Tolerance to Carbaryl in Honey Bees Increased by Selection[1, 2]

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ABSTRACT

Nine generations of selection for survival to carbaryl produced resistance in newly emerged queens, but only vigor tolerance in newly emerged worker bees. By the 11th generation, the maximum screening dose was quadrupled for queens and doubled for drones.

A SEARCH for resistance to carbaryl (Sevin[R2]) in honey bees was prompted by losses of bees killed by insecticides applied to control pests on agricultural crops (Levin, 1970). In some parts of the U. S., bee losses to insecticides has been a chronic problem over the past 80 years (Atkins *et al.,* 1970); but with the shift in recent years to insecticides with short residual activity but greater acute toxicity to bees, these losses have become widespread and often quite serious (Levin, 1970).

Several instances of some degree of tolerance to pesticides in honey bees have been reported previously. One case of DDT tolerance was believed to be due to intercolony variation without the influence of selection (Tahori *et al.,* 1969). Another instance of DDT tolerance was thought to represent a response to selection in an outcrossed population (Atkins and Anderson, 1962), but was not increased by two generations of selection with controlled mating (Graves and Mackenson, 1965).

However, a continuously breeding line of bees tolerant to pesticides had not been developed; the feasibility of developing such a line was the objective of this research.

Carbaryl was chosen as the agent to select for tolerance because it is quite toxic to bees (Atkins *et al.,* 1970) and has been responsible for considerable bee losses (Anonymous, 1967).

Preliminary Screening for Carbaryl Tolerance

The attempt to develop a carbaryl tolerant line began with a survey to identify any colonies whose worker bees were more tolerant than average. The survey was conducted during one season (1968) until several colonies were discovered whose bees survived the screening procedure better than most bees.

The methods used for the initial screening were as follows. Quart paper cartons with plastic screen windows on the tops and sides were filled about ¼ full with bees brushed from honey combs. Very young bees were avoided. The caged bees were fed water with a saturated cellulose sponge, and sugar syrup containing carbaryl (5% suspension of 10% wettable powder) from plastic bags with fine punctures. These feeders were placed on the top screen. Most worker bees were killed by this mixture, but some bees from certain colonies survived longer than most, and did so repeatedly. Worker samples of one of these colonies (colony A in Table 1) were used subsequently as a comparison in each test.

Out of the 101 colonies screened, 4 were selected for further breeding (Table 1). These 4 were of diverse stock, none of which had been selected for pesticide tolerance and, where information was available, all had susceptible close relatives. One stock (Table 1, (1)) was started from 2 queens whose colonies survived or escaped being killed by Azodrin. However, this stock was about as susceptible to carbaryl as most bees.

Table 1. Bee stocks screened for tolerance to carbaryl.

Stock[1]	Numbers of progenies: Total tested	Relative tolerance to carbaryl: Least	Some	Most	Breeder queen selected
(1) Arizona	13	11	2		
(2) Baton Rouge	18	12	5	1	A
(3) Baton Rouge	27	21	5	1	D
(4) California x YDGkYR	15	8	6	1	C
(5) GkYD x natural	4	2	1	1	B
(6) Midnite outcrossed	17	8	9		
(7) New Jersey outcrossed	2		2		
(8) YDGkYR	1		1		
(9) YRYD outcrossed	4	3	1		
Totals	101	65	32	4	

[1] Progenies in each stock were related, except for (3).

Maintaining the Selected Gene Pool with Indirect Selection

Propagation and selection in progenies from the four selected queens were necessary to determine if carbaryl tolerance could be increased. Also, since all four queens were nearly 2 years old, and one of them was becoming a partial drone layer, further propagation was necessary to ensure survival of these stocks.

The breeding scheme used for keeping this gene pool was a circular mating scheme (Fig. 1), in which all four lines should have similar gene assortments in three generations. The breeders for each generation were selected by a progeny test of their worker bees. The queens of each mating group whose newly emerged (0-24 hour) workers best survived topical application of 0.1 μg carbaryl per bee became both queen mother and drone mother for the next generation. Whenever possible, 12-17 instrumentally inseminated queens comprised each mating group. However, only two queens were available in B X A in 1969, because queen B was lost. Also, some queens were lost before their progeny could be tested, and more were lost before the start of the next generation. The worst such losses occurred in DC X B in 1970. The gene pool crosses sufficed to keep the selected stocks from being lost. However, by the third generation each line seemed similar to the others in body color and handling qualities.

The indirect selection of the gene pool crosses apparently did not increase tolerance to carbaryl (Fig. 1). However, the proportion of susceptible progenies with no survivors of a treatment with 0.1 μg carbaryl per bee declined from 50% in the first generation to 15% in the second generation and 7% in the third generation. By the third generation the survival to carbaryl at 0.1 μg per bee was similar in each of the four groups.

The gene pool crosses were discontinued in 1972, by which time increased tolerance to carbaryl was developing in a directly screened line.

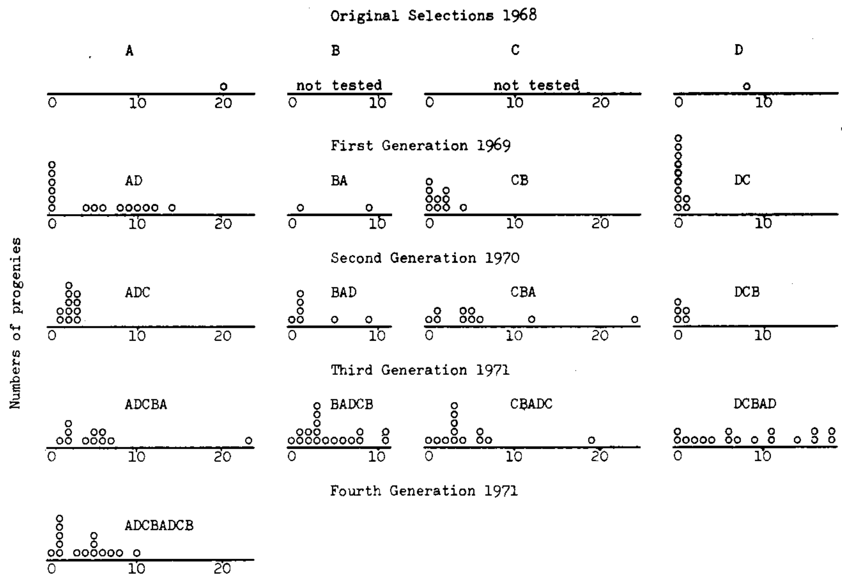

Fig. 1 Frequency distributions of worker progenies in the gene pool crosses (indirect selection). Number of worker bees alive at 24 hours out of 50 per progeny treated with 0.1 μg carbaryl per bee.

Table 2. Numbers of queens and drones treated and surviving exposure to carbaryl in 11 generations of selection.

Batches with:	Numbers (and %) of Queens: Treated	Alive at 24 hr	Alive at 1 week	Mated	Laying
Survivors not producing progeny	1,147	123 (11)	0	..	..
Survivors producing progeny	2,821	383 (14)	176 (6)	142 (5)	83 (3)

Batches with:	Numbers (and %) of Drones: Treated	Alive at 24 hr	Amount of semen in μl (av. %)[1]
Survivors not producing progeny	3,676	219 (6)	0
Survivors producing progeny	21,914	2,851 (13)	303 (1)

[1] Av. % would indicate the numbers of drones producing semen at insemination, if each drone produced 1 μl. The numbers of drones producing semen was not always recorded, but the amount of semen was.

Selecting Surviving Queens and Drones by Direct Screening

Direct screening was conducted through 11 generations, from 1969 to 1975.

In screening, the bees were exposed to carbaryl by topical application. The same procedure was used for queens, drones, and workers. Bees were permitted to emerge from sealed brood within cages in an incubator at 95° F and 65% RH. When 0-24 hours old, each bee, without being anesthetized, had a 2-μl drop of carbaryl in acetone placed on the top of its thorax by a microapplicator. The desired concentration of carbaryl was achieved by appropriate dilution of a stock solution made up at 10 μg per μl.

Each treated bee spent the first 24 hours after treatment caged individually in an incubator at 80° F and 65% RH. The cage consisted of a 1-ounce size paper souffle cup inverted over a paper-covered board. Included with the bee was a feeder made of a piece of cellulose sponge about ¼ x ¼ x ¼ inch saturated with dilute honey. For convenient handling, 25 of the cup-cages were arranged on a paper-covered substrate board. Since queens and drones could upset the cups, a restraining board was placed over each 25 cup-cages.

The queens and drones alive after 24 hours were reared to maturity, the drones within cages in strong, well fed, queenless nursery colonies, and the queens in individual nuclei (Mackensen and Tucker, 1970). Matings were done by instrumental insemination.

The level of survivorship attempted was 30% or less. If more than 30% of any batch of drones or queens survived, they were usually discarded on the arbitrary supposition that susceptible bees were surviving. The dosage of carbaryl to achieve the desired proportion had to be determined anew for each separate progeny. Typically, samples of each progeny were subjected to too high a dose at first, and then to lower doses, until an adequate level of survivorship ensued. No attempt was made to "fine-tune" the screening beyond increments or decrements of 0.05 or 0.1 μg per bee.

The level of survivorship achieved was usually well below 30% (Table 2). The average survival of queens and drones at 24 hours after treatment was 14% and 13% respectively. The carbaryl could have been responsible for perhaps another 5% or more mortality beyond 24 hours, since the further survival of treated queens and drones at insemination was that much worse than usually expected for untreated bees. However, loss of queens and drones beyond 24 hours after treatment could also be due to neglect or maltreatment by the bees of the nucleus or nursery colony. Of those alive at 24 hours, 46% of the queens and about 20% of the drones survived to the age of sexual maturity (7 days for queens, 14 days for drones). Only about half the drones produced semen. Further losses of the treated mated queens continued beyond initial laying. The incidence of attempted supersedure was so high as to make destruction of supersedure cells on a 10-day schedule necessary for stock maintenance. Indeed, the bees of one colony even started supersedure queen cells from larvae rather than eggs while their queen was present and laying; here supersedure was prevented by destroying queen cells every seventh day.

The breeding system combined screened descendents of the four original queens into one line, and then inbred it. Combining the selections took four generations. The inbreeding through four more generations was by grandmother to granddaughter matings, and the last three generations before the project was closed were propagated by aunt-niece matings. Some unscreened queens served as breeders in generations I, IX, and X.

During the course of the selection program the level of the screening dose for each generation gave an indication of progress for selection (Table 3). The highest dosage of carbaryl at which survival to reproduction was feasible in the offspring of the original four selections was 0.15 to 0.25 μg per queen and 0.15 to 0.5 μg per drone. The main differences were between progenies of the four queens; those of A and B seemed slightly more tolerant than those of C and D. The screening doses stayed about the same through generation VI. However, in generations VII to XI it was increased to 1.0 microgram per bee for both queens and drones.

The apparent lack of progress in selection for the first six generations could have been due to the breeding system. Although these were apparently assortative matings, the four different original selections could have brought together a genetic heterogeneity that needed sorting out before greater tolerance could be achieved. Further delaying the sorting out process could have been the grandmother to granddaughter matings, which may set gains back by two generations.

In the later generations, the screening dose was not strongly reduced by either the lack of screening of queens or by outcrossing. A IX generation, natural mated supersedure daughter of a queen which survived 0.6 μg carbaryl and whose mates survived 0.4 μg, produced drones which survived 1.0 μg, and daughter queens which survived 0.8 μg, and both of these were capable of producing offspring. This relatively high survival (0.8) of the outcrossed daughter queens suggests nonadditive inheritance of carbaryl tolerance. Alternatively, their supersedure queen mother could have mated with carbaryl-tolerant drones; however, not many such drones were allowed to fly. Another unscreened queen, a daughter of 0.8 x 1.0, and mated to 1.0, produced daughter queens able to survive 1.0 and produce progeny.

An increase in tolerance to carbaryl in IX-generation newly-emerged queens was indicated by their dosage mortality tests compared to those of newly-emerged queens from unselected stock from the same apiary (Fig. 2). The increase was not significant below LD_{35}, but was 3½ times greater at LD_{50} and 10½ greater at LD_{90}. The flatter slope for the tolerant line (1.7 vs 4.1 for the susceptible comparison) probably indicates a mixture of susceptible and tolerant queens in the IX generation.

The increase in tolerance in newly emerged worker bees was slight (Fig. 3). A sample from the IX generation was only 40% more tolerant than a sample from the most tolerant of the I generation, comparing their LD_{60}. The IX generation slope was steeper

Table 3. Screening doses of carbaryl and proportional survival of queens and drones in 11 generations of selection.

Generation	Queens: Number treated	Queens: % survivors at 24 hr	Queens: Fate[2]	Screening dose[1] μg/bee	Drones: Number treated	Drones: % survivors at 24 hr	Drones: Fate[2]
I	0	..	M	0.01[1]	0	..	M
	10	40	X	0.125	..	..	..
	142	17	MN	0.15	136	14	Z
	293	9	M	0.2	658	13	Z
	217	8	MN	0.25	504	3	Z
	47	2	Z	0.3	875	9	M
	..	..	..	0.4	1,711	10	MN
	..	..	..	0.5	459	5	MN
	..	..	..	1.0	30	3	Z
II	8	50	X	0.2	..	..	
	63	16	MN	0.25	..	..	
	309	7	M	0.3	..	..	
	26	12	M	0.35	..	..	
	65	0		0.4	2,216	16	M
III	77	6	MN	0.2	..	..	
	48	4	M	0.25	..	..	
	16	0		0.3	242	11	Z
	..	..		0.35	414	6	M
	4	0		0.4	379	1	Z
	..	..		0.5	888	3	M
IV	25	8	X	0.2	..	..	
	53	6	M	0.25	..	..	
	28	4	MN	0.3	1,834	8	M
V	48	17	MN	0.2	..	..	
	77	6	M	0.25	..	..	
	25	0		0.3	..	..	
	13	0		0.35	52	31	MN
	..	..		0.4	2,404	7	M
VI	40	15	M	0.2	..	..	
	78	6	Z	0.25	..	..	
	11	0		0.3	..	..	
	..	..		0.4	553	26	MN
	..	..		0.5	864	14	M
VII	1	100	X	0.3	..	..	
	50	14	MN	0.35	..	..	
	44	5	M	0.4	..	..	
	..	..		0.5	28	86	X
	..	..		0.6	973	11	M
	..	..		0.8	342	18	MN
VIII	4	0		0.35	..	..	
	30	33	MN	0.4	2,397	7	M
	45	24	MN	0.5	425	4	MN
	44	9	M	0.6	193	3	MN
IX	0	..	M	0.01[1]	0	..	M
	9	33	X	0.6	..	..	
	587	24	M	0.8	..	..	
	951	12	MN	1.0	2,219	24	M
X	0	..	M	0.01[1]	..	..	
	26	54	M	0.8	..	..	
	172	4	Z	1.0	2,793	24	M
XI	24	29	M	1.0	390	11	M
	17	0		1.6	..	..	
	34	0		2.0	..	..	

[1] Unscreened drones and/or queens contributed to the selected line in generations I, IX and X.

[2] The fates of queens and drones alive at 24 hours were:
M = Matings contributed to later generations.
MN = Matings that did not contribute to later generations.
X = Discarded.
Z = Died before maturity.

(5.1) than the I generation (4.5), so that the differences above LD_{70} were not significant. The gain in nine generations of selection (0.10 to 0.14 μg per bee) was about the same as the range of variation in the foundation stock (0.06 to 0.10 μg per bee) (Table 4).

The progress toward resistance in newly emerged queens (Fig. 2) was considerably greater than that for newly emerged worker bees (Fig. 3). Following the classification of resistance of Hoskins and Gordon (1956), one would conclude that the queens had developed resistance, but that the workers had developed only vigor tolerance. This difference must not be explained only in terms of size and weight. For although the queen is about twice as heavy as a worker bee, the differences in dosages survived increased beyond this. More perplexing is the difference in slope of the dosage mortality lines. Perhaps it is due to differences in metabolic rate.

The LD_{50} values for older worker bees (Table 4, (1), (2), (3)) in the present work were about half those found previously with comparable conditions (0.5 to 0.6 vs 1.1 to 1.3 by Stevenson, 1968, and 1.123 by Georghiou and Atkins, 1964). However, much more data would be required to support any contention that this study dealt with relatively susceptible bees.

This study demonstrated a certain degree of tolerance to carbaryl gained by selection. Furthermore, the fact that outcrossing and lapses in selection did not substantially reduce the gain in the last three generations could suggest that one or a few major dominant genes are important for resistance to carbaryl.

Another selection strategy may be fruitful if we are dealing with one major factor gene present in all bee populations in low frequency. In this strategy, one would start at a relatively high level (e.g., 1 μg per drone for newly emerged drones) and treat small samples (50-100 drones) of several hundred colonies of diverse origins. If it were to succeed, this strategy would be far less work than the meticulous management needed for maintenance of several generations of inbreds accompanying gradual selection.

Further selection beyond that achieved in the present feasibility study seems necessary to determine if very young worker bees can become resistant to carbaryl. If this cannot be achieved, then even if the older worker bees were resistant, the young bees in the colony would still be vulnerable to pollen contaminated with carbaryl (Morse, 1961; Moffett *et al.*, 1970).

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FOOTNOTES

[1] In cooperation with Louisiana Agricultural Experiment Station.

[2] Mention of a proprietary product does not constitute endorsement by the USDA.

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Table 4. Topical toxicity of carbaryl to worker bees.[1]

Source of worker bees (daughters of)	Age	LD_{50}	Slope
1. A[2]	Mixed ages	0.61	3.4
2. Unselected	Mixed ages	0.59	2.5
3. Unselected	Mixed ages	0.46	2.7
4. A[2]	0 - 24 hr.	0.10	4.5
5. D[2]	0 - 24 hr.	0.06	3.9
6. Generation I	0 - 24 hr.	0.10	5.1
7. Generation I	0 - 24 hr.	0.07	3.9
8. Generation II, outcrossed	0 - 24 hr.	0.09	7.2
9. Generation IX	0 - 24 hr.	0.14	5.1
10. Generation IX	0 - 24 hr.	0.13	5.4

[1] Based on at least 4 dosage levels and at least 25 bees per treatment. The data were analyzed by computer (Daum, 1970).

[2] See Table 1.

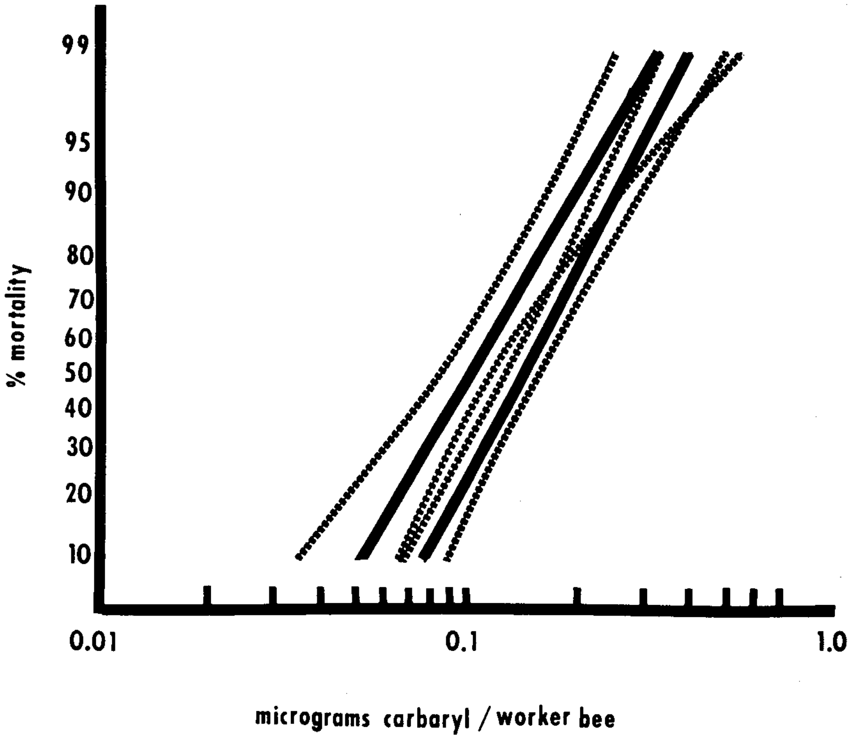

Fig. 3 Log - probability plots with 95% confidence intervals (dashed lines) for newly emerged worker bees of generations I (left) and IX (right). These are plots for the data for (4) and (9) in Table 4. The data for this plot were analyzed by computer (Daum, 1970).

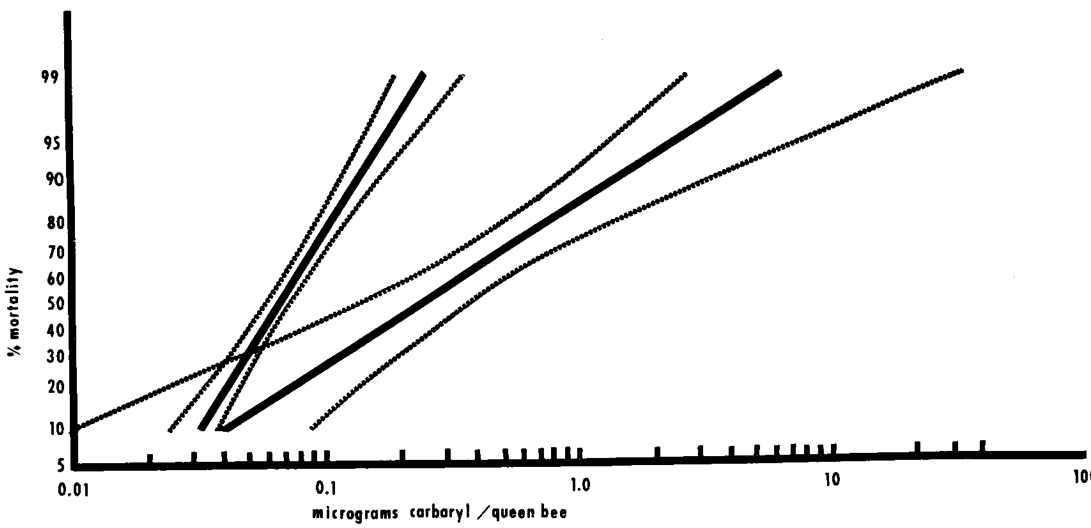

Fig. 2 Log - probability plot of dosage-mortality response and 95% confidence intervals (dashed lines) of newly emerged queen bees; unselected on left and IX generation-selected on right. The unselected queens were exposed to 6 doses, at least 50 queens per dose; a total of 700 queens including those exposed to only solvent. The selected queens were exposed to 10 doses, at least 50 queens per dose; a total of 939 queens. The data for this plot were analyzed by computer (Daum, 1970).