*Reprinted from the*
ANNALS OF THE ENTOMOLOGICAL SOCIETY OF AMERICA, VOL., 75, NO. 4, JULY 1982

# Field Test of Honey Bee (Hymenoptera: Apidae) Colony Defensive Behavior[1]

ANITA M. COLLINS AND KIM J. KUBASEK

Bee Breeding and Stock Center Laboratory, ARS, USDA, Baton Rouge, Louisiana 70808; and Department of Experimental Statistics, Louisiana State University, Baton Rouge, Louisiana 70893

**ABSTRACT**

Ann. Entomol. Soc. Am. 75: 383–387 (1982)

A method is described for evaluating honey bee colony defensive behavior. Three types of stimuli are presented successively: a synthetic alarm pheromone (chemical), a marble shot at the colony (physical), and mechanically jiggled blue suede targets (visual-tactual). The presentation of each stimulus in order elicits four discrete steps of defensive behavior: alerting, activating, attracting, and culminating.

Stinging by the honey bee, *Apis mellifera* L., is only one of many defensive behaviors. Like many other complex behaviors, this one can be subdivided into simpler units. Collins et al. (1980) proposed a four-step model for honey bee defensive behavior: alerting, activating, attracting, and culminating. A worker bee initially becomes alerted to a disturbance and may (1) release alarm pheromones which alert other bees, (2) move away from the area of disturbance, or (3) proceed to the next step of the sequence. During the second step, activating, the bee makes random movements that may bring her into contact with the source of disturbance. If the bee perceives stimuli to which she can orient (step three), she can proceed to express any one or more of the culminating actions of defensive behavior. These include such threat displays as flying at the intruder, loud buzzing, and burrowing into clothes, hair, or fur; actual physical contact through stinging, biting, or hair-pulling; or leaving the area of disturbance.

Through repeated experience, many beekeepers can identify the most defensive colonies in their apiary. Several quantitative approaches have been taken to compare nondefensive and defensive bees. Stort (1974) jerked a black leather ball in front of the colony entrance for 60 sec and measured the time to the first sting, time for the colony to become very aggressive, number of stings in the observer's gloves, number of stings in the leather ball, and the distance the bees followed the experimenter after a test. The National Academy of Sciences Committee on the African Honey Bee (Michener 1972) used a slight modification of this test with a square of suede cloth jiggled from a string in front of the entrance. Their measures were similar to Stort's. Boch and Rothenbuhler (1974) blew human breath into the colony entrance, opened the colony and placed a cork with isopentyl acetate on the entrance board, and counted the bees responding to each separate test.

The defensive behavior test reported here provides a procedure that elucidates the sequential nature of the defensive behavior of honey bees. This test can be used for quantitative comparisons of the defensiveness of various bee colonies.

## Materials and Methods

Seventy-two colonies of bees from several commercial sources were randomly chosen at six locations. They were divided into 12 experimental groups (six colonies per group) representing all possible combinations of the three types of stimuli and their controls. The normal 37.5-cm-wide hive entrance was reduced to 12 cm by stapling a screen and cardboard strip to the hive front. Each colony was tested twice between 30 June and 3 July, after the major honey flow. The light level (footcandles) at the entrance and the ambient temperature (°C) was measured at each colony before testing.

A series of stimuli was presented to each colony to stimulate defensive behavior in a stepwise fashion based on the model of Collins et al. (1980).

### *Chemical Stimulus*

A mixture of alarm pheromones associated with the honey bee sting (Blum et al. 1978) was diluted 1/99 (vol/vol) in paraffin oil. The basic pheromone mixture included *n*-butyl acetate (1.5%), isopentyl acetate (32%), isopentyl alcohol (14.5%), *n*-hexyl acetate (4%), *n*-octyl acetate (16.8%), 2-nonanol (10%), *n*-decyl acetate (1.5%), benzyl acetate (15.7%), and benzyl alcohol (4%) based on proportions found by Blum (unpublished data) in natural sting extracts. The control was paraffin oil alone.

Each test was initiated by spraying the diluted pheromone mixture or paraffin oil control just above the colony entrance by using a hand pump sprayer and squeezing to greatest draw each time. A picture of the entrance, flight board, and lower front of the colony was taken before spraying (pre) and at 15, 30, and 60 sec. In addition, the speed of the bees' reaction to the alarm pheromone was measured as the time interval from spraying until bees other than foragers began to come out and cluster around the entrance. The response of the bees to this portion of the test involved primarily the alerting and activat-

[1]In cooperation with La. Agric. Exp. Stn. Received for publication 14 July 1981.

ing steps of the model.

*Physical Stimulus*

At 60 sec (immediately after the fourth photograph), a glass marble (18.5 g, 2.3 cm in diameter) was shot at the colony directly above the entrance with a commercially available slingshot. One person did all the shooting, drawing to a standard length. The purpose of this stimulus was to further arouse the bees to respond to the visual stimulus. The measurement of the response was from a picture taken at 90 sec. Defensive behavior steps involved some alerting, but primarily activating. Controls did not receive a jolt from a marble.

*Visual Stimulus*

Visual and tactual stimuli were provided by two dark blue squares (5 by 5 cm) of suede leather, the distinctive leather smell of which provided a further chemical stimulus. These two targets, one directly in front of the colony entrance and one 45 cm away on a line perpendicular to the entrance, were clipped on the arms of a battery-powered mechanical apparatus (Fig. 1) and jiggled vertically through 20 cm, 120 times per minute. New targets were used for each colony, and the size was sufficient to allow bees ready access to sting at all the levels of response observed. Controls were either targets present without being jiggled or the jiggler in operation with no targets.

Blue color and the jerky motion are two prime stimuli for eliciting stinging (Free 1961). The bees flew out to and around the suede targets, landed on them, and stung and bit them. Stings readily remained in the suede, and the number of stings incurred during a 30-sec presentation of the targets was counted. This portion of the behavior sequence involved the attracting and culminating steps. A final picture at 120 sec completed the data collection.

In addition to the observations made during the test, each colony was inspected shortly after the experiment was completed and the population ($cm^2$ of bees on each frame) was estimated. Also, a notation was made of the approximate cavity volume of the colony, based on number and size of hive bodies (with combs) present. These measures were made to determine if colonies with larger populations are more defensive simply because there are more bees available, and if increased volume of comb might also increase defensiveness like empty comb increases foraging (Rinderer and Baxter 1978).

The analyses of number of bees were by least-squares analysis of variance and Duncan's multiple range test. The number-of-stings data were ranked, and a Kruskal-Wallis test (Hollander and Wolfe 1973) was applied to the results of an analysis of variance. Relationships between characters were measured by Pearson's Correlation.

## Results

In no instance did bees sprayed with the paraffin oil control show an alerting response. Seven (10%) of the colonies did not respond to the alarm pheromone within the first 60 sec of a test, but the remaining 64 (90%) had a mean response time ($\pm$SD) of 13.6 $\pm$ 8.95 sec. One colony died during the experiment.

Mean numbers of bees in the pretest picture for each of the 12 stimulus conditions ranged from 40.4 to 103.5 but were not significantly different ($F = 0.878$, 11 and 127 df).

The mean number of bees congregating outside the colony entrance at each of six times for both of the levels of chemical treatment is presented in Fig. 2. A split-plot analysis of variance verified the significance of the effect of chemical treatment ($P < 0.0002$) and the effect of interaction between chemical treatment and time ($P < 0.0001$). The effect of

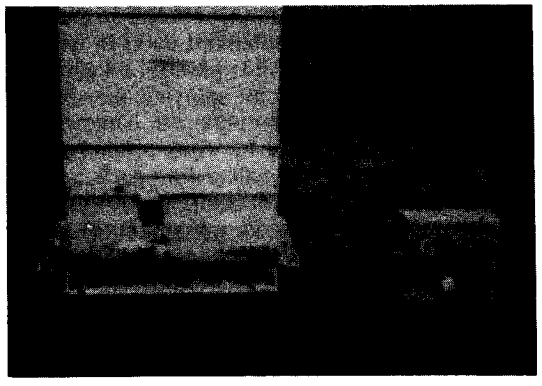

FIG. 1.—Mechanical jiggler with two suede targets in place in front of a colony.

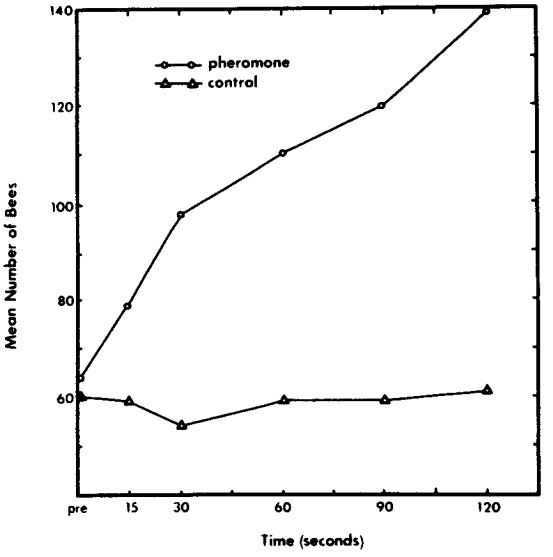


FIG. 2.—Influence of chemical and time during the test sequence on the mean number of bees at the colony entrance.

time alone was statistically insignificant due to the large amount of chemical by time interaction. Bees did not respond to the paraffin oil, but in the presence of alarm pheromone, increasing numbers of bees congregated outside the hive entrance.

Table 1 lists the mean number of bees in the 90 sec (30 sec after physical stimulus) photo for all combinations of chemical and physical treatments. A standard analysis of variance indicated a highly significant ($P < 0.0001$) effect of chemical treatments. The underlying variance was too great to detect a significant difference between physical treatments. If a colony had been alerted by alarm pheromone, a jolt from a marble increased the number of bees at the hive entrance 30 sec later.

The mean numbers of bees in the 120-sec photograph for all the combinations of chemical, physical, and visual treatments are shown in Table 2. Only the chemical stimuli had significantly different effects; there was too much variation between similarly treated colonies for the physical and visual categories to be statistically significant. The values for number of bees responding to a moving target did not include the number of bees in the air or on the target, which were considerably greater than with nonmoving targets or no targets. If these values had been included, the pheromone-marble-moving target count would probably have been as great or greater than pheromone-marble-nonmoving target.

Table 3 presents the mean number of stings for each combination of the three types of stimuli. Because the data did not appear to meet the normality requirements for analysis of variance, the data were analyzed with the "nonparametric" Kraskal-Wallis statistic (Hollander and Wolfe 1973). This analysis indicated that chemical, physical, and visual treatments all had significant effects on the number of stings in the targets. A moving target increased the incidence of stinging in conjunction with marble or pheromone, with an additive increase if both marble and pheromone were used. Classes with jiggler apparatus only were not included because no targets were present to collect stings.

Table 4 presents the Pearson correlation coefficients for all pairs of the parameters measured, using only the categories tested with full stimulus conditions before each measurement. Population size correlated positively with volume and the first two photograph bee counts, and negatively with number of stings. Volume was also negatively correlated with number of stings. Temperature was positively correlated with light and negatively with speed of reaction. Speed of reaction was negatively correlated with numbers of bees in all the pictures, all of which were positively correlated.

## Discussion

The field test of colony defensive behavior developed in this study involved the sequential presentation of a chemical, a physical, and a visual stimulus. Artificial honey bee sting alarm pheromone sprayed above the entrance aroused the bees and continued to attract bees to the area outside the entrance. A physical jolt to the colony (i.e., hit by a large marble) aroused the bees to a level at which they were more likely to sting.

The presence of a blue suede target in front of the entrance increased the number of bees outside; however, movement was necessary to stimulate a greater stinging response. Perhaps the presence of a visual stimulus alone provides cues for bees to orient to the area, or influences the bees to engage in scenting by sting protrusion or Nasonov fanning. This aspect needs to be studied in greater detail.

Free's 1961 conclusions that movement is a very

**Table 1.–Mean number of bees at the hive entrance at 90 sec for all combinations of chemical and physical treatments; least-squares means (± SE) with the same letter are not significantly different ($P < 0.05$)**

| Physical stimulus | Chemical stimulus | |
|---|---|---|
| | Pheromone | Control |
| Marble | 147.2 ± 8.5a | 55.2 ± 8.2c |
| Control | 102.7 ± 8.0b | 60.0 ± 8.0c |

**Table 3.–Mean number of total stings in the two targets for all treatment categories; least-squares means (± SE) with the same letter are not significantly different ($P < 0.05$)**

| Chemical stimulus | Physical stimulus | Visual stimulus | |
|---|---|---|---|
| | | Moving target | Nonmoving target |
| Pheromone | Marble | 16.0 ± 3.7a | 1.3 ± 3.7b |
| | Control | 9.1 ± 3.7ab | 0.3 ± 3.7b |
| Control | Marble | 7.4 ± 3.7ab | 0.4 ± 3.7b |
| | Control | 0.7 ± 3.4b | 0.3 ± 3.4b |

**Table 2.–Mean number of bees at the hive entrance at 120 sec for all chemical, physical and visual stimulus combinations. Least-square means (± SE) with the same letter are not significantly different ($P < 0.05$)**

| Chemical stimulus | Physical stimulus | Visual Stimulus | | |
|---|---|---|---|---|
| | | Moving target | Nonmoving target | No target |
| Pheromone | Marble | 139.5 ± 27.7b | 242.2 ± 27.7a | 113.7 ± 30.3bc |
| | Control | 138.4 ± 27.7b | 129.9 ± 27.7b | 104.0 ± 27.7bc |
| Control | Marble | 61.3 ± 27.7bc | 66.9 ± 27.7bc | 72.4 ± 27.7bc |
| | Control | 45.1 ± 25.6c | 78.1 ± 25.6bc | 67.8 ± 27.7bc |

Table 4.—Pearson's correlation coefficients for all traits, using observations after pheromone treatment only (90 sec, 120 sec, and number of stings from pheromone, marble, moving target treatment only)[a]

| Determination | Population | Vol | Temp (°C) | Light | Speed of reaction | No. of bees in picture | | | | | | No. of stings |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Pretreatment | 15 sec | 30 sec | 60 sec | 90 sec | 120 sec | |
| Population ($cm^2$ of bees) | 1.00 | 0.27* | −0.00 | −0.10 | −0.06 | 0.38** | 0.36** | 0.22 | 0.19 | 0.17 | 0.14 | −0.71* |
| Vol (liters) | | 1.00 | 0.17 | 0.23 | −0.08 | 0.07 | 0.23 | 0.17 | 0.07 | 0.09 | 0.10 | −0.76* |
| Temp (°C) | | | 1.00 | 0.45** | −0.43** | 0.15 | 0.19 | 0.06 | −0.09 | −0.01 | −0.12 | −0.03 |
| Light (footcandles) | | | | 1.00 | −0.12 | −0.21 | −0.05 | 0.004 | 0.02 | 0.11 | 0.16 | 0.07 |
| Speed of reaction (sec) | | | | | 1.00 | −0.38** | −0.36** | −0.34** | −0.37** | −0.37** | −0.28** | −0.18 |
| No. of bees in picture | | | | | | | | | | | | |
| Pretreatment | | | | | | 1.00 | 0.91** | 0.74** | 0.60** | 0.63** | 0.59** | −0.04 |
| 15 sec | | | | | | | 1.00 | 0.85** | 0.67** | 0.66** | 0.66** | −0.22 |
| 30 sec | | | | | | | | 1.00 | 0.85** | 0.79** | 0.73** | 0.02 |
| 60 sec | | | | | | | | | 1.00 | 0.94** | 0.91** | 0.08 |
| 90 sec | | | | | | | | | | 1.00 | 0.97** | 0.18 |
| 120 sec | | | | | | | | | | | 1.00 | −0.47 |
| No. of stings | | | | | | | | | | | | 1.00 |

[a] *, Significant at $P < 0.05$; **, significant at $P < 0.01$.

important stimulus for inducing stinging behavior is supported by these data. The number of bees in the 120-sec picture for nonmoving targets with marble and pheromone appeared to be greater than for moving targets of the same class. This can be explained by noting that when moving targets are presented, many bees fly off the entrance to the targets and are not included in the pictures. If these bees had been counted, moving and nonmoving targets would probably have had similar numbers of bees in the 120-sec picture. The absence of a method to account for flying bees was the greatest inadequacy of the field test.

The positive correlation between population size (6,500 to 27,500 $cm^2$ of bees) and colony volume (20 to 100 liters) reflects the honey bee management practice of providing more populous colonies with more comb space. Population size is not a major factor in the number of bees responding as measured by the photographs, although values for the precount and 15-sec count did reflect the numbers in the colony. The quantity of bees visible at any time during the test was indicative of the response level during the entire sequence, but the mere presence of bees did not presage a corresponding magnitude of stinging.

Surprisingly, the number of stings in the target was negatively correlated with population and volume. Several laboratory measures of response to alarm pheromone are significantly correlated (0.56) with laboratory measures of honey production, indicating that better honey producers are more defensive (Collins and Sylvester, unpublished data). If this is the case, we might expect a greater proportion of the population in the more defensive colonies to be foraging when the population sizes were measured, giving misleadingly low estimates of population for these colonies, which would account for the negative correlation.

Or perhaps there is adaptive value for colonies of the smaller sizes in this study (6,500 $cm^2$ of bees, 20 liters) to be more defensive. The information currently available is insufficient to draw any conclusions.

Colonies that responded quickly also had more bees accumulating at the entrance, but did not necessarily sting more. The relationship of speed of reaction to temperature during this test (25 to 34°C) was consistent with laboratory observations of response to alarm pheromones made by Collins (1981) (26 to 35°C), whereas light levels were only important as they related to temperature. Overall, the testing sequence described provides a procedure to evaluate the level of defensive behavior expressed by a colony of bees, and to subdivide that behavior into its component steps.

**Acknowledgment**

Sincere thanks go to Rex Nelson, Baton Rouge, La., for executing the original target jiggler design

in a more durable form. Thanks also to N. E. Gary, G. Waller, and N. Koeniger for critical review of the manuscript.

## REFERENCES CITED

**Blum, M. S., H. M. Fales, K. W. Tucker, and A. M. Collins. 1978.** Chemistry of the sting apparatus of the worker honeybee. J. Apic. Res. 17: 218–221.

**Boch, R., and W. C. Rothenbuhler. 1974.** Defensive behavior and production of alarm pheromone in honeybees. Ibid. 13: 217–221.

**Collins, A. M. 1981.** Effects of temperature and humidity on honeybee response to alarm pheromones. Ibid. 20: 13–18.

**Collins, A. M., T. E. Rinderer, K. W. Tucker, H. A. Sylvester, and J. J. Lackett. 1980.** A model of honeybee defensive behavior. Ibid. 19: 224–231.

**Free, J. B. 1961.** The stimuli releasing the stinging response of honeybees. Anim. Behav. 9: 193–196.

**Hollander, M. and D. A. Wolfe. 1973.** Nonparametric statistical methods. John Wiley and Sons, New York. 503 pp.

**Michener, C. D. 1972.** Report of the committee on the African honey bee. National Technical Information Service. Springfield, Va. 95 pp.

**Rinderer, T. E., and J. R. Baxter. 1978.** Effect of empty comb on hoarding behavior and honey production of the honey bee. J. Econ. Entomol. 71: 757–759.

**Stort, A. C. 1974.** Genetic study of aggressiveness of two subspecies of *Apis mellifera* in Brazil. 1. Some tests to measure aggressiveness. J. Apic. Res. 13: 33–38.