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Disappearing Disease: III. A Comparison of Seven Different Stocks of the Honey Bee (*Apis mellifera*)

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INTRODUCTION

"Disappearing disease" of the honey bee is a mysterious phenomenon. Strong colonies suddenly become weak. Few or no dead bees are seen; bees simply disappear over a few weeks' time. Many colonies are lost completely.

Reports of disappearing bees have come from a number of countries over more than 100 years. An early example is "the disease of 1868" which struck in Indiana, Kentucky, and Tennessee (1, 23). Other examples have come from Australia in 1872 and 1910 (3), from Louisiana and Texas in 1963-64 (20), from California in 1964-65 (8), from Mexico in 1977 (19), from the Rio Grande Valley in Texas in 1974 (11), and from various additional locations in the United States (24). Judging from these reports, there can be no doubt that many beekeepers have suffered devastating losses of bees.

An extensive review of literature on disappearing disease (DD) is given by Wilson and Menapace (24). Various explanations of the cause of the losses have been advanced but most have had little support. Furthermore, there is no reason to believe that all losses gathered under the umbrella of DD are due to the same cause. In fact, many of the earlier cases seem to have occurred in the fall or early winter, whereas some of the later cases (24) have occurred in the spring.

A prominent hypothesis over the last few years has involved some sort of stock deterioration (24). It has been suggested that such deterioration may have resulted: 1) from the admixture of African bee genes to the gene pool of North American bees, 2) from excessive inbreeding of bee stocks, or 3) from the maladaptation to northern climates of bees reared over many generations in the South.

If such genetic weaknesses exist, it should be possible to obtain evidence of them by a careful comparison of DD with non-DD stocks in the same location. Furthermore, such an investigation should reveal something about the range of variation in North American bees. Is there sufficient variation to insure success for a program of genetic selection, or are our bees reduced to a uniform genetic mediocrity? Do we have the genetic variation to deal successfully with Africanized bees? This investigation was designed to compare several stocks of bees with respect to colony population; amount of brood, honey, and pollen; presence of common bee diseases; and the possible presence and causes of DD.

MATERIALS AND METHODS

Nucleus Colonies, 1977

For the first year's work (1977), a number of three-frame nucleus colonies of three different stocks were transported by air freight to Columbus, Ohio.

One stock came from Texas from a beekeeper who stated that he lost 1,000 of 4,000 colonies moved from Nebraska. Losses began the third week in December and colonies dwindled constantly throughout the succeeding months. Some apiaries were visited on May 23, 1977, by Thomas E. Rinderer. Parent colonies were generally weak. European foulbrood, chalkbrood, and sacbrood were seen. There was evidence of abandoned brood. Adult bees were sluggish and symptoms of hairless-black syndrome were seen in every colony. Some colonies were out of honey. The beekeeper stated that he had treated colonies with tetracycline powder and fed pollen supplement patties made of soybean flour, yeast, powdered milk, and honey. In earlier years he had fed soybean flour alone, in the open, for bees to collect. He was not aware of any insecticides used recently nor at the time of loss in the vicinity. Twelve nucleus colonies from this beekeeper's hives were established in the Hard Road Apiary and designated as Hard Road DD Stock (HRDD1-12).

The second DD stock came from Kansas. The apiary was visited by W. C. Rothenbuhler on June 24, 1977, when nucleus colonies were prepared. Parent colonies were variable in amount of bees and brood, ranging from 6 to 15 combs covered by bees. Losses had occurred 5 to 6 months earlier in January and February after bees were moved from Kansas to south Texas in mid-December. Prior to the move, colonies had no brood, but started brood rearing upon arrival in Texas. The beekeeper couldn't say how much pollen was in the hives when losses occurred. Some colonies that were partially depleted of adult bees recovered later, but 15 to 18 of 125 died. Terramycin was fed. No insecticides were in use at the time of loss as far as was known. Eleven nucleus colonies were established in the Jewett Road Apiary as JRDD13-23.

The third stock came from Colorado from a bee-

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keeper who said that he had never seen DD in his apiaries. These nucleus colonies were prepared for shipment by personnel of the Laramie USDA Bee Lab and established in the Jewett Road Apiary as Jewett Road Control Stock (JRC1-12).

JRC colonies were established on May 18, 1977, HRDD colonies on May 24, and JRDD colonies on June 25. The Hard Road and Jewett Road apiaries were about 2 miles apart but similar in available nectar and pollen sources.

Although some colony inspections were made earlier, beginning in early July each colony was carefully inspected approximately every 10 days. Amounts of brood, pollen, and honey were estimated on each inspection in terms of number of Langstroth combs or tenths thereof. Number of bees was established similarly in terms of combs covered with bees. We realize that subjective elements can enter into such estimates, but any other measure would have increased the time required to inspect a colony, which would have drastically reduced the number of colonies inspected. Furthermore, independent estimates by Rothenbuhler and Kulincevic in the early part of the work were in close agreement. In addition to the estimates, brood diseases, supersedure cells, and quality of the brood were noted and recorded.

Certain measures of longevity were made. These involved caging a comb of emerging brood for about 24 hours in an incubator. Fifty emerged bees were then caught and put into a laboratory cage with food and water as described elsewhere (14, 18). The bees were inspected daily and the dead ones counted and removed. The days required for half of the bees to die were taken as the length of life for bees in that cage. The results from three such cages were averaged to obtain the laboratory longevity measure of the bees from each colony.

Samples of honey and pollen, stored by the bees in their previous locations (Texas, Kansas, and Colorado), were taken from combs of certain colonies in order to check for toxins. These materials were fed to bees in laboratory cages and mortality was recorded daily.

Eight of these colonies survived without queen supersedure through a second year. They were inspected on a 10-day schedule in 1978 and certain comparisons between years were made.

Queens Only, 1978

To obtain new stocks to be tested during the second season (1978), queens only from four different sources were moved to Columbus and introduced to our approximately uniform three-frame nucleus colonies. Twelve queens were obtained from a beekeeper in Florida who had suffered severe losses of bee populations, resulting in many weak and dead colonies. Soybean flour had been fed to colonies. A sample of worker bees from each of 17 weak colonies was sent to Truman Clark (Bioenvironmental Bee Laboratory, USDA, Beltsville, Md.) to be examined for pathogens. One of 10 bees from one colony showed a filamentous virus. Ten of the 17 colonies showed nosema infection. Nosema spores were found in 10 to 60% of the bees in each infected colony. No other pathogens were found. Queens from these weak colonies were to be discarded by the beekeeper, but instead 12 were introduced to our nucleus colonies on May 23, as HRDD1-12.

Control stock with no history of DD was obtained from Wisconsin and introduced in the same apiary as the Florida stock on May 24, as HRC13-24.

A second DD stock for 1978 was obtained from a second location in Texas. The beekeeper had seen population loss in his colonies the previous fall. There was no obvious reason for this loss. Twelve queens were shipped to us and introduced to our three-frame nuclei on June 1, as JRDD11-22.

Control stock was obtained from British Columbia and introduced to our Jewett Road nucleus colonies on June 3, as JRC1-10. These queens were reared from stock obtained from New Zealand and consequently were expected to be genetically different from North American bees.

All colonies were inspected approximately every 10 days beginning a few days after queen introduction and continuing until the end of October. The same types of data were recorded in 1978 as in 1977.

Nucleus Colonies, 1978

In the latter part of June 1978, we obtained seven nucleus colonies of DD stock from the same Florida beekeeper who had supplied a dozen DD queens. Considerable variation in quantities of bees, brood, and food was found in these colonies upon receipt (see page 17). Nevertheless, they were located in our Hard Road apiary and inspected regularly throughout the summer and early fall. The colonies were numbered HRNDD25-31.

RESULTS AND ANALYSES

General Presentation Procedures

The data collected by regular inspections of colonies throughout the summer season have been graphed (as in Fig. 1) for visual evaluation. Space limitations preclude the publication of each colony's graph. Each

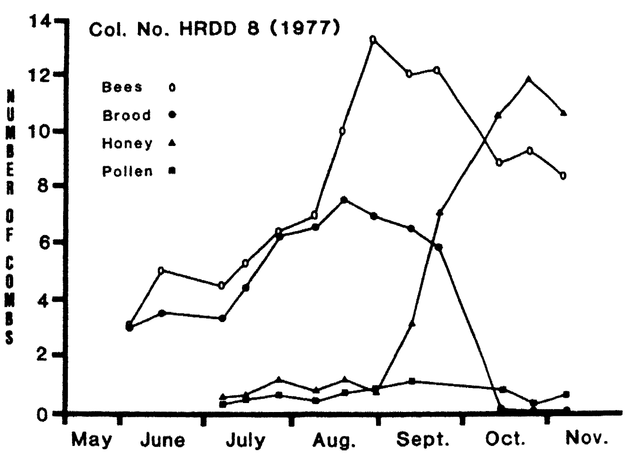

Fig. 1.—Colony HRDD8 in 1977. A typical profile.

TABLE 1.—Number of Colonies and Number of Larvae Showing Signs of Brood Disease in Inspections Conducted About Every 10 Days During Summer of 1977.

Stock	N*	Diseased	AFB*	EFB*	Sac*	Chalk*
JRC	12	Colonies	0	4	2	0
		Larvae	0	213	4	0
JRDD	11	Colonies	3	5	6	10
		Larvae	86	10	9	107
HRDD	12	Colonies	1	6	8	12
		Larvae	57	86	26	1390

*N = total number of colonies under observation, AFB = American foulbrood, EFB = European foulbrood, Sac = Sacbrood, Chalk = Chalkbrood

graph, however, has been photographed and 2 x 2 slides may be borrowed from either Ohio State author, or from the USDA Bee Laboratory in Baton Rouge. Only graphs of average or those of striking examples of some phenomenon will be presented here.

Nucleus Colonies Obtained in 1977

Observations in 1977

Well-known bee diseases: Counts of diseased brood were made and recorded during colony inspections. Results are given in Table 1. The control stock (JRC) was comparatively free of disease except for European foulbrood (EFB) which was severe in 2 colonies and light in 2 others (12 cells in one colony, 1 in the other). Colony JRCll (Fig. 2) failed to increase its bee population and eventually died. Examination of the last 13 bees from this colony by Truman Clark revealed that 12 were infected with *Nosema apis*, 7 with *Malpighamoeba mellificae*, 5 with the honey-bee filamentous virus, and 5 with organisms of septicemia.

JRDD stock showed light (less than five cells in any one colony) but widespread presence of EFB and sacbrood. The 3 colonies with American foulbrood (AFB) showed 7, 18, and 61 cells of diseased brood. Chalkbrood was present in all except one colony. Only 1 colony had more than 10 mummies, and it had 58.

Disease was even more severe in HRDD stock; all colonies showed chalkbrood. The range in total mummy count for the entire summer was 3 to 356, with an average of about 115 per colony. Fifty-seven cells of AFB were found in one colony. Sacbrood was light but present in two-thirds of the colonies. The EFB count exceeded 36 cells in each of 2 colonies but was less than 4 cells in each of 4 others.

Clearly the DD stocks had far more recognizable disease than the control stock. Such disease would be expected to have an adverse impact upon colony strength and productivity.

Pressure to supersede: Many colonies built supersedure cells during the summer. We usually destroyed such cells to prevent or delay supersedure of the queen, but their presence was recorded. Table 2 summarizes the extent of supersedure cell construction. The two DD stocks engaged in far more supersedure attempts than the control stock.

An estimate of brood quality (based on frequency of

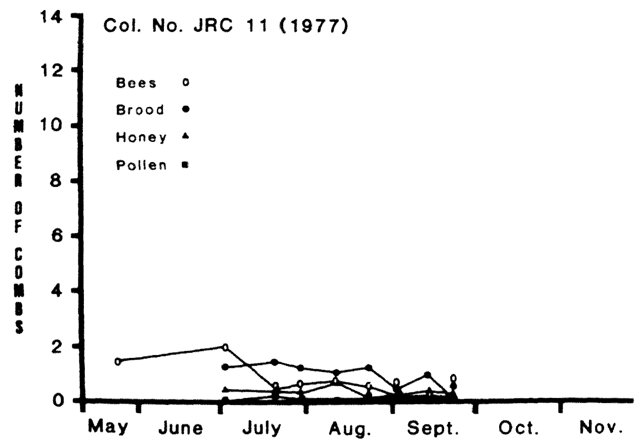

Fig. 2.—Colony JRC11, 1977. A heavily diseased colony.

cells with brood vs. empty cells in brood area) was made at each colony inspection. Great variation was seen among colonies within a stock, but few or no differences were seen among stocks. On a scale of 3 equals an excellent brood pattern with 99% of cells occupied and 0.5 equals 20% of cells occupied, the JRC stock colonies averaged 2.3, the JRDD stock 2.2, the HRDD stock 2.0. No great accuracy can be claimed for these ratings, but they suggest that the control stock may have had the best brood pattern and the HRDD stock the poorest.

Sudden reduction of adult bee population: Colonies normally reach a population peak in the summer and experience a substantial decrease in the fall period. All three stocks showed a normal or even less than normal loss (Fig. 6). A sudden and drastic reduction in colony

TABLE 2.—Tendency to Build Supersedure Queen Cells in 1977 by Three Stocks of Bees.

	JRC	JRDD	HRDD
No. of Colonies	12	11	12
No. of Colonies with Queen Cells	1	7	4
No. of Colony Inspections	97	94	94
No. of Times Queen Cells Found	1	23	16
No. of Queen Cells	2	62	28
No. of Colonies which Reared Queens	1	4	4

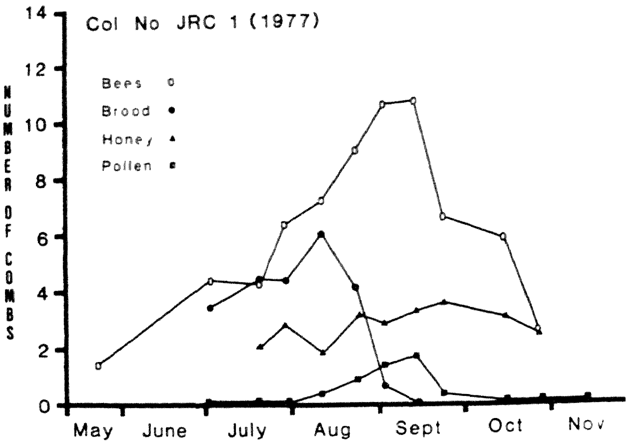

Fig. 3.—Colony JRC1, 1977. Decrease in population due to queenlessness which occurred in August.

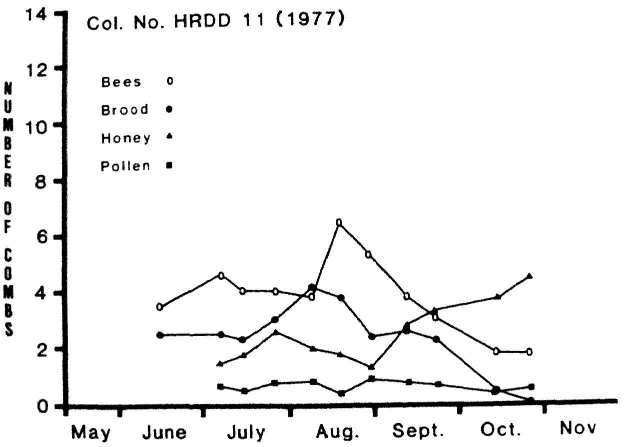

Fig. 4.—Colony HRDD11, 1977. Decrease in population due to a non-prolific queen which was superseded in September.

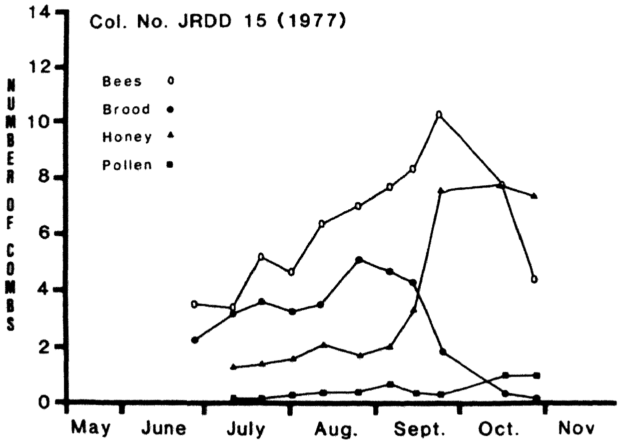

Fig. 5.—Colony JRDD15, 1977. Lost 56% of adult bees in September and October, died during winter.

population is characteristic of DD. Five colonies have been selected to show the types of population decreases that were seen. Colony JRCl (Fig. 3) decreased from about 10.5 combs of bees in early September to about 2.5 combs in late October. This was the greatest reduction seen in 1977, but it was due to queenlessness.

Supersedure accounted also for large drops, as in colony HRDD11 (Fig. 4). Brood quality was low in this colony which was bulding queen cells over several weeks. A supersedure queen was present on Sept. 22. Perhaps brood production would have increased if supersedure had occurred earlier.

Colony JRDD15 (Fig. 5) suffered a 56% decrease and died during winter for no immediately obvious reason. This reminds one of a DD loss. One other colony (JRDD14, Fig. 5A), with the original queen throughout the test period, had a 50% loss of adult bees. Inasmuch as this colony failed to build a normal-size population, it was too weak to winter and was discarded in the fall (see Table 36 for details). Colony JRCll (Fig. 2), discussed previously as diseased (page 3), suffered a decline in July, failed to increase its population, and died in September.

Test for toxic substances: Samples of pollen and honey were taken from several nuclei, just after their arrival, to be tested for presence of toxic material. Specifically, 9 samples of pollen and honey from 3 DD colonies (HRDD1, 2, 4); 12 samples of pollen only from 3 DD colonies (HRDD1, 3, and JRDD14); and 3 samples of pollen from 1 non-DD colony (control) were fed to young caged bees according to the procedures of the longevity test. Time for 50% of the bees to die ranged from an average of 21.7 to 27.0 days for tests of DD colonies. The control test required 21.3 days. There was no evidence of toxic material in the honey or pollen in five DD colonies.

Analysis of Variation in Quantity of Bees, Brood, Honey, and Pollen

Omitted colonies: For the analyses of variation, data from four colonies were omitted. Two of these, HRDD1

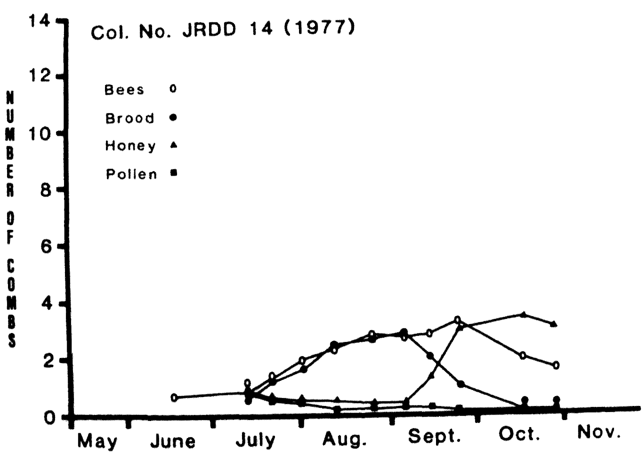

Fig. 5A.—Colony JRDD14, 1977. Failed to build a normal population, then suffered a decline. Too weak to winter, destroyed.

and HRDD12, superseded their queens early in the season. This necessitated dropping them from the test since the colonies' worker bees would not, after supersedure, represent the original stock. Colony JRC1 became queenless in August and was deleted. Colony JRC11 was deleted because it was so heavily diseased that it dropped to a population of 13 bees, all of which were diseased when examined by Dr. Clark as reported on page 3.

Variation among stocks: The average bee populations of the colonies in each of the three stocks, at each inspection, throughout the summer are presented in Fig. 6. No stock appears to be different from any other in bee population. The averages of brood present in colonies of each stock are given in Fig. 7. No striking difference is apparent. Averages of honey present are given in Fig. 8. In this case it seems that the JRC stock may have made more honey than JRDD or HRDD. No difference in pollen hoarding is apparent (Fig. 9).

A nested analysis of variance was performed to investigate differences among stocks in bees, brood, honey, and pollen. Results are given in Table 3. No differences among stocks in bees, brood, or pollen were detected.

TABLE 3.—Nested Analysis of Variance for Differences Among Stocks (JRC, JRDD, and HRDD) in Combs Covered by Bees and Combs Occupied by Brood, Honey, and Pollen.*

Bees	Brood	Honey	Pollen
F = 1.60	F = 2 07	F = 6.47	F = 1.94
df = 2, 28	df = 2, 28	df = 2, 28	df = 2, 28
p = 0.2195	p = 0.1445	p = 0.0049**	p = 0.1623

*F values, degrees of freedom, and probability values are given
**Significant at less than the 1 % level.

TABLE 4.—Duncan's Multiple Range Test at the 5% Level for Differences Among Stocks in Honey Hoarded.

Stocks	N*	Mean	Grouping†
JRC	97	6.49	A
HRDD	94	5.00	AB
JRDD	94	3.65	B

*N = Number of inspections per stock.
†Stocks with the same letter are not significantly different from each other.

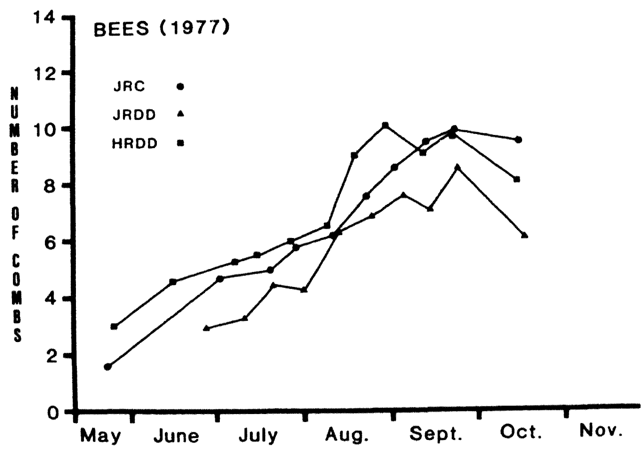

Fig. 6.—Mean number of combs per colony covered by adult bees in each of three stocks, 1977.

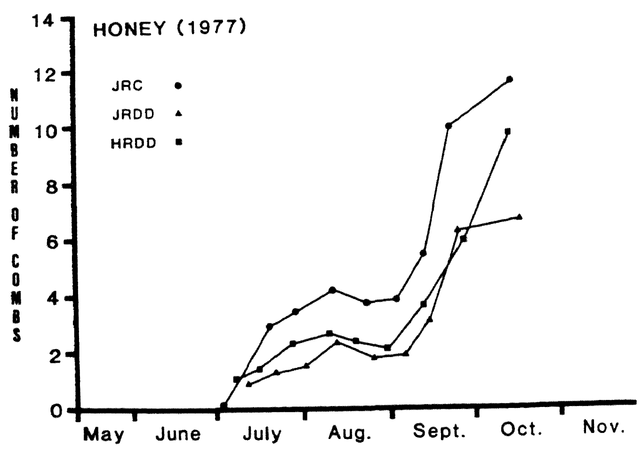

Fig. 8.—Mean number of combs of honey per colony in each of three stocks, 1977.

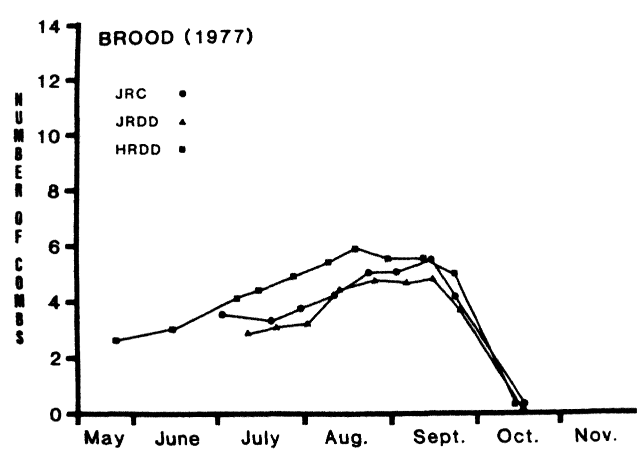

Fig. 7.—Mean number of combs of brood per colony in each of three stocks, 1977.

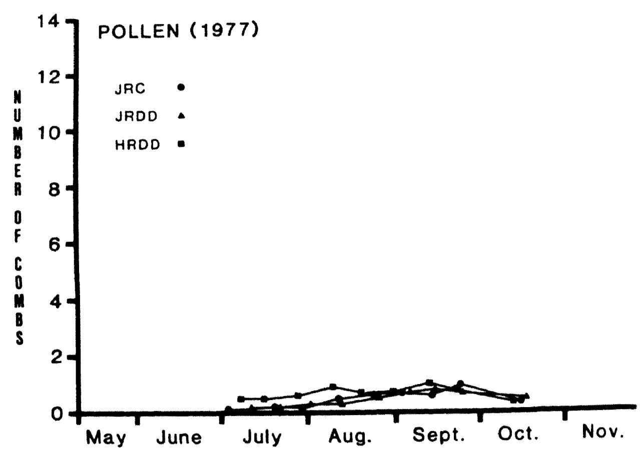

Fig. 9.—Mean number of combs of pollen per colony in each of three stocks, 1977.

TABLE 5.—Results of Several One-Way Analyses of Variance to Test for Differences in Bees, Brood, Honey, and Pollen Among Colonies within Each 1977 Stock.†

	JRC	JRDD	HRDD
Bees	F = 15.57 df = 9, 87 p = 0 0001**	F = 10 91 df = 10, 83 p = 0 0001**	F = 12.71 df = 9, 84 p = 0.0001**
Brood	F = 0 96 df = 9, 87 p = 0.4800	F = 2 05 df = 10, 83 p = 0 0377*	F = 0.47 df = 9, 84 p = 0.8900
Honey	F = 3 90 df = 9, 87 p = 0.0004**	F = 1 71 df = 10, 83 p = 0.0911	F = 2.05 df = 9, 84 p = 0.0438*
Pollen	F = 6.31 df = 9, 87 p = 0.0001**	F = 2.45 df = 10, 83 p = 0.0130*	F = 3.10 df = 9, 84 p = 0.0030**

*Significant at less than the 5% level.
**Significant at less than the 1% level.
†F values, degrees of freedom, and probability values are given.

TABLE 6.—Duncan's Multiple Range Test at the 5% Level of Probability for Differences in Combs of Bees Among Colonies of the 1977 JRC Stock.

Colony	N*	Mean	Grouping†
12	10	12.81	A
8	10	9.66	B
2	10	8.97	B
6	10	8.32	BC
9	10	7.92	BC
3	10	7.09	CD
10	10	6.67	CD
7	7	6.46	CD
5	10	5.73	DE
4	10	4.19	E

*N = number of inspections of the indicated colony.
†Colonies with the same letter are not significantly different from each other.

TABLE 7.—Duncan's Multiple Range Test at the 5% Level of Probability for Differences in Combs of Honey Hoarded Among Colonies of the 1977 JRC Stock.

Colony	N*	Mean	Grouping†
12	10	8.83	A
2	10	8.57	A
3	10	7.92	A
8	10	7.60	A
6	10	7.52	A
10	10	7.20	A
7	7	6.56	AB
9	10	5.27	ABC
5	10	3.05	BC
4	10	2.42	C

*N = number of colony inspections.
†Colonies with the same letter are not significantly different.

Stocks were different in honey production, and Duncan's multiple range test (Table 4) indicates that the JRC and JRDD stocks were different at the 5% level of probability. JRC made more honey than JRDD. The HRDD stock, lying between the other two (Fig. 8 and Table 4), cannot be distinguished from either of the others in amount of honey.

We conclude that the three stocks were similar in amount of bees, brood, and pollen, but different in amount of honey in the hives.

Variation among colonies: It is of interest to know whether or not there are differences among colonies *within* each stock. A one-way analysis of variance was used to answer this question. F values, degrees of freedom, and probability values are given for each variable in each stock in Table 5. A probability value of less than .05 is accepted as indicating a significant difference among colonies. Even though the three stocks were alike in number of combs of bees, colonies within each stock showed real differences. Likewise, no stock differences for amount of pollen were found, but real differences among colonies within each stock were shown. With respect to combs of honey, the colonies of the JRC stock and the HRDD stock showed significant differences. Only the JRDD colonies showed significant within-stock variation in amounts of brood.

To learn the source and extent of the variation among colonies within a stock, a series of Duncan's multiple range tests were performed (Tables 6-14). For instance, Table 6 shows that the colonies of the JRC stock are assembled into five groups with respect to bee populations by Duncan's test. Colony number 12, marked by A, is different from all other colonies at a 5% level of probability. The B-marked colonies differ significantly from the D-marked ones, but both overlap with some C-marked colonies. Colonies 4 and 5, marked by E, are different at the 5% level of probability from A-, B-, and C-marked colonies, but they overlap with D-marked colonies. Means in combs of bees in each colony range from 12.81 to 4.19.

JRC colonies did not show significant differences in amount of brood. They did show differences in amount of honey (Table 5), which is analyzed further in Table 7.

TABLE 8.—Duncan's Multiple Range Test at the 5% Level of Probability for Differences in Combs of Pollen Hoarded Among Colonies of the 1977 JRC Stock.

Colony	N	Mean	Grouping
8	10	1.02	A
7	7	0.77	AB
9	10	0.60	BC
6	10	0.55	BCD
10	10	0.44	CD
2	10	0.38	CD
5	10	0.32	CD
4	10	0.31	CD
3	10	0.28	D
12	10	0.26	D

Table 8 shows the nature of the variation among colonies of the JRC stock with respect to pollen hoarded.

Perusal of the remaining tables (Tables 9-14) confirms the presence of differences among colonies of the other two stocks (HRDD and JRDD).

We conclude that none of the stocks is composed of a homogeneous group of colonies, but that the colonies making up each of the three stocks are highly variable in bee populations and pollen stored. There is less variation in brood reared and honey stored since the colonies of only part of the stocks differ significantly in combs of brood and honey.

TABLE 9.—Duncan's Multiple Range Test at the 5% Level of Probability for Differences in Bee Population Among Colonies of the 1977 JRDD Stock.

Colony	N	Mean	Grouping
21	9	9.19	A
19	9	8.82	AB
23	9	7.38	BC
15	9	6.87	CD
16	6	6.70	CD
17	9	6.58	CD
20	7	6.19	CD
13	9	5.90	CD
22	9	5.73	CD
18	9	5.24	D
14	9	2.28	E

TABLE 10.—Duncan's Multiple Range Test at the 5% Level of Probability for Differences in Brood Quantity Among Colonies of the 1977 JRDD Stock.

Colony	N	Mean	Grouping
21	9	4.77	A
19	9	4.60	A
16	6	4.53	A
23	9	3.57	AB
17	9	3.56	AB
20	7	3.30	AB
22	9	3.21	AB
13	9	3.18	AB
15	9	3.01	AB
18	9	2.68	AB
14	9	1.53	B

TABLE 11.—Duncan's Multiple Range Test at the 5% Level of Probability for Differences in Pollen Hoarded Among Colonies of the 1977 JRDD Stock.

Colony	N	Mean	Grouping
21	9	0.76	A
16	6	0.75	AB
17	9	0.70	AB
22	9	0.60	ABC
23	9	0.58	ABCD
19	9	0.53	ABCD
15	9	0.52	ABCD
20	7	0.40	ABCD
13	9	0.39	BCD
18	9	0.30	CD
14	9	0.22	D

Observations in 1978 on 1977 Stock and Certain Comparisons

Wintering 1977-78: At the end of the active foraging season, 22 of the initial 35 colonies had not superseded their queens or failed in some other way, and we desired to test their overwintering ability. These colonies are listed in Table 15 along with the number of combs of

TABLE 12.—Duncan's Multiple Range Test at the 5% Level of Probability for Differences in Bee Population Among Colonies of the 1977 HRDD Stock.

Colony	N	Mean	Grouping
3	10	11.08	A
2	10	10.74	A
9	10	9.68	AB
6	10	9.60	AB
8	10	9.21	AB
7	10	7.88	BC
5	10	6.46	CD
10	7	6.26	CDE
4	10	5.64	DE
11	7	4.33	E

TABLE 13.—Duncan's Multiple Range Test at the 5% Level of Probability for Differences in Honey Hoarded Among Colonies of the 1977 HRDD Stock.

Colony	N	Mean	Grouping
9	10	7.31	A
2	10	6.97	A
7	10	6.18	AB
3	10	6.11	AB
6	10	5.68	AB
8	10	4.76	AB
10	7	4.27	AB
4	10	2.79	B
5	10	2.69	B
11	7	2.23	B

TABLE 14.—Duncan's Multiple Range Test at the 5% Level of Probability for Differences in Pollen Hoarded Among Colonies of the 1977 HRDD Stock.

Colony	N	Mean	Grouping
3	10	0.97	A
6	10	0.83	AB
10	7	0.76	AB
9	10	0.74	AB
11	7	0.70	ABC
8	10	0.68	ABC
2	10	0.59	BC
7	10	0.51	BC
5	10	0.50	BC
4	10	0.34	C

TABLE 15.—Number of Combs Covered by Bees at Beginning and End of Winter with Percent Surviving in Several Colonies of Each of Three Different 1977 Stocks.

JRC STOCK				JRDD Stock				HRDD Stock			
Colony	1977 10-27	1978 3-31	Percent Survival	Colony	1977 10-28	1978 3-31	Percent Survival	Colony	1977 10-26	1978 3-31	Percent Survival
2	9.5	2.4	25	15	4.5	0.0	0	2	10.8	5.5	51
3	8.7	0.8	9	18	4.7	0.2	4	3	12.4	6.2	50
4	4.1	0 0	0	19	9.0	0.7	8	4	6.7	1.4	21
5	5.8	1.3	22	21	9.8	2.9	30	5	7.3	1.9	26
6	9.9	5.8	59	22	5.2	1.5	29	7	6.4	0.0	0
8	10.2	6.2	61	23	8.5	0.8	9	8	9.3	3.2	34
9	8.5	5.3	62	Mean	7.0	1.0	14.6	9	9.5	3.0	32
10	5.8	2.9	50					Mean	8.9	3.5	34.0
12	11.9	7.3	61								
Mean	8.3	3.6	43.4								

bees in late October and in late March of the next year. The winter was severe for central Ohio and some colonies were too small to survive. Three colonies died and many were severely reduced in population. It appears that the JRDD stock wintered most poorly since only 15% of the bees survived, compared with 34 and 43% in the other stocks.

Since the size of the bee population going into winter may have influenced survival over winter, a least-squares analysis of covariance was used to test for stock differences. None was found. The probability value for a stock difference of the magnitude found, as a chance event, was 0.11 which might be considered close to significant.

Another kind of data on wintering was taken. On Jan. 12, 1978, the bees which had died in the hive during the past 2 months were collected from the bottom board and in front of the entrance (Table 16). It appears that JRC was wintering much better than JRDD, although no consideration has been given in these calculations to

TABLE 16.—Comparison of Number of Bees Found Dead in Each Stock After 2 Months of Winter (1977-1978) with the Number of Combs of Bees Present in Each Stock at the Beginning of Winter.

Stock	Combs of Bees in Colonies	Dead Bees Collected	Expected Loss*	Deviation from Expectation
JRC	74.4	714	1454	−740
JRDD	41.7	1476	815	+661
HRDD	62.4	1298	1219	+79
Total	178.5	3488		

*To calculate expected loss, it was assumed that bees would die in proportion to the number of combs of bees for that stock.

TABLE 17.—Number of Colonies and Number of Larvae Showing Signs of Brood Disease in Inspections Conducted About Every 10 Days in the Summers of 1977 and 1978.

			AFB		EFB		Sac		Chalk	
Stock	N*	Diseased	77	78	77	78	77	78	77	78
JRC	6	Colonies	0	0	2	4	1	5	0	2
		Larvae	0	0	13	1649	1	90	0	2
JRDD	4	Colonies	1	2	1	3	1	4	4	4
		Larvae	7	461	2	8	1	20	16	81
HRDD	6	Colonies	0	0	2	1	4	2	6	1
		Larvae	0	0	4	1	13	6	808	16
Total	16	Colonies	1	2	5	8	6	11	10	7
		Larvae	7	461	19	1658	15	116	824	99

*N = number of colonies inspected throughout both seasons.

the different population sizes at the beginning of winter.

Well-known bee diseases: Sixteen colonies were inspected for disease in both 1977 and 1978. The incidence of disease in the same colonies was different in 1978 than in 1977. Table 17 shows the number of colonies in each stock found with diseased larvae each year, and the number of diseased larvae in those colonies. Both AFB and sacbrood increased somewhat in the second year. The spectacular changes were a tremendous increase in EFB and a similar decrease in chalkbrood in the second year. In 1977 a total of 5 colonies showed 19 cells of European foulbrood. In 1978 8 colonies showed 1658 cells of EFB. The heaviest increase occurred in the control stock. Three colonies had more than 100 cells each, and 1 colony had more than 1200 cells. The number of colonies with EFB increased in the JRDD stock but the infection was light. By contrast, chalkbrood decreased from 10 colonies showing 824 dead larvae in 1977 to 7 colonies showing 99 larvae in 1978. The decrease occurred in the HRDD stock, which changed from 6 colonies showing 808 dead larvae to 1 colony showing 16. One or more unidentified aspects of the environment impacting differently upon different colonies would seem to be involved in the apparent decrease of chalkbrood and increase of European foulbrood. These environmental aspects could include weather, pathogens, and drugs (not fed in our apiaries).

Pressure to supersede: Some of the colonies still in the testing program built supersedure cells in 1978. Table 18 shows that all stocks were involved and no stock was clearly worse than another in tendency to supersede in the second year.

Comparison of 1977 Measurements of Bees, Brood, Honey, Pollen, and Longevity with Those of 1978

Graphical comparisons: Eight colonies (four DD's from two stocks and four controls) survived with original queens and provided data over two summer and fall seasons. Figures 10 and 11 show the average performance of the eight colonies throughout each year. From the graphs, two major results are apparent: 1) in 1977, the rate of adult population buildup was much slower than in 1978; 2) in the fall period of 1978, a much greater drop in bee population occurred than in 1977 (44% and 14%, respectively).

Population buildup and longevity: How can the 1977 slow rate of population buildup be explained?

TABLE 18.—Tendency to Build Supersedure Queen Cells in 1978 by Remaining Colonies of the Three Stocks of Bees Obtained in 1977.

	JRC	JRDD	HRDD
No. of Colonies	7	4	6
No. of Colonies with Queen Cells	6	2	5
No. of Colony Inspections	80	48	68
No. of Times Queen Cells Found	12	4	10
No. of Queen Cells	38	19	68*
No. of Colonies which Reared Queens	4	1	5

*26 cells were swarm cells.

One reasonable hypothesis is that the adult bees were dying at a young age so that birth rate only slightly exceeded death rate during the buildup period. By contrast, in 1978 bees were living much longer and birth rate greatly exceeded death rate for a period of time. Support for this hypothesis is found in the results of laboratory tests of longevity. Bees up to 1 day old were collected (from caged brood) at the dates given below. The average time required for 50% mortality in 3 samples of 50 bees from each of the 8 colonies are the following with their standard errors:

1977

First test, Aug. 4: 20.6 days ± 1.3

Second test, Sept. 23: 22.5 days ± 0.7

1978

Single test, June 16: 42.3 days ± 1.3

Why bees should live twice as long in 1978 as in 1977 is not clear. All conditions of the experiment were similar as far as we can judge, except the pollen supply and the time of test—different dates in two different years. Colonies had about twice as much pollen in 1978 as in 1977, and amount of pollen per comb of bees in the early part of the year was greater in 1978 than in 1977. We advance the hypothesis that the difference in rate of

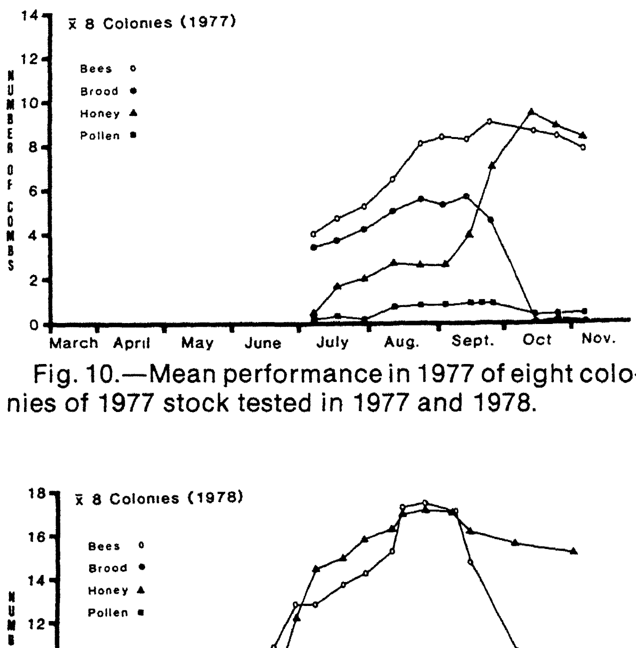

Fig. 10.—Mean performance in 1977 of eight colonies of 1977 stock tested in 1977 and 1978.

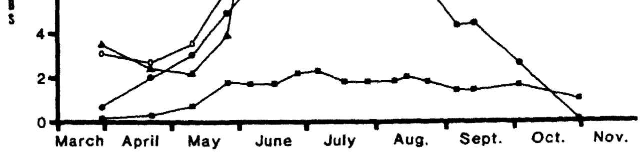

Fig. 11.—Mean performance in 1978 of eight colonies of 1977 stock tested in 1977 and 1978.

buildup is due to a difference in longevity which is caused in part by a difference in pollen availability to the young bees of the colony.

Sudden reduction of adult bee population—midpoint of brood rearing and brood reared in September: The difference between the 2 years in the fall decrease of bee population is explained hypothetically by the following: a different level of brood rearing in the month of September and a different average age of adult bees in the fall period.

If one adds the number of combs of brood found at regular inspections throughout the bees' active season, and divides the cumulated sum of brood by two, the midpoint of brood rearing is obtained. Such a midpoint of brood rearing will most likely fall between two inspection dates. By interpolation, the date of the midpoint may be found. These midpoints are different for different colonies, and presumably the earlier the midpoint, the more old bees in the colony, and the older the average age of the colony's bees.

The midpoint of brood rearing, to the nearest day, for the eight colonies pooled in 1978 was July 11 (range: June 26 to July 23). Their midpoint in 1977 was August 17 (range: August 14 to August 20). Consequently, on Sept. 15 (or any other date in the fall period), the average age of adult bees was greater in 1978 than 1977. Other things being equal, the death rate of old bees is greater than that of young bees. So a decrease of population is expected.

If one adds to an increasing death rate a decreasing "birth" rate (due to a decreasing amount of brood), the population is further decreased. In September 1978, brood rearing averaged about 3.9 combs per colony per inspection. By contrast, it was maintained at a higher level in September 1977, about 5.3 combs. The 1978 brood rearing decreased the birth rate which contributed to population decline, whereas the 1977 brood rearing tended to hold the birth rate constant and to stabilize the population in September and October.

Among those colonies of the stock acquired in 1977 and tested a second time in 1978, none lost all of its bees in the second year. The largest loss (60%) occurred in Colony HDD5 (Fig. 12), which decreased from 20.6 combs of bees to 8.2 combs in a little more than 7 weeks, extending from Sept. 8 to Oct. 30. The fastest drop occurred in Colony JDD21 (Fig. 13), which decreased from 23.7 combs of bees to 12.8 combs (46%) in a period of about 3.5 weeks from Sept. 8 to Oct. 2. Colony JC5, from the control stock, showed a 51% drop over about a 10-week period, which was the largest loss in the control stock (more information in Table 36). None of these colonies died, so whether or not they displayed DD in a mild form remains an open question.

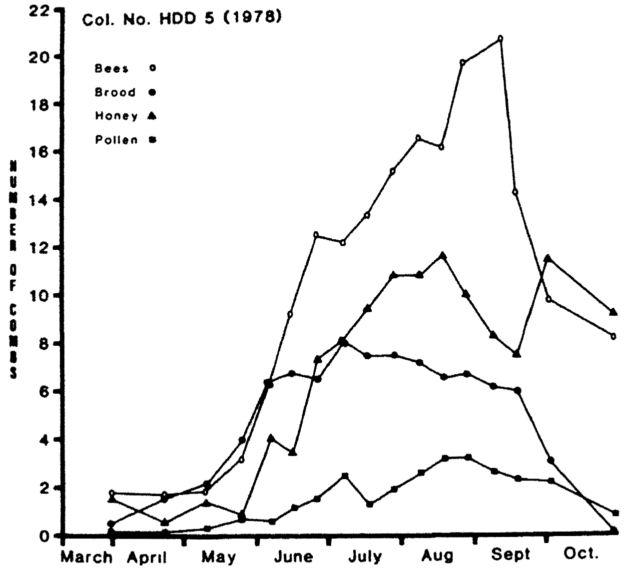

Fig. 12.—Performance in 1978 of Colony HDD5 (1977 Colony HRDD5). Sudden large drop in population.

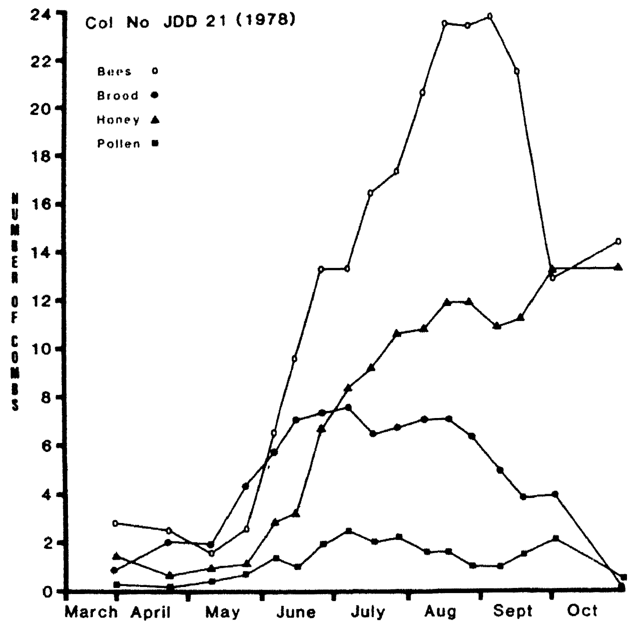

Fig. 13.—Performance in 1978 of Colony JDD21 (1977 Colony JRDD21). Sudden drop in population. Low in honey, high in bees compared with JC8 (Fig. 16).

Differences between two colonies: A striking example of a difference in the performance of two colonies in the same apiary in 1977, and again in the same apiary in 1978, can be seen in their records. In 1977, JRDD21 (Fig. 14) at its peak had 12 combs of bees but never got above 10 combs of honey. JRC8 (Fig. 15) at its population peak had about the same number of combs of bees but got almost 14 combs of honey.

In 1978, the differences between these colonies were repeated and magnified. JRDD21, now renumbered as JDD21 (Fig. 13), had 24 combs of bees and only 13 combs of honey. JRC8, now renumbered as JC8 (Fig. 16), had fewer bees (17 combs) and twice as much honey (30 combs). This is an example of the tremendous variation that can be found among colonies in the same apiary. Since the relative performance of each colony in the

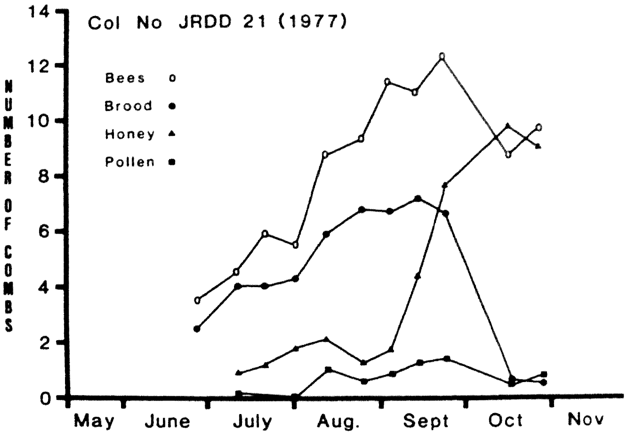

Fig. 14.—Performance in 1977 of Colony JRDD21. Low in honey, high in bees compared with JRC8 (Fig. 15).

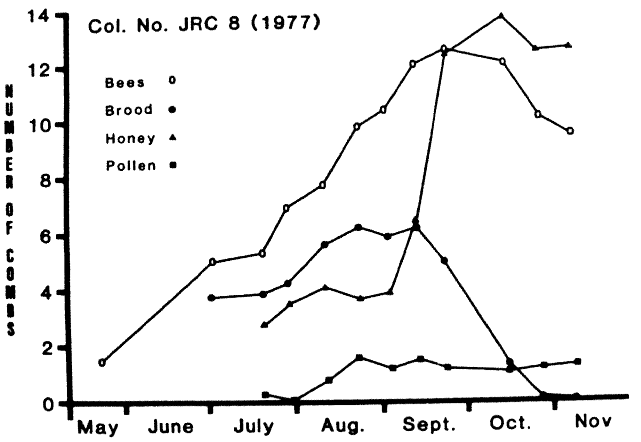

Fig. 15.—Performance in 1977 of Colony JRC8. High in honey, low in bees compared with JRDD21 (Fig. 14).

second year was similar to its performance in the first, a genetic basis of these differences is indicated.

Colonies Developed from Mated Queens Obtained in 1978

Queens were introduced over a 12-day period, beginning May 23, to nucleus colonies which were made as uniform as possible. Inspections of all colonies during the period June 2-9 revealed the following averages and standard deviations in bee populations for each stock:

HRDD1-12	4.8 ± 0.6 combs of bees on June 2
HRC13-24	4.3 ± 0.5 combs of bees on June 3
JRDD11-22	4.1 ± 0.6 combs of bees on June 7
JRC1-10	4.3 ± 0.9 combs of bees on June 9

Although the stocks were not identical in bee population, they differed by less than one comb of bees. Each DD stock and its control differed by a half comb in one case and by 0.2 of a comb in the other. This amount of variation seems acceptable and not likely to confuse results.

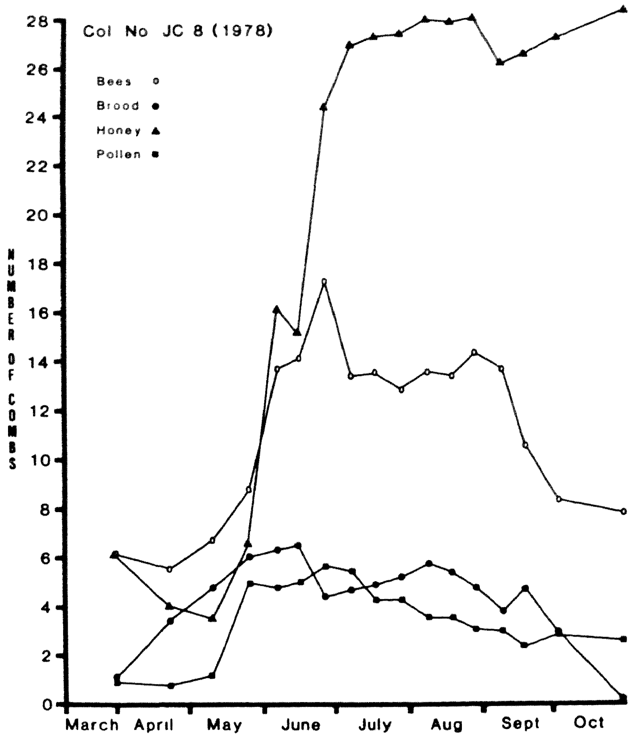

Fig. 16.—Performance in 1978 of Colony JC8 (1977 Colony JRC8). High in honey, low in bees compared with JDD21 (Fig. 13).

Well-known Bee Diseases

A summary of the presence of disease in 1978 is presented in Table 19. Both EFB and sacbrood were present in some colonies of all stocks. Nine of 21 control colonies and 9 of 24 DD colonies showed EFB. Fifteen of 21 control colonies and 12 of 24 DD colonies showed sacbrood. If all diseases are considered, control colonies showed more larvae killed by disease. Nevertheless, it is not suggested that there were any differences among stocks in incidence of these four diseases.

A suggestive fact is that chalkbrood occurred only in the Hard Road apiary—the site of heavy chalkbrood infections in 1977 (Table 1). There is no reason to think that the nuclei to which the queens were introduced in 1978 were previously contaminated by the chalkbrood fungus. Whether the 1978 colonies became contaminated by spores obtained from the locality, from spores transmitted by infected queens (10), or from some other source is an open question.

In general, disease was not as severe in the 1978 stocks as it was in those obtained in 1977. One possibility is that less disease was brought into our test apiaries by the 1978 queens than by the 1977 nuclei.

Pressure to Supersede

A summary of queen-cell construction by the stocks acquired in 1978 is presented in Table 20. About half of the colonies attempted supersedure. Supersedure was

TABLE 19.—Number of Colonies and Number of Larvae Showing Signs of Brood Disease in Inspections Conducted About Every 10 Days in the Summer of 1978.

Stock	N*	Diseased	AFB	EFB	Sac	Chalk
HRC	12	Colonies	2	3	9	7
		Larvae	93	11	68	279
HRDD	12	Colonies	0	5	8	3
		Larvae	0	16	27	4
JRC	9	Colonies	0	6	6	0
		Larvae	0	82†	145	0
JRDD	12	Colonies	1	4	4	0
		Larvae	215	12	12	0

*N = number of colonies in each stock. Stock obtained in 1978.
†One colony had 74 of these EFB-killed larvae.

TABLE 20.—Tendency to Build Supersedure Queen Cells by the Newly Acquired Stocks in 1978.

	HRC	HRDD	JRC	JRDD
No. of Colonies	12	12	9	12
No. of Colonies with Queen Cells	5	6	3	5
No. of Colony Inspections	174	172	98	156
No. of Times Queen Cells Found	19	23	4	8
No. of Queen Cells	31*	30	10	10
No. of Colonies which Reared Queens	2	4	2	1

*One colony had 24 of these cells in four inspections.

prevented in about half of these colonies, but one or more colonies succeeded in their supersedure attempt in every stock.

Analysis of Variation in Quantity of Bees, Brood, Honey, and Pollen

Omitted colonies: The queen in JRC 2 failed to lay eggs and this colony was discarded. Two colonies that superseded early (JRC 6 and JRC 9) were deleted from this analysis.

Variation among stocks: Figure 17 shows the average bee population of the colonies of each stock at each inspection. It appears that the JRC stock may differ from the others, which appear to be similar. Stocks appear similar in amount of brood (Fig. 18) and amount of honey (Fig. 19), but differ in pollen (Fig. 20).

To test the validity of these impressions, a nested analysis of variance was performed. Results of the calculations are given in Table 21, which shows stocks to be different only in pollen hoarded. JRDD was highest, HRC was lowest, and JRC and HRDD were interme-

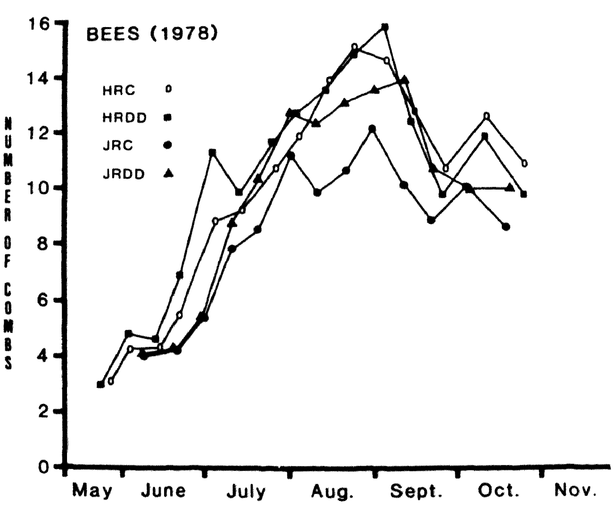

Fig. 17.—Mean number of combs per colony covered by adult bees in each of four stocks, 1978.

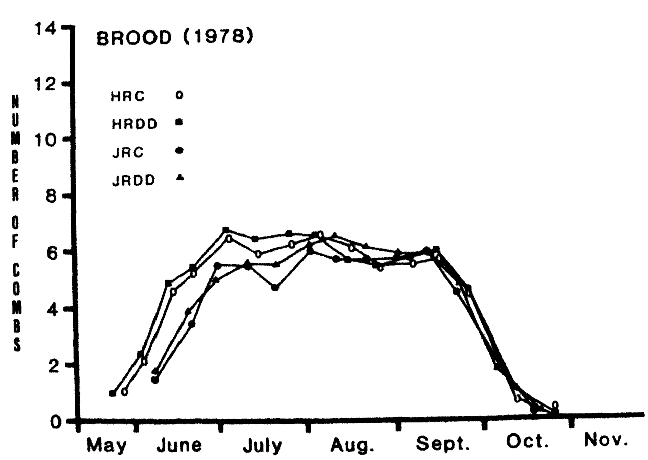

Fig. 18.—Mean number of combs of brood per colony in each of four stocks, 1978.

diate and not different in their mean values (Table 22).

Variation among colonies within each stock: As in the 1977 stocks, the 1978 stocks showed a considerable amount of variation among colonies within each stock. Results of calculating one-way analyses of variance are given in Table 23. Colonies of the HRC stock showed significant variation (at the 1% level) in bees, honey, and pollen. The HRDD stock showed significant variation among colonies only in pollen hoarded. The JRC stock colonies differed significantly in pollen. The JRDD stock colonies differed in combs of bees and combs of

TABLE 21.—Summary of Results of Nested Analysis of Variance for Differences Among 1978 Stocks (JRC, JRDD, HRC, and HRDD) in Combs Covered by Bees and Combs Occupied by Brood, Honey, and Pollen.

	Bees	Brood	Honey	Pollen
F	1.40	0.07	1.67	16.71
df	3, 39	3, 39	3, 39	3, 39
p	0.258	0.974	0.189	0.0001**

**Significant at less than the 1 % level.

TABLE 22.—Duncan's Multiple Range Test at the 5% Level for Differences Among 1978 Stocks in Pollen Hoarded.

Stock	N*	Mean	Grouping†
JRDD	156	1.43	A
JRC	91	1.12	B
HRDD	172	0.998	B
HRC	174	0.782	C

*N = number of colony inspections of the indicated stock.
†Stocks with the same letter are not significantly different.

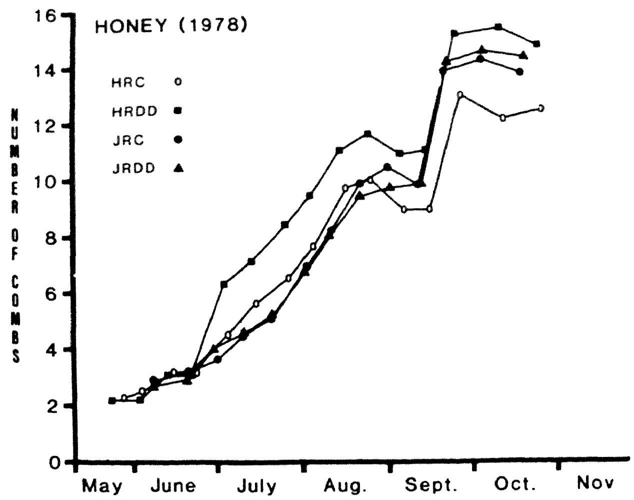

Fig. 19.—Mean number of combs of honey per colony in each of four stocks, 1978.

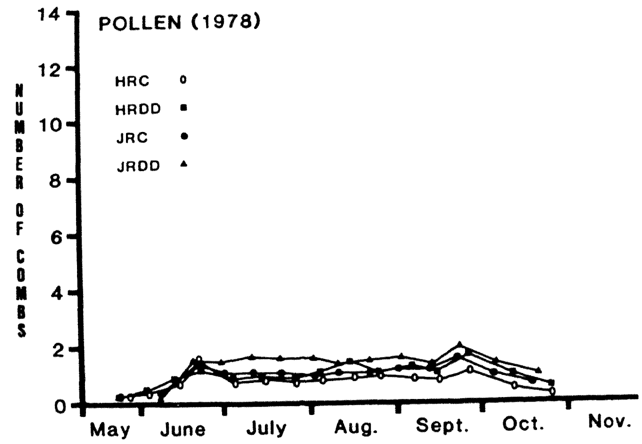

Fig. 20.—Mean number of combs of pollen per colony in each of four stocks, 1978.

TABLE 23.—Summary of Results from One-Way Analyses of Variance of Combs of Bees, Brood, Honey, and Pollen. Testing Was for Differences in Means Among Colonies within Each of the Indicated Stocks. The Mean for Each Colony Was Derived from 11 to 15 Observations as Shown in the Following Tables.

		Stocks			
		HRC	HRDD	JRC	JRDD
Bees	F	2.79	1.63	0.94	1.88
	df	11, 162	11, 160	6, 84	11, 144
	p	0.002**	0.093	0.469	0.046*
Brood	F	1.25	0.92	0.71	1.32
	df	11, 161	11, 160	6, 84	11, 144
	p	0.261	0.527	0.640	0.216
Honey	F	2.66	1.42	1.80	1.57
	df	11, 162	11, 160	6, 84	11, 144
	p	0.0004**	0.170	0.109	0.114
Pollen	F	4.04	2.89	3.24	2.50
	df	11, 162	11, 160	6, 84	11, 144
	p	0.0001**	0.002**	0.006**	0.007**

*Significant at less than the 5 % level.
**Significant at less than the 1 % level.

pollen hoarded. There was about the same amount of variation among colonies of the control and DD stocks.

From the standpoint of the characteristics measured, all stocks showed variation among colonies in pollen hoarded, two of the four in combs covered by bees, one in honey hoarded, and none in amount of brood.

TABLE 24.—Duncan's Multiple Range Test at the 5% Level of Probability for Differences in Bee Population Among Colonies of the 1978 HRC Stock.

Colony	N	Mean	Grouping
21	15	11.92	A
22	15	11.35	A
19	15	11.34	A
24	15	11.05	A
18	15	10.86	A
13	15	9.84	AB
20	15	9.55	AB
16	15	9.53	AB
15	15	9.37	AB
14	12	8.75	ABC
27	15	6.57	BC
23	12	5.81	C

TABLE 25.—Duncan's Multiple Range Test at the 5% Level of Probability for Differences in Honey Hoarded Among Colonies of the 1978 HRC Stock.

Colony	N	Mean	Grouping
22	15	9.49	A
18	15	8.69	AB
19	15	8.57	AB
13	15	8.47	AB
21	15	8.41	AB
24	15	7.79	AB
20	15	7.11	ABC
15	15	6.28	ABC
14	12	5.50	BC
16	15	5.48	BC
17	15	5.43	BC
23	12	4.15	C

TABLE 26.—Duncan's Multiple Range Test at the 5% Level of Probability for Differences in Pollen Hoarded Among Colonies of the 1978 HRC Stock.

Colony	N	Mean	Grouping
13	15	1.15	A
23	12	1.03	AB
20	15	0.97	ABC
18	15	0.89	ABCD
17	15	0.80	BCD
15	15	0.79	BCD
16	15	0.73	BCDE
14	12	0.72	BCDE
24	15	0.69	CDE
19	15	0.59	DE
22	15	0.58	DE
21	15	0.47	E

The means of bees, brood, honey, and pollen for each colony and the groupings of colonies by Duncan's multiple range test are given in Tables 24 to 30.

Sudden reduction of adult bee population: Fig. 17 shows the growth and decline of bee populations in the four stocks. All stocks dropped in population during early September, three gained in late September, and the same three dropped again in October. Individual colonies decreased to a greater or lesser extent. Two of the largest drops occurred in HRC21 (Fig. 21) and HRDD10 (Fig. 22), but the colonies were in no way

TABLE 27.—Duncan's Multiple Range Test at the 5% Level of Probability for Differences in Pollen Hoarded Among Colonies of the 1978 HRDD Stock.

Colony	N	Mean	Grouping
7	15	1.31	A
2	15	1.21	AB
12	15	1.19	AB
3	15	1.16	AB
6	15	1.11	AB
4	15	1.09	AB
10	15	0.92	ABC
11	15	0.87	BC
5	15	0.81	BC
1	11	0.77	BC
9	11	0.76	BC
8	15	0.65	C

TABLE 28.—Duncan's Multiple Range Test at the 5% Level of Probability for Differences in Pollen Hoarded Among Colonies of the 1978 JRC Stock.

Colony	N	Mean	Grouping
3	13	1.42	A
1	13	1.25	AB
8	13	1.22	ABC
5	13	1.21	ABC
7	13	0.96	BC
10	13	0.88	BC
4	13	0.88	C

TABLE 29.—Duncan's Multiple Range Test at the 5% Level of Probability for Differences in Bee Population Among Colonies of the 1978 JRDD Stock.

Colony	N	Mean	Grouping
14	13	11.67	A
13	13	11.64	A
16	13	11.38	AB
20	13	10.97	AB
22	13	10.31	ABC
18	13	10.12	ABC
12	13	10.05	ABC
21	13	9.87	ABC
17	13	9.12	ABC
11	13	8.41	ABC
19	13	8.07	BC
15	13	7.47	C

depleted. The drop in population in these colonies seems to be due to a decrease in brood rearing coupled with an accumulation of old bees due to earlier heavy brood rearing. Every normal queenright colony in this study seems to have undergone a drop in population in the fall period.

Colony HRC17 (Fig. 23) suffered a devastating loss of bees, going from 9.9 combs of bees to 1.9 combs for an 81% loss. The colony was dead on Dec. 1. Bees could be seen dying in this colony in late October, and an examination of the bees by Truman Clark revealed the presence of the honey-bee filamentous virus. Presence of dying bees is supposedly not characteristic of disappearing disease, but certainly this colony was destroyed and a virus was found in the bees. Some bee loss by beekeepers may be due to infection by this pathogen.

Wintering 1978-79

At the end of the active 1978 season, 36 of the original 46 colonies had not superseded or failed in some other way and were prepared for overwintering. Sizes of the colony population going into and coming out of winter are given in Table 31. Considered from the standpoint of percentage of bees (not colonies) surviving, the JRC stock was clearly poorest, the JRDD and HRC stocks were perhaps not different, whereas the HRDD stock was obviously best. This consideration, however, ignores the lack of uniformity at the start of winter. To take into account the variation in number of combs of bees at the start of winter, an analysis of covariance was done on the wintering results (Table 32). Highly significant differences among stocks were found. A least significant difference test specified which stocks were different (Table 33). The mean of each of the four stocks was adjusted to allow for the different numbers of bees in 1978 at the start of winter. The table shows that the

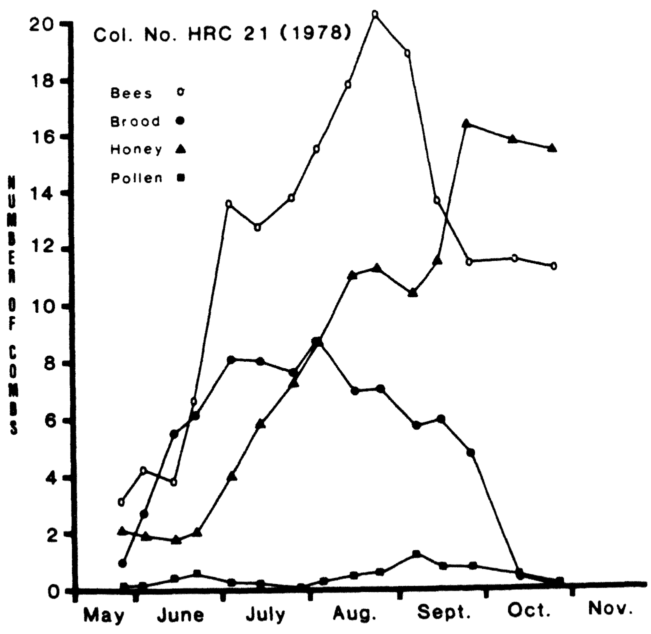

Fig. 21.—Colony HRC21, 1978. Decrease in population, colony survived.

TABLE 30.—Duncan's Multiple Range Test at the 5% Level of Probability for Differences in Pollen Hoarded Among Colonies of the 1978 JRDD Stock.

Colony	N	Mean	Grouping
15	13	1.90	A
12	13	1.72	AB
20	13	1.60	AB
16	13	1.59	AB
13	13	1.48	AB
21	13	1.38	AB
22	13	1.38	AB
11	13	1.36	ABC
19	13	1.33	BC
18	13	1.28	BC
17	13	1.25	BC
14	13	0.84	C

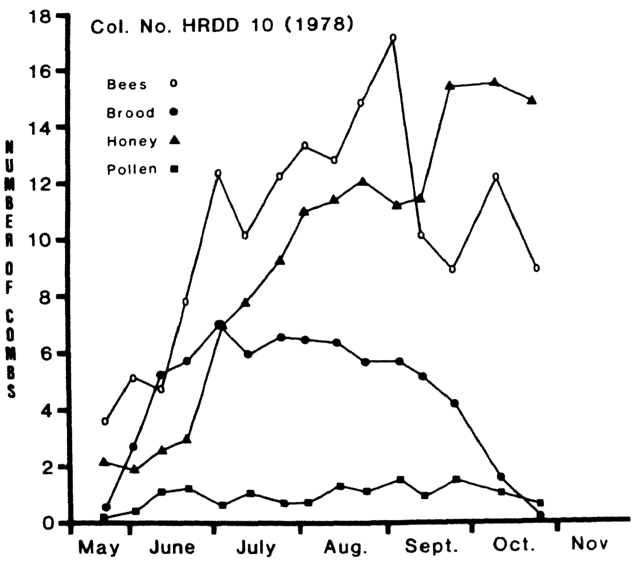

Fig. 22.—Colony HRDD10, 1978. Decrease in population, colony survived.

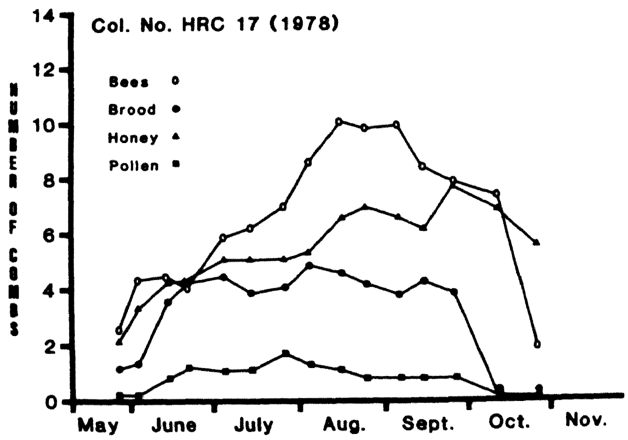

Fig. 23.—Colony HRC17, 1978. Decrease in population, colony dead by Dec. 1.

TABLE 31.—Number of Combs Covered by Bees at Beginning and End of Winter with Percent Surviving in Several Colonies of Each of Four Different 1978 Stocks.

HRDD Stock				HRC Stock			
Colony	1978 10-25	1979 3-22	Percent Survival	Colony	1978 10-25	1979 3-22	Percent Survival
2	8.5	4 2	49	13	12.1	6.1	50
3	8.4	5.5	65	15	11.6	2.6	22
4	7.9	3.7	47	16	10.9	3.5	32
5	12.7	10.0	79	18	9.9	4.3	43
7	9 9	6.5	66	19	11.5	5.4	47
8	9.6	4.6	48	20	10.0	3.5	35
10	8.9	4.0	45	21	11.3	4.0	35
11	11.5	6.4	56	22	11.0	8.3	75
12	12.4	6.8	55	24	11.3	7.1	63
Mean	10.0	5.7	56.7	Mean	11.1	5.0	44.7

JRDD Stock				JRC Stock			
Colony	1978 10-20	1979 3-21	Percent Survival	Colony	1978 10-18	1979 3-23	Percent Survival
11	8.9	1.9	21	1	8.3	0.0	0
12	11.0	5.2	47	3	7.7	2.5	32
13	11.6	6.0	52	4	8.1	2.2	27
14	12.9	5.1	40	5	5.8	0.4	7
16	11.9	10.0	84	7	11.1	3.1	28
17	7.1	2.7	38	8	9.8	2.7	28
18	9.8	3.8	39	10	9.6	1.4	15
19	7.2	0.9	12	Mean	8.6	1.8	22.4
20	12.0	4.8	40				
21	9.6	3.5	36				
22	10.6	3.4	32				
Mean	10.2	4.3	40.1				

TABLE 32.—Analysis of Covariance of Wintering Data.

Source	Sum of Squares	df	Mean Square	F	P
Total	194.90	35			
Stock	33.06*	3	11.02	4.85	0.0070**
No. in 78	56.29*	1	56.29	24.75	0.0001**
Error	70.49	31	2.27		

*For **stock** and **No. in 78**, the sum of squares is the partial sum of squares. $R^2 = 0.64$.
**Significant at less than the 1 % level.

TABLE 33.—Results of a Least Significant Difference Test of Wintering Ability of Four Stocks of Bees.

Stock	N	Least Squares Mean and SE*	Grouping†
HRDD	9	5.82 ± 0.50	A
HRC	9	4.16 ± 0.53	B
JRDD	11	4.16 ± 0.46	B
JRC	7	2.93 ± 0.62	B

*Approximate LSD at 0.05 level with 31 df equals 1.50.
†Stocks with the same letter are not significantly different.

TABLE 34.—Number of Colonies and Number of Larvae Showing Signs of Brood Disease in Inspections Conducted About Every 10 Days During Summer of 1978.

Stock	N*	Diseased	AFB	EFB	Sac	Chalk
HRNDD	7	Colonies	0	3	5	6
		Larvae	0	21	27	110

*N equals number of colonies inspected.

HRDD stock wintered best, and that the other stocks were similar to each other at the 5% level. At the 10% level, the JRC stock differed also from the HRC and JRDD stocks.

Nucleus Colonies Obtained in 1978

Studies of seven nucleus colonies obtained from Florida in 1978 are pertinent. In Florida they had not increased in bee population over a period of several weeks, according to the owner, but in Ohio bee populations increased steadily as shown in Fig. 24. Upon their arrival, only one of the seven had as much as 0.1 of a comb of pollen. The remainder had a few cells or none. A pollen shortage, deficiency in pollen quality, and/or some other environmental factor were hindering colony buildup in Florida. Since the same mated queens were heading these colonies in both Florida and Ohio, a genetic basis cannot be invoked to explain the different responses in the two locations.

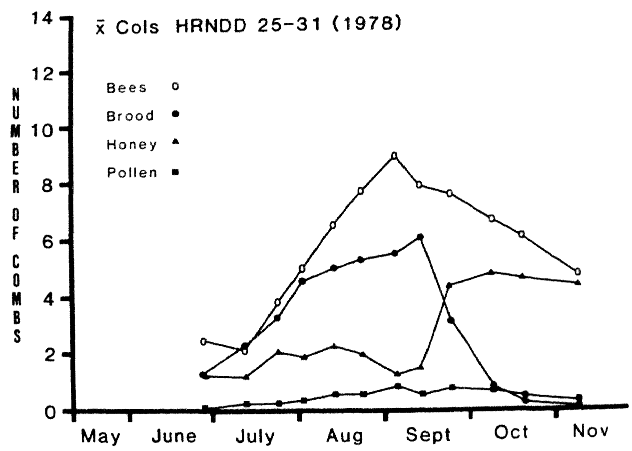

Fig. 24.—Mean performance of seven colonies obtained as nuclei in 1978.

Well-known Bee Diseases

Table 34 shows that three colonies had a few cells of EFB and five colonies had a few cells of sacbrood. Six of the seven colonies had chalkbrood, but only one colony was heavily infected. This one had 93 of the 110 chalkbrood mummies found in 12 inspections of these colonies. Compared with the 1978 HRDD colonies (queens from Florida) these nuclei had somewhat more disease.

Pressure to Supersede

Only one queen cell was built during the observations on these seven colonies.

Sudden Reduction of Adult Bee Population

Fig. 24 shows that the average fall loss of adult bees in the seven colonies was about 46%. Considerable variation existed in the group since the range was 27.6 to 66.7%.

In the analysis of the data from the eight colonies tested in both 1977 and 1978, it was suggested that population loss in the fall period was related to the average age of the colony's bees in the fall period and to the amount of brood reared in September. Table 35 presents the data on brood rearing and population loss in the seven colonies to see whether or not there was any relationship. Neither midpoint of brood rearing nor amount of brood in September were significantly correlated with percent loss of population in the fall. Nevertheless, if colonies are arranged according to percent loss, two midpoint groups may be identified—one earlier and one later. The later group of three colonies had, on the average, slightly more brood in September than the earlier group of four. It is possible that the higher losses in this group of seven colonies occurred in colonies with older bees, but we are unable to draw that conclusion from the small differences and limited data.

TABLE 35.—Patterns of Brood Rearing in the Summer and in September, and Percent of Adult Bee Population Lost in the Fall Period by the Seven Nucleus Colonies from Florida in 1978.

Colony Number	Percent Loss in Fall	Midpoint of Brood Rearing to Nearest Day	Total Brood Found in Three Inspections in September
29	67	Aug. 19	14.0
25	54	Aug. 18	15.9
26	52	Aug. 14	13.9
27	44	Aug. 16	14.5
28	39	Aug. 19	14.9
31	37	Aug. 20	15.0
30	28	Aug. 20	14.7

Colony HRNDD29 displayed what was perhaps the closest approach to the classical disappearing phenomenon (Fig. 25). The population dropped steadily from about nine combs of bees to about three in 2 months' time. No dead bees were seen in the early 5 or 6 weeks of the disappearance, and only a few after that period. Bees from this colony were sent in mid-September to Truman Clark and were examined by him for pathogens, including the honey-bee filamentous virus. No pathogens were found in either blood or guts. Even though

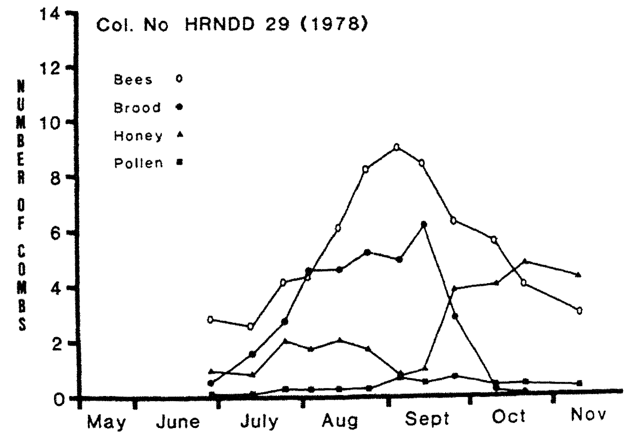

Fig. 25.—Colony HRNDD29, 1978. Decrease in population, colony dead in early December.

no virus was found, we cannot be certain that it was absent. This examination was perhaps too early in the demise of the colony to reveal dependably the virus in a small sample of bees. Unfortunately, bees for a later examination were not sent. The colony died in early December.

A Chance Observation

In the fall of 1980 we found some very weak colonies in place of the moderate-sized colonies seen earlier. Upon complete examination, these colonies were found to be honey bound. Brood rearing was stopped much too soon because of lack of space for egg deposition. Since the average population size was 1.5 combs covered by bees, and the largest was 4 combs, no colony of this group could be wintered.

DISCUSSION AND CONCLUSIONS

Variation in North American Stocks of Bees

There seems to be a strong feeling among some beekeepers that DD is due to some hereditary deterioration. The same idea has been expressed by some scientists (24). Our investigations were designed to reveal such a deterioration of certain stocks of bees if it does indeed exist.

Little Evidence of Differences Among Stocks

In 1977, two stocks from beekeepers who reported having DD and one stock from a beekeeper who had not experienced such a phenomenon were brought together for testing. No stock was found in a summer and fall of systematic observation to be statistically different from any other in combs of bees, brood, or pollen. In combs of honey (Table 4), the JRC stock exceeded the JRDD stock (at the 5% level of probability), but did not differ from the HRDD stock. Among the two DD stocks and two control stocks tested in 1978 (Table 21), a difference was found only in amount of pollen stored. The JRDD stock stored more pollen than any other, whereas the HRC stock stored less. JRC and HRDD stocks were similar to each other, intermediate to and different from the other two.

We conclude that no stock of the three in 1977 and none of the four in 1978 is substantially different from any other with which it was compared.

Much Evidence of Differences Among Colonies within Stocks

Even though there is a paucity of evidence for differences among stocks, is there any evidence of differences among colonies within stocks? Every one of the seven stocks had significant differences (at the 5% level or less) among colonies in amount of pollen (Tables 5 and 23). The same is true of combs of bees with the exception of the 1978 HRDD and JRC stocks. Amount of brood was about the same among the colonies of each stock. Three of the seven stocks showed colonies to be significantly different in honey stored. From this evidence, one can conclude that none of these so-called stocks were really genetic stocks showing genetic homogeneity among their colonies. The 1978 HRDD stock came closest, since no significant differences were found among its colonies for bees, brood, and honey. Only number of combs of pollen differed among HRDD colonies. JRC colonies also differed only in pollen, but the number of colonies tested was small.

The tremendous difference in honey production by two colonies in the same apiary described earlier (page 10) merits emphasis. In 1977, the two colonies reached about the same population peak. One colony (JRDD21) stored 10 combs of honey, the other colony (JRC8) stored 14 combs. In 1978, the same two colonies were tested again. As in the previous year, both were located in the same apiary. The *stronger* one stored 13 combs of honey, whereas the *weaker* colony of the 2 stored 30 combs. The 1978 repetition of the 1977 results argues for a genetic basis of this difference in honey hoarding.

A great deal of the phenotypic variation among colonies in this study seems likely to be the result of genetic variation. These results suggest that there is ample genetic variation in North American bees to insure the success of a breeding program. There is no evidence in this study of a necessity to import stock for breeding purposes. Tucker (22) has expressed a similar opinion based on his experience.

Disappearing Disease

Was DD Seen?

The question remains: Were any cases of DD seen in this investigation? Since one of the most obvious characteristics of DD is a drop in population size, the data were examined for population decreases. Every stock showed a decrease in colony population in the fall period (Figs. 6, 17, 24). It is the natural consequence of old bees dying at a faster rate than young bees are being "born". This is one kind of disappearing phenomenon, but it normally stops short of destroying the colony. Some 20, 30, 40, or even 50% of bees in a colony may be lost. If a population of sufficient size is left, the colony will survive the winter.

Table 36 lists those colonies which lost 50% or more of their adult bees by other than queenlessness or supersedure. Out of some 70 colonies tested (8 tested twice),

TABLE 36.—List of Cases (1977 and 1978) in which 50% or More of the Adult Bees of a Colony Were Lost. No Cases of Population Loss Due to Queenlessness or Supersedure Are Included.

Stock and Year Obtained		Year Tested	Colony Number	Percent Lost	Combs of Bees Left	Figure	Disposal
DD	Kansas 1977	1977	JRDD14	50	1.6	5A	Too weak to winter
DD	Kansas 1977	1977	JRDD15	56	4.5	5	Dead in winter
C	Colorado 1977	1977	JRC11	75	0.5	2	Died in September
C	Colorado 1977	1978	JC5	51	7.5	—	Wintered*
DD	Texas 1977	1978	HDD5	60	8.2	12	Wintered
C	Wisconsin 1978	1978	HRC17	81	1.9	23	Dead Dec. 1
DD	Florida 1978	1978	HRDD10	51	8.3	—	Wintered
DD	Florida 1978	1978	HRNDD25	54	4.5	—	Wintered
DD	Florida 1978	1978	HRNDD26	52	4.2	—	Wintered
DD	Florida 1978	1978	HRNDD29	67	3.0	25	Dead in Dec.

*Wintered means the colony survived the winter season.

only 10 colonies lost as much as 50% in the summer or fall period. Losses of 50% or more occurred in both DD and control stocks. Five of the 10 colonies survived the following winter and are listed in the table as *wintered.* We have no evidence that their population decline was due to more than a high death rate among old bees and a declining birth rate in the fall period.

Among the five colonies which did not survive, two failed to build a normal population and eventually suffered a population decline of 50% or more. One of these colonies, JRDD14 (Fig. 5A), was too weak to winter (1.6 combs of bees) and was disposed of. The other colony, JRC11 (Fig. 2), was afflicted with several diseases and died in September 1977. The other three colonies built up normally, but suffered abnormally high fall losses and, as a consequence, died over winter. HRC17 (81% loss, Fig. 23) was infected with the filamentous virus (6). Conceivably this virus could affect a whole apiary or even a region. Colony JRDD15 (1977, 56% loss, Fig. 5) and colony HRNDD29 (1978, 67% loss, Fig. 25) lost their populations for unknown reasons. Since most of the bees simply disappeared, these were designated as two cases of DD. It is suggested further that the disappearing phenomenon occurred to a greater or lesser extent in all other colonies.

Environmental Factors Responsible for DD

That DD was dependent *primarily* upon environmental and not genetic factors in our apiaries is suggested by the data from eight colonies which performed differently in the 2 years of observations (Figs. 10 and 11). We postulated that the more rapid buildup in 1978 was due to greater longevity in that year, and that the greater population decline in 1978 was due to the presence of more old bees in September 1978 than were present in September 1977. Longevity itself may have been dependent upon nutrition (13). Among some colonies which died, pathogenic microorganisms were present. Whether or not these factors (which we have invoked as explanations) can influence population levels is subject to experimental testing.

Factors Affecting Birth and Death Rates

Birth and death rates are constantly changing in the bee colony, and a theoretical consideration of some of the factors which may be involved in specific cases should be useful. Some which affect birth rate are: oviposition rate, number of nurse bees, food supply, and brood diseases. Rate of oviposition is affected by the quality of the queen, availability of comb space for eggs, and genotype of the queen (5). If the number of nurse bees is low, or if nurse bees are attracted to processing nectar, birth rate is adversely affected. Both honey and pollen are necessary for brood rearing. The absence of sufficient pollen is not always detected, and the effect of such a deficiency lowers birth rate and raises death rate. Quality of the protein food source is a more subtle factor. Most pollen substitutes fail to support brood rearing as well as pollen supports it, and low quality pollens fall short of better pollens (9). Presence of American foulbrood, European foulbrood, and chalkbrood can reduce birth rate drastically.

Death rate is increased by factors which shorten life and decreased by factors which lengthen life. Factors which affect length of life include: adult diseases, food, genotype, and workload (foraging, processing nectar, and brood rearing). Paralysis and nosema certainly shorten life. Food eaten during larval life affects longevity. Eischen (7) showed that more nurse bees per larva led to longer life of the resulting adult bees. Kulincevic, Rothenbuhler, and Rinderer (17) found the same, and found also that diets (different protein sources) during larval life affected adult longevity. Kulincevic and Rothenbuhler (unpublished) found that pollen-fed bees in laboratory cages lived about twice as long as non-pollen-fed bees. Genetic differences affect longevity (15). The amount of work the bee does would affect length of life. Foraging is hazardous. In our experience, bees prevented from foraging, by confinement to a flight cage, lived much longer than those allowed free flight. Processing nectar and rearing brood take their toll in life expectancy.

When all of these factors come into play, a system of great complexity is observed. If several of these factors work on population in a negative direction, or if one factor works strongly, disappearing disease is the result. The beekeeper's task is to be aware of the possibilities and prevent the unwanted development.

Speculations on Causes of Some Early Cases of DD

Australia, over many years, has experienced severe "disappearing trouble". It appears when they have a winter nectar flow from two species of *Eucalyptus* and no pollen flow. Death rate goes up because of an accumulation of old bees and poor nutrition, while birth rate goes down because of lack of pollen and perhaps lack of egg laying space (3, 4, 12).

In the "Disease of 1868" (1), whole apiaries were lost. "The hives were left, in most cases, full of honey, but with no brood and little pollen." Again it seems brood rearing for some reason was terminated too early.

About 40% of colonies were lost in Iowa in the winter of 1871-72 (2, quoting E. S. Tupper). "The dry weather of last season checked brood rearing early, . . . Then the honey harvest through the autumn was unusually good, and the bees gathered it late." Again, no late brood rearing (for one or more reasons), old bees, and a heavy flow increased death rate and decreased birth rate.

Speculations on Causes of DD in Apiaries Where Stocks Were Obtained

The fact remains that four beekeepers from whom we obtained our DD stocks reported having DD, and three beekeepers from whom we obtained control stocks had no DD. In our possession, DD stocks did not differ from control stocks with respect to DD. Consequently, we have *no evidence* that the differences in incidence of DD in the beekeepers' apiaries were due to genetic differences. On the contrary, we believe that the differences between DD and control stocks observed by beekeepers were due primarily to differences in the environments. These would include food availability, pathogens, management, and a host of other environmental elements. Furthermore, the DD reported by one beekeeper may differ greatly from that reported by another, and may be due to entirely different causes. In view of the situation, Shimanuki (21) has suggested that we drop the name disappearing disease and, until more information is available, use instead spring, fall, or winter dwindling syndrome.

The beekeeper who provided the 1978 HRDD and the HRNDD stocks had a number of factors affecting birth and death rates in his apiaries. Samples of bees from 17 weak colonies in his apiaries were examined by Truman Clark. Ten colonies showed nosema and 1 of 17 showed the filamentous virus. Another study (16) has suggested that pollen was inadequate, and feeding old soybean flour, which the beekeeper did, was detrimental. Although the beekeeper was discarding queens out of the conviction that they were of poor quality, genetically or otherwise, the queens heading weak colonies in Florida performed very satisfactorily when transferred to our apiaries. The cause of DD in this case seems to be primarily poor nutrition, which led to a low birth rate by inhibiting brood rearing and a high death rate by shortening adult bee life. In a few colonies, adult diseases may also have increased death rate by shortening adult bee life.

The primary cause of the failure of colonies to build up in the apiaries from which the 1977 HRDD stock came seems to be disease. EFB, sacbrood, and chalkbrood were seen. Some colonies showed all three. All 12 nuclei that we received showed chalkbrood (Table 1). Furthermore, adult bees were sluggish and showed symptoms of hairless-black syndrome. Brood was abandoned in some colonies. Birth rate was low and death rate high—a situation in which colonies cannot build up.

The cause of the DD in the case of the other two stocks is unclear. The 1978 JRDD stock suffered fall depopulation. It may have been an exaggeration of the normal fall loss due to an older than usual average bee age and too-early cessation of brood rearing, which in turn can be caused by a variety of factors. The 1977 JRDD stock in our possession showed a considerable amount of disease and attempts to supersede were frequent (Table 2). Since the beekeeper did not know whether or not colonies in his apiaries had pollen during the time of loss, one cannot make an informed guess as to the cause of colony loss.

From our data we can offer no support for the genetic hypothesis of DD.

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