[Proceedings of the Iowa Academy of Science](https://scholarworks.uni.edu/pias)

Volume 77 | Annual Issue Article 32

1970

Some Factors Affecting the Nest-Cleaning Behavior of Honey Bees (Apis mellifera)

Harold A. Borchers
Bermidji State College

Recommended Citation

Borchers, Harold A. (1970) "Some Factors Affecting the Nest-Cleaning Behavior of Honey Bees (Apis mellifera)," *Proceedings of the Iowa Academy of Science, 77(1),* 210-218.
Available at: https://scholarworks.uni.edu/pias/vol77/iss1/32

Some Factors Affecting The Nest-Cleaning Behavior of Honey Bees *(Apis mellifera)*[1]

HAROLD A. BORCHERS[2]

Abstract. Glass-paned observation hives were used to study the behavior of nestcleaning in American Foulbrood-resistant honey bees. Transplanted-dead brood in comb inserts was used to determine the rate of removal of dead brood.

Dead brood located within the brood nest was removed faster than dead brood located in other areas. Strong nectar flow appeared to exert a positive influence on the rate of removal of dead brood. Comparisons made between the responses of honey bees toward brood killed by American Fouldbrood and brood killed by hydrogen cyanide showed inconsistencies that indicate the possibility of unknown environmental factors.

Nest-cleaning behavior is of great importance to those animals which construct nests or use natural shelters for the rearing of young in repeated cycles. An accumulation of organic material in the form of feces or dead young would preclude the use of the nest by reducing the amount of space available. If the young die from a disease the nest would build up a continuing source of infection.

The honey bee, *Apis mellifera,* is a social insect which constructs a nest of wax cells for the rearing of brood and the storage of food. The brood is reared in a part of the hive called the brood nest. An important activity of adult bees is to to clean these cells after new adult bees emerge. If an egg fails to hatch, or a larva or pupa dies in its cell, a special sanitation problem is presented. When a colony is infected with *Bacillus larvae,* the causative organism of American foulbrood (AFB), dead larvae and pupae may accumulate in large numbers. For many years it was thought that colonies infected with AFB could not recover from the disease. Furthermore, it was assumed that bees would not remove AFB-killed larvae or pupae from brood cells even though individuals dead of other causes were observed not to accumulate in appreciable numbers.

Park (1936, 1937) tested colonies of bees that had been diseased and had later recovered. He established that AFB-killed larvae and

[1] This investigation was conducted at the Iowa State University Apicultural Laboratory during the summer and fall of 1962 under research grant MH 03882-03 from the National Institute of Mental Health, Public Health Service, made to Iowa State University, and at The Ohio State University Bee Laboratory during the summer of 1963 under research grant MH 07927-0- National Institute of Mental Health, Public Health Service, made to The Ohio State University.

[2] Associate Professor of Biology, Bemidji State College, Bemidji, Minnesota 56601.

pupae were often removed from brood combs. He suggested that the resistance of certain lines of bees to AFB was due at least in part to the nest-cleaning behavior of the adults.

Woodrow and Holst (1942) and Rothenbuhler (1964) found resistant colonies removed AFB-killed brood much faster than susceptible colonies. Jones and Rothenbuhler (1964) showed that resistant and susceptible lines of bees removed brood killed by hydrogen cyanide gas.

Thompson, (1964), working with AFB-killed brood, obtained varied responses toward the removal of dead brood. This variation seemed to be correlated with the presence or absence of a nectar flow.

Although nest-cleaning behavior is known to be inheritable, several environmental factors are thought to affect the behavior of nest-cleaning. Jones and Rothenbuhler (1964) suggested that position of dead brood in the hives may affect the rate of dead-brood removal.

Testing of nest-cleaning behavior of colonies is considerably easier with HCN-killed brood (Jones and Rothenbuhler, 1964). The use of HCN-killed brood eliminates the lengthy, involved procedure needed to obtain test material. Furthermore the danger of contamination to equipment and colonies is also avoided. Although the response of resistant bees to HCN-killed brood appears to be similar to the response toward AFB-killed brood, the question arises as to whether or not AFB-resistant honey bees respond in the same way to AFB-killed brood as they do to HCN-killed brood.

The purpose of this investigation was threefold:

1. To determine what effect location of dead brood within the colony in relation to the brood nest has on the nest-cleaning behavior of honey bees.
2. To determine the effect of simulated nectar flow on the removal of dead brood during a transition from a moderate nectar flow to a dearth condition.
3. To compare the response of bees to AFB-killed brood and brood killed by HCN.

Materials and Methods

Four glass-paned observation hives were used to study the behavior of nest-cleaning. Each hive consisted of two stories; the lower story contained the brood nest, the upper story contained the honey stores. The four observation hives were located inside a shelter equipped with a thermostatically controlled heater and fan to maintain a constant temperature of about 85°F. Bees were allowed to move freely in and out of the hives through entranceways. Each entranceway was painted with different colored patterns to aid bees in orientation (von Frisch, 1950).

The Brown resistant line of honey bees was used for all investigations reported here. The Brown line was developed from four queens obtained from Mr. Edward G. Brown, Sioux City, Iowa, in 1954 (Rothenbuhler and Thompson, 1956). Each observation hive contained adult Brown worker bees with Brown brood of all ages in the lower story. A commercial hybrid queen headed each colony and was confined to the lower story by a queen excluder. The colonies were so manipulated every 19 days that new Brown brood of all ages from the parent colony replaced the commercial hybrid brood before it emerged. Thus it was possible to maintain four colonies which were headed by commercial hybrid queens but contained adult Brown workers from a common origin.

Two types of test combs (referred to as inoculation-comb inserts) were used. Squares containing only larvae or pupae killed by HCN were used in some experiments, whereas some experiments utilized HCN- and AFB-killed brood combined in the same square.

To obtain inoculation squares containing HCN-killed brood, an entire brood comb containing sealed brood from a commercial hybrid line was exposed to an environment of Cyanogas for about 12 hours. After aerating the combs, sections of approximately 2 inches square were then cut from the sealed brood comb. Square sections were cut from the desired areas of the combs in the test colonies and replaced with the inoculation-comb insert.

Inoculation-comb inserts containing both HCN- and AFB-killed brood in each square were obtained in a manner similar to that used by Schulz-Langner (1960).

Sealed brood comb drawn on Dadant and Sons plastic foundation was cut into sections 2 inches square. Each section of comb was separated from the plastic foundation on each side with a sharp knife. Every other row on each side was opened at the base and the pupae removed. American foulbrood material (the remains of larvae or pupae killed by *Bacillus larvae*) was placed into each of these empty cells with a microspatula. The two halves of the inoculation square were then placed together with the plastic midrib in place and sealed at the edges with melted beeswax. An inoculation square of this type contained about 50 cells with AFB material and 50 cells with HCN-killed pupae. These composite inoculation squares were then placed into combs of the test colonies.

The effect of varied nectar flow on nest-cleaning behavior was tested by feeding simulated nectar (60% sugar-in-water solution) to some test colonies and not to others. To facilitate feeding, yet discourage robbing by bees from other colonies, the feeders were attached to the bottom of the fed colonies at the opposite end of the entranceway.

Observations were made at intervals on each day during the experiments. The condition of cells in the inoculation square was

Proceedings of the Iowa Academy of Science, Vol. 77 [1970], No. 1, Art. 32

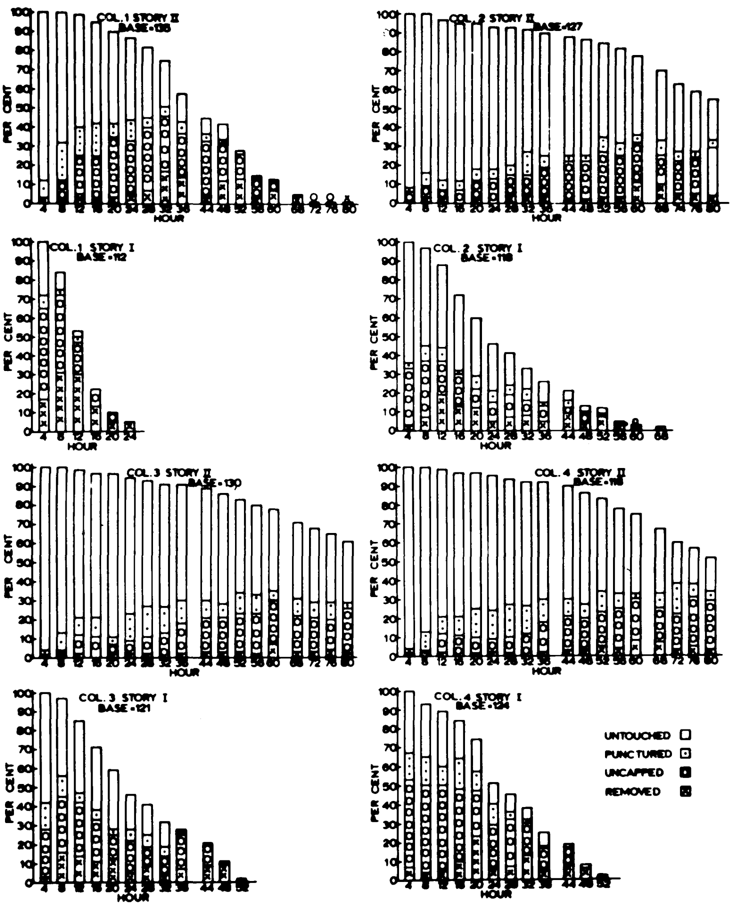

Figure 1. Nest-cleaning response of Brown honey bees to HCN-killed brood in upper and lower stories of four test colonies.

recorded during each observation period. The condition of cells was designated as untouched, punctured, uncapped, or empty. Any cell having more than one-half of the cap removed was considered uncapped. A cell having a hole less than half the area of the cap was considered to be punctured. Cells having caps with no holes were considered untouched. No larva or pupa was considered removed until the cell was completely empty.

The per cent untouched, punctured, uncapped, and emptied cells at each observation time was plotted or bar graphs as in Figure 1. The base counts of brood are shown on the graphs so that raw data may be computed by multiplying the base times the percentage for a given attribute at any observation time. Chi-square computations were made using the adjusted chi-square formula for small sample sizes (Snedecor, 1946). Responses toward two treatments were compared at the time at which the fastest response had at least 75% of the dead brood removed. The .05 level of confidence was used as the level of significance.

Investigations, Results, and Analyses

I. *The Effects of Location of Dead Brood in the Colony on the Nest-cleaning Behavior of Honey Bees.*

Eight experiments were conducted to test the hypothesis that dead brood located within the brood nest would be uncapped and removed faster than dead brood in the honey stores of the upper story. In each of the experiments inoculation squares containing approximately 100 dead brood were placed into the center of the honey stores and brood nest of each test colony. The response of one of these experiments is shown in Figure 1 where it can be seen that in the colonies tested, dead brood was removed faster from the brood nest than from the honey stores.

The Chi-square values in Table 1 indicate significant differences in the rate of removal of dead brood from the brood nest vs. the honey stores of all test colonies.

Table 1

Chi-square values for the comparison of HCN-killed brood removed from brood nest vs. honey stores (see Figure 1).

Test colony	d.f.	χ^2
Colony 1	1	123.80*
Colony 2	1	102.07*
Colony 3	1	130.59*
Colony 4	1	111.67*

* Significance at .05 level of confidence.

The results of all eight experiments showed that dead brood located in the brood nest was removed faster than dead brood located in the honey stores. In each experiment the Chi-square comparisons indicated significant differences between the rate of removal of dead brood from the brood nest vs. the honey stores.

II. *The Effect of Simulated Nectar Flow on the Removal of Dead Brood From the Brood Nest.*

Three experiments were conducted during the late summer and fall of 1962 to test the hypothesis that dead brood would be removed more rapidly from colonies having a simulated nectar flow than in colonies having a slight flow or none at all. The first experiment was conducted during a moderate nectar flow in early August. The second experiment was conducted in late September

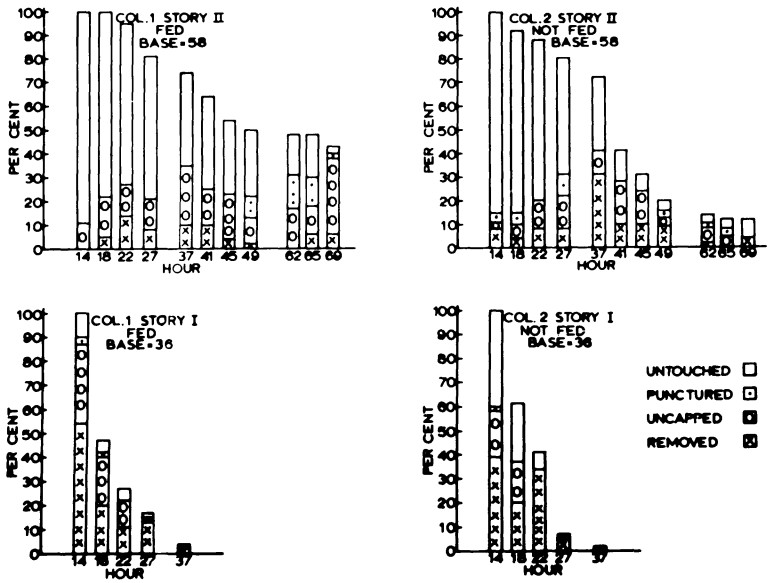

Figure 2. Nest-cleaning response of Brown honey bees to HCN-killed brood in fed and non-fed colonies during a moderate nectar flow.

when there was little or no nectar flow. The third experiment was conducted in mid-October when there was a complete dearth of nectar. In each of the three experiments two colonies were fed sugar sirup and two colonies were not fed.

Figures 2 and 3 show the graphical results of the first and last experiment of this series. An examination of the graphs in Figure 2 shows that there are no consistent differences in the speed of dead-brood removal between the fed colonies and the non-fed colonies during a moderate nectar flow. Figure 3 shows the striking difference in the response of fed colonies vs. those not fed during a complete dearth of nectar. The Chi-square values in Table 2 indicate a significant difference in the rate of removal of dead brood from fed colonies vs. non-fed colonies during the October 1962 dearth. The second experiment of this series showed results intermediate between the first and last. These data support the hypothesis that nectar flow exerts a positive influence on the nest-cleaning behavior of honey bees.

Table 2

Chi-square values calculated from pooled data of fed vs. non-fed test colonies during a complete dearth of nectar (see Figure 3).

Test colony	d.f.	χ^2
Colonies 1 and 3 vs. Colonies 2 and 4	1	126.84*

* Significance at .05 level of confidence.

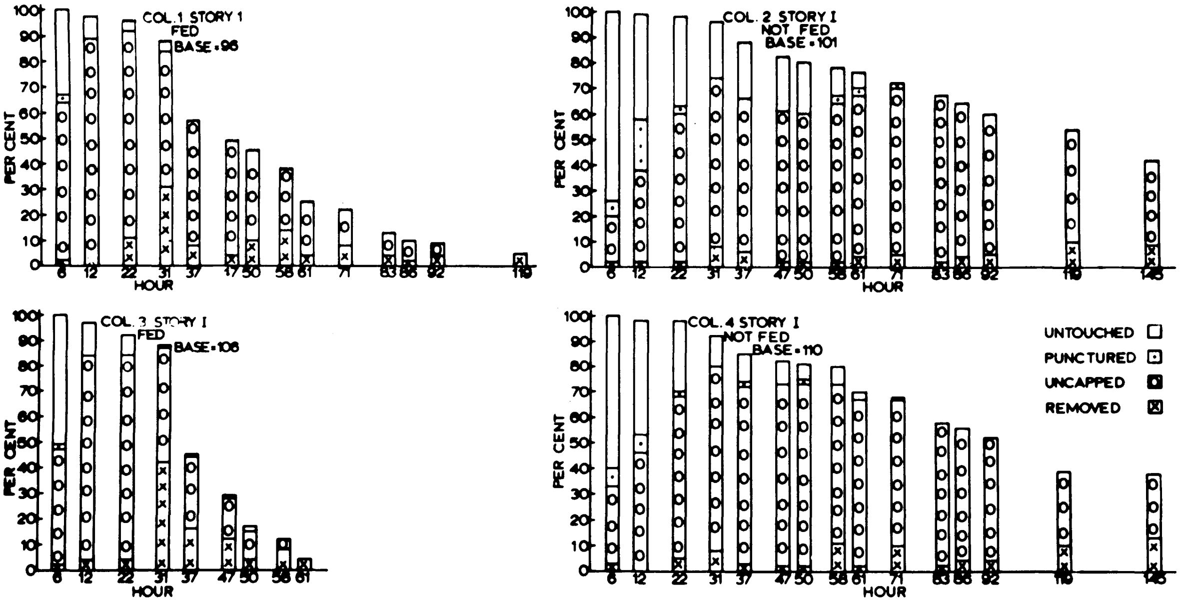

Figure 3. Nest-cleaning response of Brown honey bees to HCN-killed brood in fed and non-fed colonies during a complete dearth of nectar.

III. *The Comparative Response of Honey Bees to AFB-killed Brood and to Brood Killed by HCN.*

Investigations of nest-cleaning behavior of honey bees have been conducted using brood killed by *Bacillus larvae* (Woodrow and Holst, 1942; Rothenbuhler, 1964; Thompson, 1964) and by HCN (Jones and Rothenbuhler, 1964); however, the responses toward these types of dead brood were not compared in the same experiment. The use of composite inoculation squares containing both types of dead brood enables one to compare the responses of the bees toward dead brood resulting from two types of killing agents, a pathogen and HCN.

Four experiments were conducted to investigate these comparative responses. In all experiments composite inoculation squares (described above) containing AFB-killed brood and HCN-killed brood were used.

The results obtained from these experiments were far from definitive. Table 3 shows the responses for each experiment. The HCN-killed brood was removed significantly faster than AFB-killed brood in three out of four experiments. Analysis of uncapping responses showed that AFB-killed brood were uncapped faster in Experiments I and III, yet in Experiment III the HCN-killed brood was removed faster. In some cases the differences were so small that the uncapping response was about equal for both kinds of dead brood. In other cases there were great differences.

The inconsistency of the results suggests that there may be some unknown environmental factor or factors that determine which substances will be removed first. However, it should be pointed out that the task of removing a pupa or larva killed by HCN is not equal to the task of removing the remains of a larva or pupa killed by AFB. Once a brood cell is uncapped a worker bee can drag an HCN-Killed larva or pupa out in one piece in a matter of seconds. On the other hand, the sticky, viscous nature of the AFB material precludes the rapid removal of the material from the cell. Further work is visualized utilizing experimental techniques which may equalize the task of removing the cell contents.

Table 3

Comparative response to HCN- and AFB-killed brood.

Experiment	Fast Removal		Fast Uncapping	
	HCN	AFB	HCN	AFB
I		+		+
II	+		+	
III	+			+
IV	+		+	

Acknowledgments

Investigations were conducted under the direction of Dr. Walter C. Rothenbuhler, Director of The Ohio State University Bee Laboratory. I wish to thank Mr. Victor Thompson of The Ohio State University Bee Laboratory for his assistance in design and construction of research facilities. The assistance of Mrs. Walter Momot of Columbus, Ohio, contributed significantly to the success of these experiments.

Literature Cited

FRISCH, K. VON. 1950. *Bees, their vision, chemical senses and language.* Cornell University Press, Ithaca, New York.

JONES, D. L. & W. C. ROTHENBUHLER. 1964. *Anim. Behav.* XII, 4:584-588.

PARK, O. W. 1936. *Amer. Bee J.* **76**:12-15.

————————. 1937. *J. Econ. Entomol.* **30**:504-512.

ROTHENBUHLER, W. C. & V. C. THOMPSON. 1956. *J. Econ. Entomol.* **49**: 470-475

ROTHENBUHLER, W. C. 1964. *Anim. Behav.* XII 4:578-583.

SCHULZ-LANGER, ELISABETH. 1960. *Z. f. Bienenforsch.* 5 (1):1-7.

SNEDECOR, G. W. 1946. *Statistical methods.* 4th ed. Iowa State College Press, Ames, Iowa.

THOMPSON, V. C. 1964. *J. Apicult. Res.* 3 (1):25-30.

WOODROW, A. W. & E. C. HOLST. 1942. *J. Econ. Entomol.* **35**:327-330.