Journal of Chemical Ecology, Vol. 14, No. 2, 1988

NEST PLUNDERING ALLOMONES OF THE FIRE BEE *Trigona (Oxytrigona) mellicolor*[1,2]

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(Received September 1, 1986; accepted February 28, 1987)

Abstract—Ten volatile compounds derived from the cephalic glands of the fire bee *Trigona (Oxytrigona) mellicolor* were bioassayed for possible allomonal activities facilitating nest plundering. Two diketones, (*E*)-3-heptene-2,5-dione and (*E*)-3-nonene-2,5-dione, caused the honeybee *Apis mellifera* to display avoidance behavior and reduced defensive behavior. These diketones are produced in relatively large quantities in fire-bee cephalic glands.

Key Words—Fire bee, *Trigona (Oxtrigona) mellicolor*, *Trigona (Oxtrigona) tataira*, honeybee, *Apis mellifera*, Hymenoptera, Apidae, mandibular gland secretion, allomone, nest plundering, diketones, (*E*)-3-heptene-2,5-dione, (*E*)-3-nonene-2,5-dione.

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0098-0331/88/0200-0495$06.00/0

INTRODUCTION

The exocrine secretions of stingless bees in the genus *Trigona* possess varied functions in the chemical ecology of these apids. *T.* (*Oxytrigona*) *mellicolor* produces a remarkable mandibular gland secretion (Kerr and Costa Cruz, 1961) which causes blistering lesions of up to 2 cm in diameter, over four times the length of the bee which inflicted it, when applied to the skin of humans (Wille, 1961; T.E. Rinderer, field observation). The pain caused by such lesions, in combination with the reddish-orange color of *T. mellicolor*, explains why the bee is frequently called the fire bee.

The fire bee is also remarkable for its ability to remove honey from the nests of honeybees (*Apis mellifera*) imported from both the palearctic and Ethiopian realms (Moure, 1946; T.E. Rinderer, field observation). During nest plundering, the fire bee produces a cephalic secretion which has a strong but, to humans, pleasant floral odor (Moure, 1946; Bian et al., 1984). Yet, during a nest-plundering episode, honeybees do not defend their nest but remain motionless on the comb, hang in a cluster of bees outside the entrance of the colony, or appear to "wander" in a seemingly disorientated manner over the surface of the comb. Cephalic extracts contain a variety of compounds that we were interested in evaluating as potential allomones that might facilitate nest plundering. This report describes the results of a honeybee bioassay to determine the allomonal activities of the components of fire-bee cephalic secretions identified by Bian et al. (1984).

METHODS AND MATERIALS

Assay for Primary Effects. Thirteen observation-hive colonies were prepared from established field colonies in Baton Rouge, Louisiana. These colonies were derived from sister queens of general European commercial stock that were permitted to open-mate from the same apiary. Each observation-hive colony contained the original queen, about 0.25 kg (2500) of her adult worker bee daughters, one brood comb (16 × 45 cm) containing the queen's worker brood (about half sealed and half unsealed) and one comb (16 × 45 cm) half-filled with honey and pollen. The observation hives were then left undisturbed for four days to allow them to return to normal organization and regulation (egg laying, colony defense, foraging, etc.).

Ten chemicals [(*E*)-3-nonene-2,5-dione, or (*E*)-3-n, (*E*)-3-heptene-2,5-dione, or (*E*)-3-h, 3-hepten-2-one, dodecyl acetate, 2-decanone, tetradecyl acetate, hexadecyl acetate, pentadecane, 2-heptanone, and tetradecane] found in fire-bee cephalic gland secretions and synthesized commercially or in our laboratories (Bian et al., 1984) were prepared separately as 1:500 dilutions by

weight in paraffin oil. Additionally, a mixture containing equal proportions of each by weight was prepared as a 1:500 dilution by weight in paraffin oil. In the bioassay, the diluted compounds were presented to the observation-hive colonies as 0.5 ml of solution in 7 × 11-mm "sleeve-type" rubber septa. Two control treatments were incorporated into the design: rubber septa with no added materials and rubber septa containing 0.5 ml of paraffin oil.

The effects of the 13 treatments (exposure to 10 compounds, a mixture of the 10 chemicals, and two controls) were tested in a randomized Latin-square design with the 13 colonies. The tests were conducted on 13 consecutive days, each rain-free and with similar temperatures (ca. 30°C).

For each test, a rubber septum was held in a firm steel wire loop, filled with 0.5 ml of the appropriate solution, and inserted from the top of the hive to the center area of one side of the brood comb. Prior to the test, this side of the observation hive was fitted with a standard grid to facilitate later measurements. After 2 min, the septum was withdrawn and the number of stings implanted in it was counted. The entire procedure was recorded on video tape for later evaluation. The maximum diameter (measured through the center of the top surface of the septum) of the area surrounding the septum that had no bees at the end of the 2 min was measured using the grid image to standardize measurements. Ten seconds after the septum was in place, the reaction of the first bee to approach the vial was observed and recorded. Such reactions were observed for a total of 10 bees identified at 10-sec intervals. Reactions included, from the least defensive to the most defensive: (1) rapidly withdrawing from the area, (2) slowly withdrawing from the area, (3) remaining in the area, (4) investigating the septum, (5) "buzzing" wings in response to the septum or its contents, and (6) attacking the septum either by biting or stinging it.

Bee responses were converted to a composite defensive scale by summing the numerical values (1 through 6) of the responses of the 10 bees and then rank-transforming the 169 sums (Conover and Iman, 1981).

These scores, the numbers of stings, and the diameters of areas without bees surrounding the septa at the end of the tests, were submitted to analyses of variance according to the Latin-square design for the main factors of chemicals, days, and colonies. Videotaping errors resulted in a few missing cells for defensive behavior and diameter of area. These missing cells were estimated using the method of Steel and Torrie (1980). The error degrees of freedom were reduced appropriately to reflect the number of observed values. Mean separation evaluations were based on Duncan's multiple-range tests.

Additional information was obtained by ranking the responses to the independent variables for each dependent variable. These ranks were then analyzed with a Friedman two-way analysis of variance in order to test the null hypothesis that the independent variables had generally similar effects for the three dependent variables in the analysis (Siegel, 1956).

Assay for Synergistic Effects. Seven additional observation-hive colonies were prepared and tested in a 7 × 7 Latin-square design by the procedures described for the first experiment. Based on the results of the first experiment, seven chemicals and mixtures were used; (1) (*E*)-3-n, (2) (*E*)-3-h, (3) 3-hepten-2-one, (4) a mixture of 1 and 2, (5) a mixture of 1 and 3, (6) a mixture of 2 and 3, and (7) a mixture of 1, 2, and 3.

RESULTS

Primary Effects. Significant and consistent trends were revealed by the analysis (Table 1). Two of the dependent variables, the number of stings in the septum and the intensity of the defensive responses, differed significantly among

TABLE 1. HONEYBEE RESPONSES TO FIRE BEE CEPHALIC COMPOUNDS[a]

Treatment	*N*	Diameter (cm) of comb area cleared of bees	Number of stings	Defensive scale
(*E*)-3-Nonene-2,5-dione	13	5.83 ± 0.22a	1.03 ± 0.66a	69.3 ± 11.7ab
(*E*)-3-Heptene-2,5-dione	13	5.62 ± 0.22a	1.07 ± 0.86ab	55.5 ± 9.8a
Mix of all chemicals	13	4.41 ± 0.14b	1.20 ± 0.76abc	58.3 ± 13.0a
3-Hepten-2-one	13	4.20 ± 0.15bc	1.61 ± 0.86cd	105.2 ± 13.3bc
Dodecyl acetate	13	4.15 ± 0.15bc	1.28 ± 0.81abc	71.7 ± 11.9abc
2-Decanone	13	4.11 ± 0.15bc	1.24 ± 0.61abc	78.0 ± 13.2abc
Tetradecyl acetate	13	3.99 ± 0.15bcd	1.54 ± 1.16cd	91.9 ± 14.7abc
Hexadecyl acetate	13	3.92 ± 0.10bcd	1.49 ± 0.76bcd	89.6 ± 16.7abc
Pentadecane	13	3.89 ± 0.10cd	1.56 ± 0.76cd	102.3 ± 12.4bc
2-Heptanone	13	3.78 ± 0.08cd	1.63 ± 0.86cd	90.3 ± 13.3abc
Paraffin oil control	13	3.77 ± 0.07cd	1.87 ± 1.32d	108.1 ± 15.1c
Septum control	13	3.59 ± 0.07d	1.34 ± 0.96abc	83.2 ± 10.6abc
Tetradecane	13	3.57 ± 0.07d	1.63 ± 0.97cd	101.6 ± 14.3bc

Analyses of Variance

Source of Variation	*df*	*F*	*P*	*F*	*P*	*F*	*P*
Chemicals	12	20.63	0.0001	3.19	0.0005	2.45	0.006
Days	12	1.03	0.4230	7.36	0.0001	3.23	0.0004
Colonies	12	3.07	0.0005	4.14	0.0001	3.96	0.0001
Sampling	132 (128)[b]						

[a] Average diameter of an area without honey bees surrounding an empty septum, a septum containing paraffin oil, a septum containing paraffin oil with each of ten chemicals, or a septum containing paraffin oil and all 10 chemicals; average number of stings delivered to the septa containing the treatments; average indexes of graded defensive responses (explained in text). For each dependent variable, values are significantly different if not followed by the same letter as determined by Duncan's multiple-range tests. Each set of statistical values from the analysis of variance in the lower part of the table is for the column above.

[b] Sampling df for number of stings and (diameter and defensive scale).

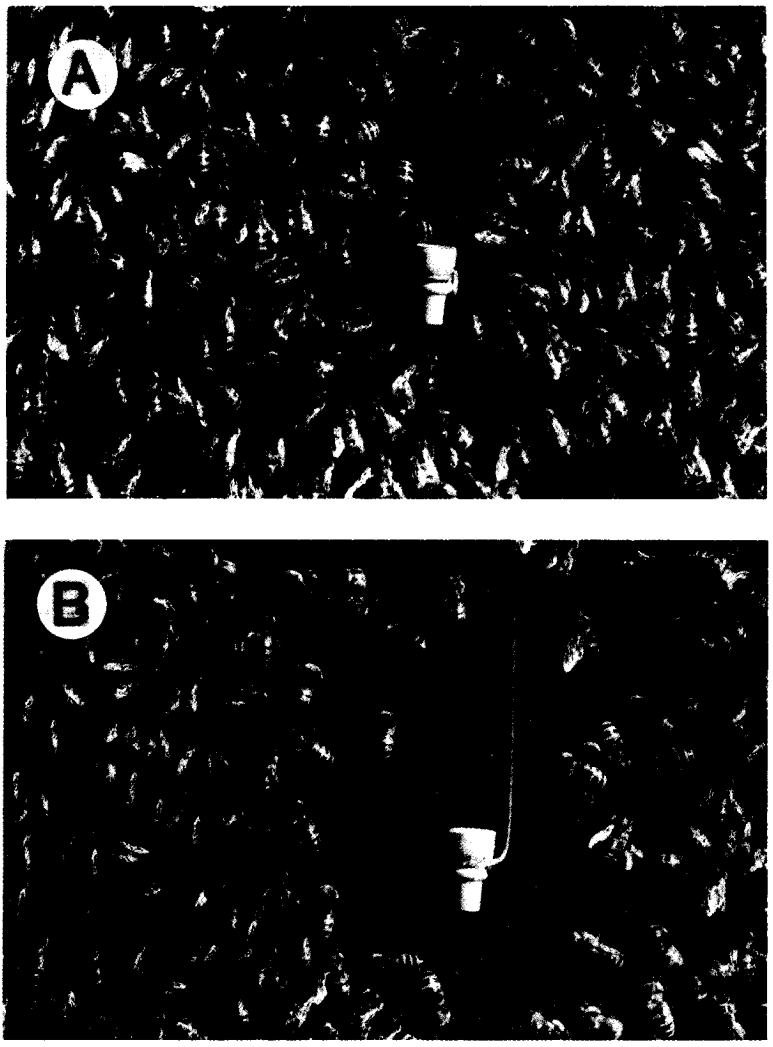

FIG. 1. (A) Area surrounding rubber septum containing paraffin oil vacated by bees. (B) Area surrounding rubber septum containing (*E*)-3-nonen-2,5-dione vacated by bees. Both A and B are responses after 2 min.

treatments, days, and colonies. The diameter of the area vacated by bees differed among treatments and colonies.

Analysis of the differences among treatments by Duncan's multiple-range tests identified strong allomonal activities by (*E*)-3-n and (*E*)-3-h. These two compounds cleared an area with a significantly larger diameter than was cleared by the other treatments (Figure 1). Also, of all the remaining treatments, the

mixture of compounds (which contained these two, but at one tenth the concentration) was most similar to them. Additionally, the septa containing these two compounds received the fewest numbers of stings, and the septa containing the mix of compounds received the next fewest. These three treatments also provoked the least intense defensive responses as measured by the defensive scale.

The Friedman two-way analysis of ranks confirmed the consistent effects of the two unsaturated diketones and the mixture containing them ($X_r^2 = 152.12$, $df = 12$, $p = 0.001$). (*E*)-3-n and (*E*)-3-h and the mixture of compounds had combined ranks of 6, 6, and 7, respectively. The next closest grouping was hexadecyl acetate and pentadecane with combined ranks of 13 and 14. The remaining treatments had combined ranks of 22–35.

Synergistic Effects. No significant or even suggestive differences were found to support the hypothesis that synergistic effects are elicited by mixtures of these compounds.

DISCUSSION

Generally, the chief honeybee response to the diketones was negative chemotaxis. Reductions in stinging and defensive behavior most probably stem from this negative chemotactic response rather than a second specific response. The reactive nature of the diketones (Michael addition) suggest that they may function as irritants, especially to sensitive antennal chemoreceptors.

The diketones also may have an important role in the defensive secretion of *T. mellicolor*. When added to the formic acid in this secretion (Roubik et al., 1987), the diketones may increase its ability to produce burning sensations and blistering either by increasing its solubility in epidermal lipids or facilitating nucleophilic additions by protonation of the carbonyl groups.

The possible roles of the diketones in both nest robbing and colony defense suggest that the existence of one behavioral potency made possible the evolution of the other. *T. mellicolor* has often been observed robbing the nests of *Apis mellifera* but not the nests of sympatric neotropical bees (Roubik et al., 1987). This simply may be a consequence of *A. mellifera* nests being more easily observed. However, *A. mellifera* lacks the elaborate entrance structure typical of neotropical stingless bee nests. Perhaps the importation of *A. mellifera* to the Americas provided a novel opportunity for the development of *T. mellicolor* nest plundering of comparatively unprotected nests. The possibility exists that nest plundering is a simple extension of other foraging-related behavior. Perhaps *T. mellicolor* has expanded the use of its nest-defense secretions to repel competitors from rich floral patches. If so, the use of the secretions in plundering the nests of recently introduced honeybees would be a further expansion of an already existing behavior.

Because the two diketones are so effective at repelling honeybees, they may have desirable commercial uses. Formulations may be developed to remove bees from hive chambers prior to honey harvesting or to reduce the incidence of persons being stung while handling bees.

Acknowledgments—We thank Robert Spencer for excellent technical assistance and an anonymous reviewer for especially useful suggestions.

REFERENCES

BIAN, Z., FALES, H.M., BLUM, M.S., JONES, T.H., RINDERER, T.E., and HOWARD, D.F. 1984. Chemistry of cephalic secretion of fire bee *Trigona* (*Oxytrigona*) *tataira*. *J. Chem. Ecol.* 10:451–460.

CONOVER, W.J., and IMAN, R.L. 1981. Rank transformations as a bridge between parametric and nonparametric statistics. *Am. Stat.* 35:124–132.

KERR, W.E., and COSTA CRUZ, C. DA. 1961. Funcoes diferentes tomadas pela glandula mandibular na evolucao das albelhas em geral e en "*Trigona* (*Oxytrigona*) *tataira*" em especial. *Rev. Bras. Biol.* 21:1–16.

MOURE, J.S. 1946. Sobre a abelha tataira ou cagafogo. *Chacaras Quintais* 73:612–613.

ROUBIK, D.W., SMITH, B.H., and CARLSON, R.G. 1987. Formic acid in caustic cephalic secretions of stingless bee, *Oxytrigona* (Hymenoptera: Apidae). *J. Chem. Ecol.* 13:1079–1086.

SIEGEL, S. 1956. Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, New York.

STEEL, R.G.D., and TORRIE, J.H. 1980. Principles and Procedures of Statistics: A Biometrical Approach. McGraw-Hill, New York.

WILLE, A. 1961. Las abejas jicotes de Costa Rica. *Rev. Univ. Costa Rica* 22:1–30.