was made for each day's virus feeding, and each experiment required 3 days of feeding, six virus suspensions were made. For the first experiment, approximately 744 sick bees were utilized on each of the 3 days; for the second, approximately 558 bees were utilized daily.

Each virus suspension was prepared by grinding, in sterile distilled water, with a mortar and pestle, the heads of bees displaying hairless-black syndrome collected from several free-flying colonies. The resulting slurry was mixed with additional sterile distilled water and subjected to clari-

fying centrifugation at 480, 9750, and 9750g for 15, 60, and 60 min, respectively. Centrifugation at 73,950g for 120 min produced virus pellets which were resuspended in 2.0 ml sterile distilled water. A 0.1 ml sample of the suspension was mixed with 0.1 ml of a suspension containing a known concentration of latex balls and 0.1 ml of 1% phosphotungstic acid. These mixtures were sprayed on coated electron microscope grids and the resulting droplets were observed with a Zeiss EM9-S electron microscope. Relative counts of virus particles and latex balls were used to calculate the concentration of virus particles following the method of Williams and Backus (1949). Subsequent dilutions of the virus suspensions were based on these calculations.

Over the first 3 days of each experiment, test samples were fed a total dose of  $4 \times 10^9$ ,  $4 \times 10^7$ ,  $4 \times 10^6$ ,  $4 \times 10^4$ , or 0 virus particles

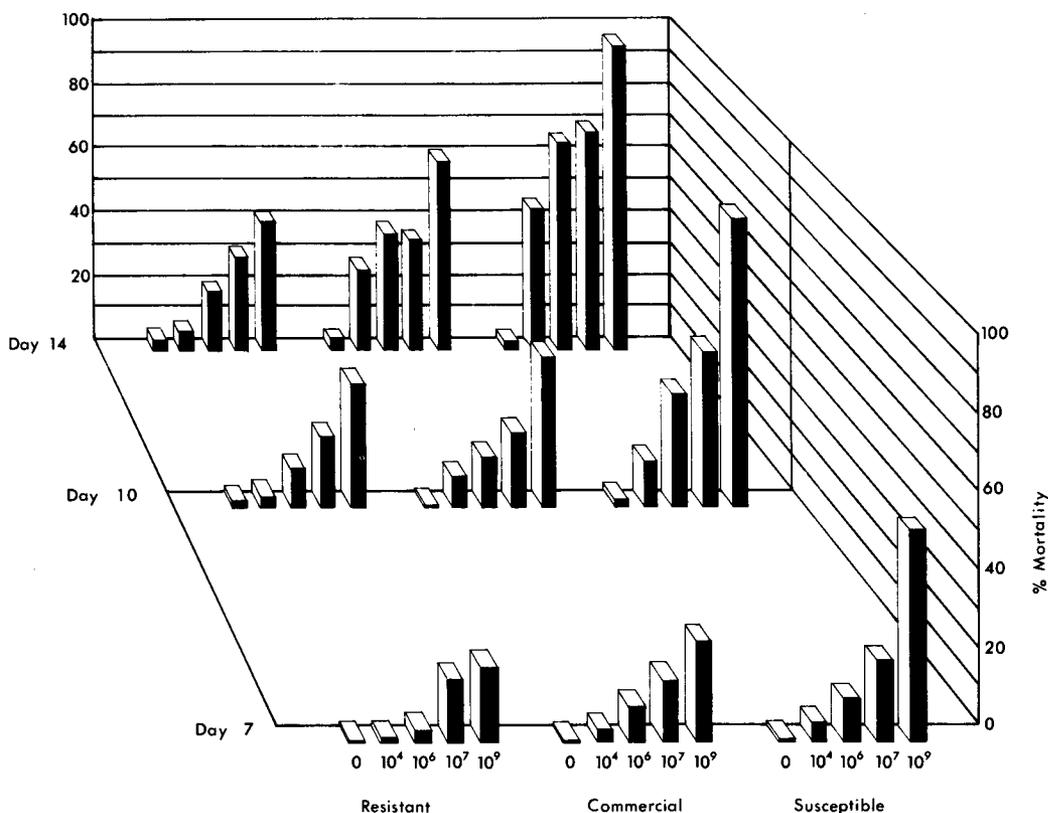


FIG. 1. Average mortality for groups of 50 bees representing resistant, commercial, and susceptible genetic stocks at 7, 10, and 14 days after initial exposure to various doses of a nonoccluded virus.

per bee. To induce the bees to ingest the water-suspended pathogen, it was mixed with an equal volume of a saturated solution of ribonuclease-free sucrose. Each day, 2 ml of this inoculum were given in a small, open container placed on the floor of each test cage. On each of 14 days, starting with the first day inoculum was administered, each test cage was observed, dead bees were removed, and mortality was recorded. Total cumulative mortality data at days 7, 10, and 14, were analyzed by a least-squares analysis of variance and least significant difference tests.

### RESULTS, ANALYSES, AND DISCUSSION

Results of the experiment are presented in Figure 1 and Table 1. Mortality in control groups was less than 4% in all cases. From Figure 1 it appears that higher doses cause higher mortality in all three stocks; that the cumulative mortality increases from day 7 to day 14; and that the susceptible, commercial, and resistant stocks display, respec-

tively, greatest, intermediate, and least mortality.

The analysis of variance presented in Table 2 reveals no differences in replications but highly significant differences among doses, among stocks, and in dose by stock interactions.

The origins of the differences among stocks can be identified by referring again to Table 1 and employing least-significant difference tests. When the resistant and commercial stocks are considered by day and dose, least significant difference analyses reveal the commercial stock to be significantly different ( $P < 0.05$ ) from the resistant line at doses  $4 \times 10^9$ ,  $4 \times 10^6$ , and  $4 \times 10^4$  for day 14. The susceptible line consistently has significantly higher mortality than either the commercial or the resistant stock. At day 7, the susceptible line is significantly different ( $P < 0.01$ ) from the other two stocks at the  $4 \times 10^9$  dose. By day 10, it is significantly different ( $P < 0.01$ ) from both other stocks at the doses  $4 \times 10^9$ ,  $4 \times 10^7$ , and  $4 \times 10^6$ , as well as from the resistant line at the dose  $4 \times 10^4$  ( $P < 0.05$ ).

TABLE 1  
Line by Dose Least Squares Means of Mortality

Line	Virus dose per bee	Number of replicates per dose <sup>a</sup>	Least squares means of mortality with standard errors		
			Day 7	Day 10	Day 14
Resistant	$4 \times 10^9$	5	9.60 ± 1.48	17.80 ± 1.36	19.99 ± 2.27 <sup>b</sup> s**; <sup>c</sup> *
	$4 \times 10^7$	5	7.60 ± 1.48	10.20 ± 1.36	14.59 ± 2.27 s**
	$4 \times 10^6$	5	1.10 ± 1.48	5.80 ± 1.36	9.19 ± 2.27 s**; <sup>c</sup> *
	$4 \times 10^4$	5	0.80 ± 1.48	1.20 ± 1.36	2.79 ± 2.27 s**; <sup>c</sup> *
	0 <sup>c</sup>	10	0.50 ± 1.05	1.00 ± 0.96	1.69 ± 1.61
Commercial	$4 \times 10^9$	5	12.60 ± 1.48	21.60 ± 1.36	29.80 ± 2.27 r*; <sup>s</sup> **
	$4 \times 10^7$	5	7.60 ± 1.48	10.60 ± 1.36	16.80 ± 2.27 s**
	$4 \times 10^6$	5	4.60 ± 1.48	7.20 ± 1.36	18.20 ± 2.27 r*; <sup>s</sup> **
	$4 \times 10^4$	5	1.80 ± 1.48	4.80 ± 1.36	12.00 ± 2.27 r*; <sup>s</sup> **
	0 <sup>c</sup>	10	0.10 ± 1.05	0.29 ± 0.96	1.00 ± 1.61
Susceptible	$4 \times 10^9$	4	27.32 ± 1.67 r**; <sup>c</sup> **	41.88 ± 1.54 r**; <sup>c</sup> **	47.38 ± 2.56 r**; <sup>c</sup> **
	$4 \times 10^7$	4	10.32 ± 1.67	22.13 ± 1.54 r**; <sup>c</sup> **	33.38 ± 2.56 r**; <sup>c</sup> **
	$4 \times 10^6$	4	5.32 ± 1.67	16.38 ± 1.54 r**; <sup>c</sup> **	32.13 ± 2.56 r**; <sup>c</sup> **
	$4 \times 10^4$	4	2.57 ± 1.67	6.38 ± 1.54 r*	21.13 ± 2.56 r**; <sup>c</sup> **
	0 <sup>c</sup>	8	0.44 ± 1.19	1.00 ± 1.09	1.25 ± 1.82

<sup>a</sup> Bees per replicate = 50.

<sup>b</sup> r, c, and s indicate a significant difference from resistant, commercial, and susceptible stocks, respectively (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ).

<sup>c</sup> Control groups.

TABLE 2  
F-Values from the Least-Squares Analysis of Variance

Source	Day 7	Day 10	Day 14
Among replicates	2.44	0.92	1.39
Among doses	62.93**	191.39**	101.09**
Among stocks	14.54**	74.48**	69.66**
Stock by dose interactions	6.57**	14.39**	8.21**

\*\* $P < 0.01$ .

At day 14, the susceptible line is significantly different ( $P < 0.01$ ) from the resistant and the commercial stocks at all doses except the control dose.

It is concluded that the isolated virus causes mortality in all three stocks and this mortality increases with higher doses. It is further concluded that the three stocks of bees, resistant, commercial, and susceptible, are differentially resistant to a specific virus.

This experiment provides two pieces of information. First, the dosage response of all three stocks ties mortality to observed virus particles. Second, it provides positive confirming evidence not only that the lines developed by Kulinčević and Rothenbuhler (1975) are differentially resistant but also that this differential resistance is to a virus.

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