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## A CHEMICAL ALARM RELEASER IN HONEY BEE STINGS (*APIS MELLIFERA* L.)<sup>1</sup>

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Chemicals that function to communicate alarm among the members of hymenopteran colonies have been discovered in recent years by several investigators. These chemicals, released by disturbed insects and detected in the gaseous state by other members of the colony, have been shown to elicit various forms of alarm behavior, differing according to species, but always related to the defense of the colony. Certain species of ants in particular have been shown to employ such "alarm releasers" (Wilson, 1958; Butenandt and Lindauer, 1959; Brown, 1960; Ghent, 1961). Preliminary evidence presented by Huber (1814) suggests that the honey bee (*Apis mellifera*) also possesses such a mechanism.

It is a common observation among beekeepers that the probability of being stung increases after the first sting. The frequency of stinging often appears to rise exponentially: for example, a beekeeper working with colony after colony becomes increasingly liable to be stung. This phenomenon suggests that bees possess some means of labelling an intruder, presumably by applying an alarm releaser to the victim during the act of stinging.

A characteristic, pleasantly sweet scent is noticeable at the site of stinging. This scent, although not detectable from undisturbed bees, becomes quite concentrated in a closed container of agitated bees and is most perceptible from newly-inserted stings. One exception is that the stings of recently emerged bees bear no such scent. When the bee stings, the barbed sting and its basal motor apparatus are torn from the departing worker and remain imbedded in the skin. The fragrant

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substance associated with the sting would, therefore, seem to be an efficient tagging mechanism. Subsequent stings would then replenish and augment this chemical label.

An examination of the sting apparatus to determine the source of this scent revealed several facts. The contents of neither the large poison reservoir nor the so-called "alkaline" accessory gland have any detectable odor. A minute amount of fluid, immiscible with water, is held between the bulbous base of the sting shaft and the setose lobe of membrane which folds over it. The sting odor is particularly associated with this membrane, although it is sometimes detectable on other basal structures as well.

No glandular tissue was found to be immediately associated with the setose lobe covering the bulb of the sting shaft. There are, however, two masses of glandular cells, lying against the inner surface of the quadrate plates, which secrete by individual ducts onto the outer surface of these plates (Snodgrass, 1956). There is a continuous space surrounding the sting base through which this secretion can flow, eventually to collect beneath the setose lobe.

It may be significant that under natural circumstances the under surface of this lobe is exposed only when the sting is partially extruded, or during the act of stinging. The sting is frequently extruded by alarm bees, particularly when bees in the winter cluster are disturbed.

Exposure of honey bees to stings pulled from freshly-frozen workers results in a marked change in behavior. Pieces of filter paper, bearing one or more stings, were introduced into an observation hive containing a normal colony, and the subsequent behavior observed. A sudden agitation was first observed in the vicinity of the introduced stings, spreading quickly outward to a radius of about 15-20 cm. After the first sharp wave of agitation, during which most of the bees in the area buzzed momentarily, a general orientation to the stings occurred, with many bees converging on the paper. Individual bees standing near or over the introduced stings assumed an abnormally high stance, with the antennae constantly waving, and the wings partially extended, in marked contrast to the normal posture of resting bees. These individuals successively accosted approaching workers, turning from side to side and sometimes making short flights to do so. Apparently identical behavior may be observed in guard bees at the entrance of a hive. Control pieces of filter paper, introduced into other parts of the hive, or alternately with those bearing stings, elicited no such behavior if the introduction was made carefully without mechanical disturbance.

An experiment was conducted to determine whether the presence

of a fresh sting on an introduced object increases the probability of its being stung. Captured workers were first induced to sting a disk of suede leather 1.5 cm. in diameter. This was lowered on a wire into

TABLE 1. Response to suede leather disk bearing three fresh stings compared to response to an untreated control disk.

	<i>(a) Disk Bearing Stings</i>		<i>(b) Control Disk</i>	
	No. of bees clustered on disk	No. of additional stings	No. of bees clustered on disk	No. of additional stings
Test #1	50	4	0	0
#2	12	3	0	0
#3	10	0	1	0
#4	5	1	0	0
#5	100	3	0	0
#6	30	1	0	0

Test of significance for difference in number of additional stings:  
 $\chi^2 = 12$  with 1 degree of freedom  
 $P < .001$

an observation hive and held about 5 cm. above the comb for one minute, then carefully removed. Allowing 30 seconds between introductions, this disk and other control disks bearing no stings were alternately introduced in the same manner. The number of bees which congregated on the disks during each test was estimated and the newly deposited stings were counted after each removal. The data in table (1) clearly exhibit greater attractiveness of the leather disk bearing stings, as well as a significantly ( $P < .001$ ) greater number of stings retained in it.

These results seemed to indicate that the presence of a fresh sting alone is sufficient to provoke stinging attacks. In subsequent observations, however, it was demonstrated that one or more secondary stimuli are usually necessary to elicit stinging. Although stings invariably attract bees, we observed very few cases of stinging unless the test object was moved or jarred. Indeed, bees that congregated about an introduced sting tended to fly at any moving object in the vicinity. Since the loss of the sting is often fatal to the worker, there is an obvious selective pressure against the stinging of immobile objects, even though coated with an alarm releaser.

In subsequent experiments, it was found that extracts of stings attracted worker bees, and induced a pattern of behavior which was apparently identical to that of workers exposed to fresh stings.

The alarm reaction was further characterized in an experiment relating the quantity of extract to the number of bees attracted to it. Three hundred whole uncrushed stings, pulled from freshly-frozen workers, were extracted in 1 ml. of methylene chloride. Measured

quantities were pipetted into vials (4.5 cm. long by 1.3 cm. in diameter) with an inner lining of filter paper. Each vial was placed in a cylindrical screen cage 10 cm. long by 3 cm. in diameter, which was

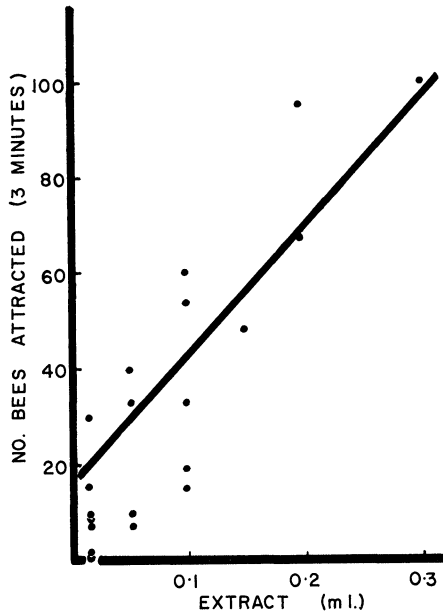


Figure 1. Attraction of worker bees to an extract of bee stings in methylene chloride. The extract was pipetted onto filter paper and introduced in screen cages laid over the combs of a colony. Bees attracted to each cage were collected and counted.

open at one end. The cages were laid in groups of four across the top bars of an open colony, immediately enclosed by an empty hive body and cover, and left in this dark chamber for 3 minutes. Each was then carefully transferred with the adhering bees into a closed container. The bees thus collected were anesthetized and counted (Fig. 1). Although the data show considerable variation, there was a positive correlation between the quantity of extract per vial and the number of bees attracted.

An extract containing about 5,000 stings in 10 ml. of methylene chloride was distilled, beginning at 35° C. and slowly rising to 90° C. The colorless distillate was collected in three approximately equal fractions. The last two fractions, distilled at temperatures over

60° C., had the characteristic bee sting scent. Some of this scent was retained by the yellow residue.

Vapor phase chromatograms of the first fraction showed evidence of only the solvent, methylene chloride. In both of the last two fractions only two components in addition to the solvent were detected: water and the scented substance presumed to be the alarm releaser. Attempts to concentrate the odoriferous component by evaporation of the methylene chloride were only partially successful. Vapor phase chromatograms indicated that, although it was slightly concentrated during evaporation, the greater part was lost.

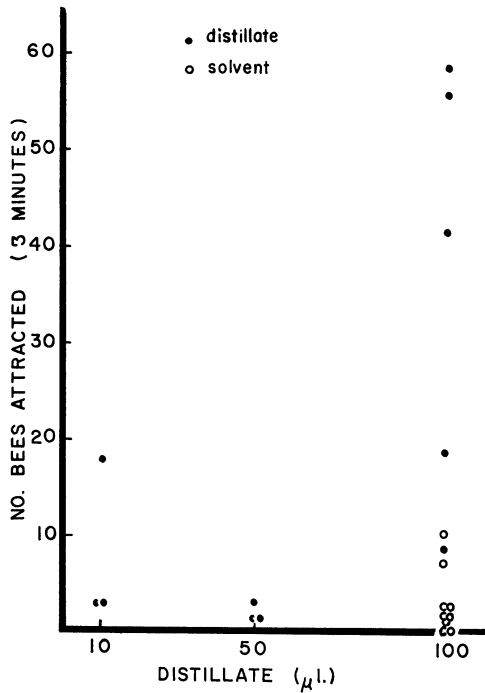


Figure 2. Attraction of worker bees to distilled extract of bee stings. The distillate was pipetted onto filter paper and introduced in screen cages laid over the combs of the colony. Bees attracted to each cage were collected and counted.

The effect of the whole distillate on bee behavior was tested by the same method as that used for the crude extract. Data on attraction

confirmed the presence of the alarm releaser in the distillate (Fig. 2). The behavior of bees exposed to the distillate was indistinguishable from that observed in the presence of fresh bee stings. Moreover, it was evident that bees attracted to filter papers bearing the distillate demonstrated an extraordinary tendency to sting upon the slightest provocation.

We have not yet succeeded in concentrating sufficient quantities of the alarm releaser to permit chemical characterization. Our observations indicate that extremely small traces of the scented substance of bee stings are detectable both by humans and by honey bee workers, and that the amount borne by single stings is in fact minute.

Alarm behavior in honey bees is governed by many factors, and the presence of the alarm releaser is not essential to stinging. Bees often attack moving objects or animals in the vicinity of the hive where no alarm releaser could possibly have been applied previously. Movement, odor, and texture probably all determine to some degree whether the response is aggressive. Unless accompanied by a supplementary stimulus, for example movement, the odor of the alarm releaser rarely precipitates stinging. The function of the alarm releaser, besides serving as an efficient recruiting mechanism, must be considered as an intensifier of the normal defensive responses of the colony.

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