Journal of Chemical Ecology, Vol. 13, No. 7, 1987

(Z)-11-OCTADECENYL ACETATE, AN AGGREGATION PHEROMONE IN *Drosophila simulans*

ANGELA M. SCHANER, ROBERT J. BARTELT, and LARRY L. JACKSON

Department of Chemistry
Montana State University
Bozeman, Montana 59717

(Received August 1, 1986; accepted October 24, 1986)

Abstract—Existence of a male-produced pheromone, which attracts both males and females in a wind-tunnel olfactometer, has been demonstrated in *Drosophila simulans* (Sturtevant). A pheromone component was identified as (Z)-11-octadecenyl acetate (Z11-18:Ac), also known as *cis*-vaccenyl acetate. The pheromone is synergized by food volatiles. In bioassay ca. 1/1000 of a mature male equivalent of Z11-18:Ac is attractive and activity increases with increased amounts of Z11-18:Ac. Flies do not begin responding to Z11-18:Ac until after they have been away from food for at least 2 hr. Z11-18:Ac is transferred from the male to the female during mating, and the female emits the majority of the transferred Z11-18:Ac within 6 hr after mating.

Key Words—Diptera, Drosophilidae, *Drosophila simulans*, aggregation pheromone, (Z)-11-octadecenyl acetate, *cis*-vaccenyl acetate.

INTRODUCTION

Aggregation pheromones have previously been studied in seven *Drosophila* taxa: *D. virilis* (Bartelt and Jackson, 1984; Bartelt et al., 1985a); *D. a. americana, D. a. texana, D. novamexicana*, and *D. lummei* (Bartelt et al., 1986); *D. hydei* (Moats et al., 1987); and *D. melanogaster* (Bartelt et al., 1985b). In all these taxa, the pheromone component(s) are produced only by mature males and attract both males and females in a wind-tunnel olfactometer. This study was undertaken to determine whether an aggregation response could be demonstrated in *D. simulans* (Sturtevant), a close relative of *D. melanogaster*. In *D. melanogaster* a major aggregation pheromone component was identified as (Z)-

0098-0331/87/0700-1777$05.00/0

11-octadecenyl acetate (Z11-18:Ac) (Bartelt et al., 1985b). This pheromone, in combination with food, substantially increased the attractiveness of food odors. Z11-18:Ac has been reported in *D. melanogaster* to mediate close-range behavior as well, by discouraging males from courting other males or recently mated females (Jallon et al., 1981). Because of the dual role of Z11-18:Ac in *D. melanogaster* and because *D. simulans* also possesses Z11-18:Ac (Jallon, 1984), it was hoped that *D. simulans* would provide further information on the function of Z11-18:Ac. Starting with crude extracts of mature flies and using bioassay to guide purification, Z11-18:Ac was identified as a major component of the aggregation pheromone of *D. simulans*. Other experiments related to aggregation in *D. simulans* are also discussed.

METHODS AND MATERIALS

Flies. D. simulans (strain 14021-0251.0) was obtained from the National *Drosophila* Species Resource Center at Bowling Green, Ohio. It was originally collected at Kenscoff, Haiti. Flies were reared on Instant *Drosophila* Medium Formula 4-24 (Carolina Biological Supply Co. Burlington, N.C.) in 1-liter jars or in 3.5 × 10-cm vials under a light–dark cycle of 16:8 hr and ambient laboratory temperatures.

Extracts and Chromatography. Flies were separated by sex when 0–6 hr old and extracted at that time or at 6–7 days of age by soaking them in hexane at room temperature for 24 hr. The crude extracts were separated by polarity on open columns of silicic acid (Bartelt and Jackson, 1984). Elution solvents were hexane; 10% ether in hexane; 50% ether in hexane; and 10% methanol in methylene chloride. Further purification of the male 10% ether–hexane fraction was done by preparative GC. Preparative and analytical GC were conducted as described previously (Bartelt and Jackson, 1984).

Bioassay. Bioassays were conducted in a wind-tunnel olfactometer (Bartelt and Jackson, 1984). The bioassay was used to screen chromatographic fractions for activity and to compare various synthetic and fly-derived preparations. Approximately 1000 flies (0–2 days old) were placed into the olfactometer 2 hr before tests would begin. Tests lasted 3 min and were separated by 7–10 min. When an experiment included more than two treatments, they were tested in pairs, in all possible combinations (a balanced incomplete block design). Generally, each experiment included a high control [fly-derived or synthetic compound(s) that bioassayed well] and a low control (solvent) in addition to the preparation(s) being evaluated. The activity level of a treatment was usually expressed relative to the high and low controls because catches to any one treatment varied from day to day, but ratios between treatments remained fairly constant. Analysis of bioassay data was by the method of Yates (1940), paired

t tests, or simple linear regression. The data were transformed to the log (X + 1) scale before analysis to stabilize variance.

Once it was established that the flies responded to Z11-18:Ac, three additional bioassay experiments were conducted involving this compound. First, the relationship between magnitude of response and dose of Z11-18:Ac was investigated. Doses ranged from 0.1 ng to 10,000 ng, by factors of 10. Each bioassay experiment contained two consecutive doses and a control. All possible pairs of consecutive doses were tested, and results are expressed relative to the activity (= 100) for 1000 ng. Second, the relationship between response to Z11-18:Ac and time away from food was studied by measuring the bioassay response, compared to controls, every 15 min throughout the day. To distinguish between time-of-day effects and those due to time away from food, four replications of the experiment were begun at 0800 hr, while four others began at 1100 hr. Third, synergism between Z11-18:Ac and food odors (from rearing medium inoculated with yeast) was investigated, throughout the day (three replications) and at the time of "peak" activity.

Z11-18:Ac Transfer. An experiment was conducted to test for the transfer of Z11-18:Ac from males to females and for the subsequent release of the ester from the females. Virgin females (6-7 days old) were mated with virgin males of the same age. Immediately after mating, females were either extracted or placed into an empty glass vial or vial with food medium for 6 hr and then extracted. The vials were then rinsed with hexane to recover any Z11-18:Ac which had been released (Bartelt et al., 1985b). To recover the Z11-18:Ac quantitively, it was necessary to rinse the food vials again with methylene chloride. The mated males were treated in a similar fashion. Each extract represented 10 fly equivalents. As controls, comparable sets of extracts were obtained from males and females which had not been allowed to mate. The Z11-18:Ac in the extracts was quantitated by GC relative to an internal standard (Bartelt et al., 1985b). The rearing medium did not contain compounds with the same GC retention as Z11-18:Ac.

RESULTS

Identification of Pheromone Component. The male crude extract was clearly active in bioassay against a control ($P < 0.001$). For example, the mean catches for male extract and control were 13.9 and 1.4, respectively ($n = 16$). Flies attracted to the male extract were 54% female. Both sexes responded similarly to all preparations discussed in this report.

After fractionation of the male extract on silicic acid, only the 10% ether-hexane fraction was substantially different from the control ($P < 0.001$) in bioassay (Table 1). By capillary GC, the male 10% ether-hexane fraction was

TABLE 1. ACTIVITY OF SILICIC ACID FRACTION AND EXTRACTS RELATIVE TO MALE CRUDE EXTRACT

Fraction or extract[a]	Relative response[b]	
	Male extract	Female extract
Hexane	6.4*[c] (4.5, 3.0, 26.5)[d]	−0.7 (0.8, 0.9, 14.5)
10% ether-hexane	85.0***(26.4, 5.5, 30.1)	26.0***(12.9, 5.8, 32.7)
50% ether-hexane[e]	0.8 (1.7, 1.6, 9.1)	3.0 (4.2, 3.4, 29.0)
10% methanol-methylene chloride	2.6 (2.1, 1.6, 21.1)	15.7 (3.9, 2.5, 11.4)
Crude extract	100.0***	63.0***(9.3, 1.4, 13.9)

[a] All extracts and fractions were used at one fly equivalent per test.
[b] Relative response = (fraction − control)/(male crude extract − control) × 100.
[c] *and*** denote significance of t tests vs. controls at the 0.05 and 0.001 levels, respectively.
[d] Even catches ($N = 8$) for test treatment, control, and male crude extract, respectively, shown in parentheses.
[e] Because 50% ether-hexane solvent was attractive to the flies, this fraction was taken almost to dryness under nitrogen and back up in hexane.

86% one compound. Previous work with *D. melanogaster* (Bartelt et al., 1985b) and *D. simulans* (Jallon, 1984) suggested this was probably Z11-18:Ac. GC retention time of fly-derived and synthetic Z11-18:Ac (Sigma Chemical Co., St. Louis, Missouri), mass spectra, and double-bond location by ozonolysis-GC confirmed this identity. Young males 0–6 hr old possessed only trace amounts of Z11-18:Ac and virgin females of any age contained none.

The crude extract, 10% ether-hexane fraction, synthetic Z11-18:Ac (99+% pure) and fly-derived Z11-18:Ac (obtained from the 10% ether-hexane fraction by preparative GC, 98+% pure) were compared by bioassay (all at 640 ng Z11-18:Ac/test, ca. 1 fly equivalent, Table 2). All were significantly greater than the control ($P < 0.001$). The synthetic and fly-derived Z11-18:Ac treatments were not significantly different, and Z11-18:Ac accounted for all the activity of the male 10% ether-hexane fraction and crude extract. In this study the 10% ether-hexane fraction, synthetic Z11-18:Ac, and fly-derived Z11-18:Ac were significantly greater than the crude extract ($P < 0.05$).

Other Studies with Z11-18:Ac. Response to Z11-18:Ac increased with dose (Figure 1, slope significant, $P < 0.005$, simple linear regression). A dose of 1 ng, comparable to ca. 1/1000 of a male equivalent was significantly greater than the control ($P < 0.01$), although 0.1 ng was not. The 1000 ng and 10,000 ng doses were not significantly different from each other ($P > 0.2$), suggesting further increases in dose would cause little, if any, increase in response.

TABLE 2. SOURCES OF Z11-18:Ac COMPARED IN BIOASSAY

Source[a]	Mean bioassay catch[b] (N = 32)
Control	4.7a
Synthetic Z11-18:Ac	24.8c
Fly-derived Z11-18:Ac	25.0c
Male 10% ether-hexane	28.5c
Male crude extract	19.1b

[a] Compared on an equal-weight basis for Z11-18:Ac (640 ng/test).
[b] Means followed by a different letter were significantly different at the 0.05 level (LSD).

The responsiveness of the flies to Z11-18:Ac increased with time away from the rearing jars (Figure 2), becoming significantly greater than the control (P = 0.01) after 2 hr. The onset of response was accompanied by a change in behavior. When first introduced into the olfactometer, the flies remained on the floor and moved about very little. Within a few hours, the flies became more

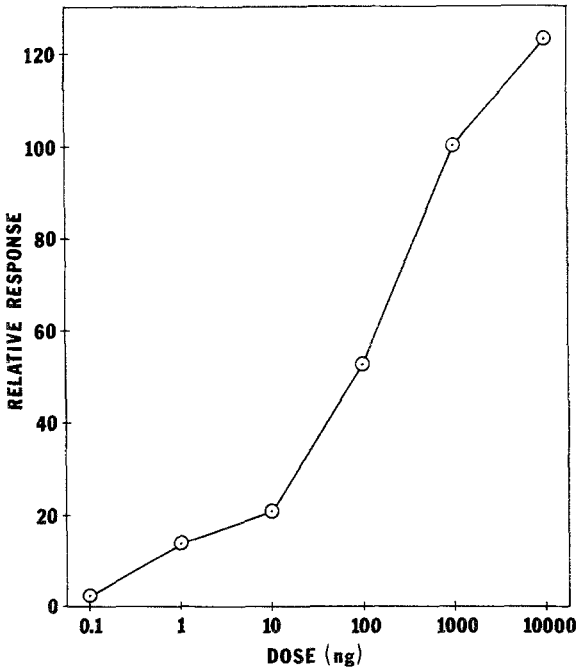

FIG. 1. Dose-response relationship for synthetic Z11-18:Ac. Relative responses are 100% for the 1000 ng dose and 0% for the controls. (See Table 1 for calculation of relative response.) Spacing along x axis is logarithmic.

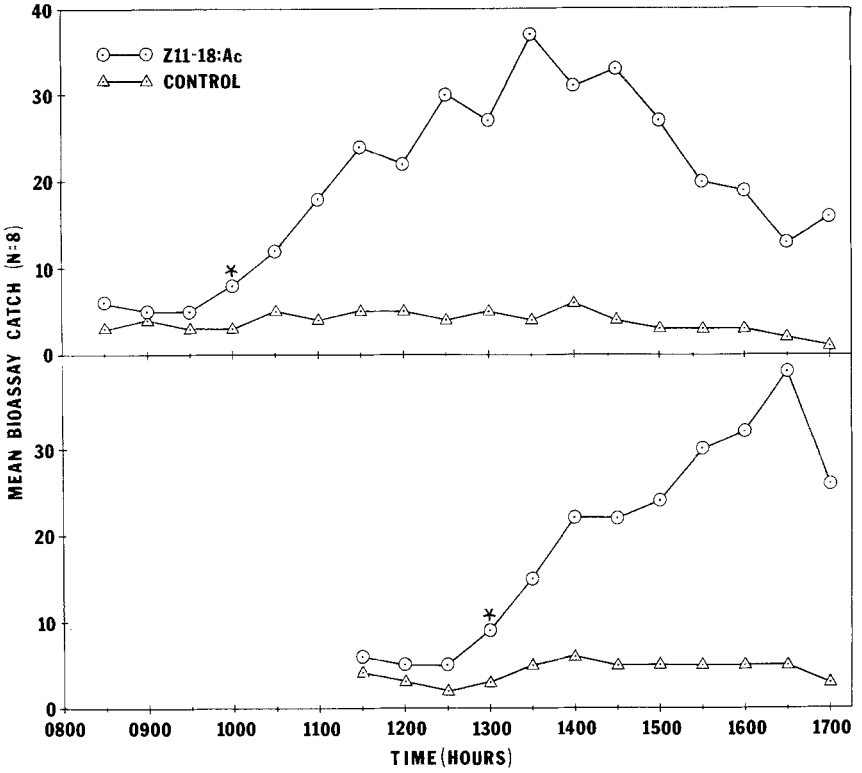

FIG. 2. Bioassay activity of Z11-18:Ac vs. time after removing flies from food. Flies were placed in the olfactometer at 0800 hr (upper panel) or 1100 hr (lower panel). Synthetic Z11-18:Ac was tested at 750 ng/test (ca. 1 fly equivalent). *Indicates when Z11-18:Ac became significantly greater than the control ($P = 0.05$).

active and spent more time on the sides of the olfactometer and in flight. This response continued throughout the day, although the number caught per test decreased with time because fewer flies were left in the olfactometer. Bioassay behavior included flying upwind with a hovering, casting flight and descending into the vial or alighting on the vial and walking inside.

When a food source (Petri plate with rearing medium and yeast) was present in the downwind end of the olfactometer, the response to Z11-18:Ac never commenced. After being tested for 6 hr, the mean catches for synthetic Z11-18:Ac (750 ng/test) and control were 0.43 and 0.29, respectively ($N = 14$). Most of the flies spent the entire day at the food plate, moving or flying very little.

The onset of the responsiveness to Z11-18:Ac appeared more related to time away from food than to time of day. The response to Z11-18:Ac became

TABLE 3. SYNERGISM OF SYNTHETIC Z11-18:AC AND FERMENTED FOOD

Treatment	Mean bioassay catch[a] (N = 18)
Control	0.8a
Synthetic Z11-18:Ac[b]	10.6b
Food[c]	43.2c
Z11-18:Ac + food	103.6d

[a] Means followed by a different letter were significantly different in the log (n + 1) scale at the 0.001 level (LSD).
[b] Synthetic Z11-18:Ac used at 750 ng/test (ca. 1 fly equivalent).
[c] "Food" is 0.5 ml of *Drosophila* rearing medium inoculated with yeast 24 hr before bioassays began and placed in the bottom of a bioassay vial.

significantly greater than the control (P = 0.01) 2 hr after placing the flies into the olfactometer, regardless of whether this was done at 0800 or 1100 hr (Figure 2).

Although successful bioassays could be conducted with *D. simulans* in the absence of food, Z11-18:Ac was a potent synergist of the fermented rearing medium (Table 3). Z11-18:Ac increased the response to the food treatment by 60 flies per test, even though Z11-18:Ac alone caught only 10 flies per test. The treatment, Z11-18:Ac plus fermented food, was so attractive that flies responded to it above control levels within minutes of placing the flies into the olfactometer (Figure 3). Yet response to this treatment, as well as to the ester and fermented food alone, did increase strongly over time, consistent with earlier results (Figure 2). Catches for Z11-18:Ac alone were relatively few in this study, presumably due to the competition from the food-containing treatments. Nevertheless, Z11-18:Ac became significantly greater than the control 2 hr after tests began (P = 0.05), in agreement with earlier results.

Z11-18:Ac Transfer. Virgin females did not possess any Z11-18:Ac. However, males transferrred approximately half of the Z11-18:Ac they possessed to the female during mating (Table 4). Within 6 hr after copulation, the female deposited the majority of the Z11-18:Ac transferred into a vial which contained rearing medium or even into an empty glass vial. The amount of Z11-18:Ac emitted by a recently mated female was ca. 10 times greater than that emitted by a virgin male. Few eggs were detected in the rearing medium vials after 6 hr, and none were in the empty vials.

Other Attractants. In bioassay, attraction to the female extract was significantly greater than the control (Table 1). Bioassay of all female silicic acid fractions indicated the 10% ether–hexane fraction was significantly above control levels (P < 0.001, Table 1), although not as active as the male-derived fraction. Thus a relatively nonpolar attractant in females of *D. simulans* is in-

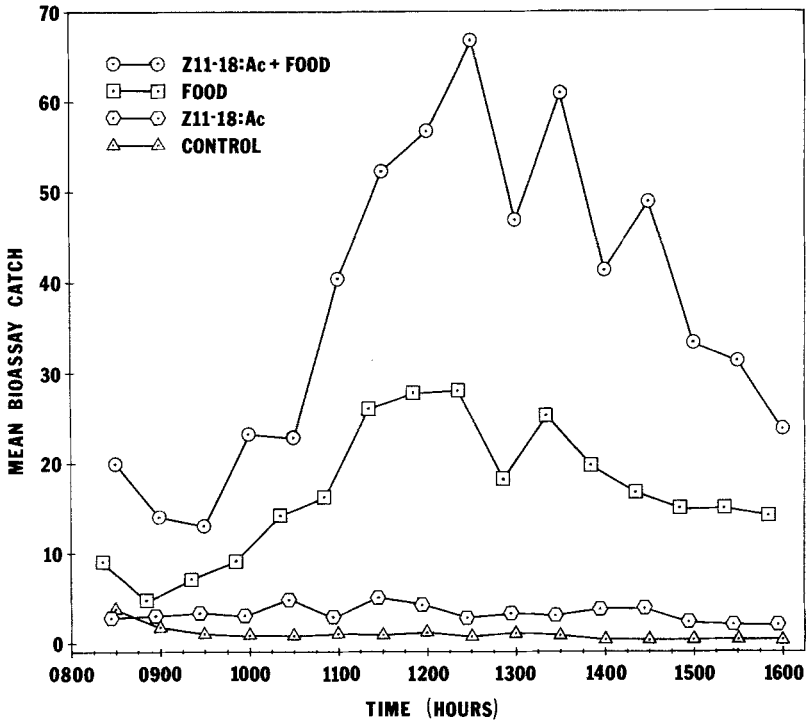

FIG. 3. Synergism of Z11–18 : Ac and fermented food over time. In each test, the treatment was bioassayed against a control. Except for controls, each point represents the mean of six catches (3 replications of experiment × 2 tests in each time interval). All control catches were combined to simplify the figure ($N = 18$); the means in any one time interval differed by no more than ±1 fly. Z11–18 : Ac was used at 750 ng/test (ca. 1 fly equivalent).

dicated (by GC no Z11–18 : Ac was detected). Identification of the attractant in this fraction is presently under investigation.

The male hexane fraction was also significantly greater than the control ($P = 0.05$) but accounted for only 6% of the activity of the male extract. The response was very subtle, and isolation of the active compound(s) under the present bioassay scheme would be very difficult.

The 10% methanol–methylene chloride fractions for both male and female were not significantly different from the control, although the extraction process for the crude extracts was geared toward the nonpolar attractants. A more thorough extraction may reveal active polar fractions, as observed in other *Drosophila* species (Bartelt et al., 1985a, 1986).

TABLE 4. MEAN AMOUNTS OF Z11-18:Ac EXTRACTED FROM MATED AND VIRGIN FLIES, AND FROM EMPTY VIALS OR FOOD VIALS WHICH THEY OCCUPIED FOR 6 HOURS

	Mean amount of Z11-18:Ac (ng/fly)			
Treatment	Mated male[a]	Mated female[a]	Virgin male	Virgin female
Flies immersed in hexane ($N = 4$)[b]				
Fly extract	386(±68)[c]	418(±112)	640(±137)	0
Flies in empty vial for 6 hr ($N = 2$ virgin, $N = 4$ mated)				
Fly extract	475(±65)	123(±81)	990(±438)	0
Vial rinse	30(±14)	158(±30)	0	0
Flies in food vial for 6 hr ($N = 2$ virgins, $N = 5$ mated)				
Fly extract	416(±89)	94(±38)	905(±78)	0
Vial rinse	48(±40)	188(±61)	15(±7)	0

[a] These flies either extracted or placed in vials for 6 hr immediately upon completion of mating.
[b] Each replication represents a group of 10 flies.
[c] Standard deviation.

DISCUSSION

Males of *D. simulans*, like *D. melanogaster*, possess Z11-18:Ac as a major pheromone component which attracts males and females in a wind-tunnel olfactometer. In *D. melanogaster*, Z11-18:Ac is found in the ejaculatory bulb, transferred to the female during mating (Brieger and Butterworth, 1970; Butterworth, 1969), and deposited by the female onto the rearing medium within 6 hr after completion of mating (Bartelt et al., 1985b). *D. simulans* is similar to *D. melanogaster* in the transfer and release of Z11-18:Ac. In both species, the mature male emits Z11-18:Ac in small quantities and a recently mated female in larger amounts. *D. simulans* is also similar to *D. virilis* in that both species must be deprived of food in order to be attracted to their aggregation pheromone in the olfactometer (Bartelt and Jackson, 1984).

Food or food odors are not needed in combination with Z11-18:Ac for a successful bioassay with *D. simulans*, as is the case for *D. melanogaster* (Bartelt et al., 1985b). Therefore, *D. simulans* would be the more convenient species in which to investigate additional, minor pheromone components.

In nature, mating, feeding, and oviposition occur at the same site. It is accepted that flies are attracted to food sites by odors (Spieth, 1974). We believe attraction to a site is a complex phenomenon, involving both food odors and fly-derived compounds. *D. simulans* males produce an attractant, Z11-18:Ac, that is a powerful synergist of food odors. One nanogram of Z11-18:Ac was sig-

nificantly more attractive than a control in our bioassay, and over a 6-hr period, a mature male or recently mated female is capable of emitting well over this amount at a food site. Since 10 times more Z11–18:Ac is emitted by recently mated females than virgin males, mating sites would be particularly attractive. Interestingly, Spence et al. (1984) found that male *D. simulans* are preferentially attracted to cylinders which had contained recently mated females compared to those with males or virgin females. Thus, a fly without a food source, moving through an area, would tend to alight preferentially at a food source which was already occupied by others of the species, especially if mating had taken place. Once at a food site, the flies would tend to remain on or near the food (Spieth, 1974). Flies in the olfactometer did not respond to Z11–18:Ac when given a food source or until deprived of food for a few hours, which indicates that responsiveness to the aggregation pheromone diminishes upon feeding.

Acknowledgments—This research was supported by NSF grant DCB-8509976 and the Montana Agricultural Experiment Station. Published as MAES Journal Series No. 1917. We are grateful to Darlene Ferderer, Jeane MacDonald, and Liane Tanico for technical assistance.

REFERENCES

BARTELT, R.J., and JACKSON, L.L. 1984. Hydrocarbon component of the *Drosophila virilis* (Diptera: Drosophilidae) aggregation pheromone: (*Z*)-10-Heneicosene. *Ann. Entomol. Soc. Am.* 77:364–371.

BARTELT, R.J., JACKSON, L.L., and SCHANER, A.M. 1985a. Ester components of the aggregation pheromone of *Drosophila virilis* (Diptera: Drosophila). *J. Chem. Ecol.* 11:1197–1208.

BARTELT, R.J., SCHANER, A.M., and JACKSON, L.L. 1985b. *cis*-Vaccenyl acetate as an aggregation pheromone in *Drosophila melangogaster. J. Chem. Ecol.* 11:1747–1756.

BARTELT, R.J., SCHANER, A.M., and JACKSON, L.L. 1986. Aggregation pheromone in five taxa of the *Drosophila virilis* species group. *Physiol. Entomol.* 11:367–376.

BRIEGER, G., and BUTTERWORTH, G.M. 1970. *Drosophila melanogaster:* Identity of male lipid in reproductive system. *Science* 167:1262.

BUTTERWORTH, F.M. 1969. Lipids of *Drosophila:* A newly detected lipid in the male. *Science* 163:1356–1357.

JALLON, J.-M. 1984. A few chemical words exchanged by *Drosophila* during courtship and mating. *Behav. Genet.* 14:441–477.

JALLON, J.-M., ANTONY. C., and BENAMAR, O. 1981. Un antiaphrodisiaque produit par les males de *Drosophila melanogaster* et transferé aux femelles lors de la copulation. *C.R. Acad. Sci. Paris* 292:1147–1149.

MOATS, R.A., BARTELT, R.J., JACKSON, L.L., and SCHANER, A.M. 1987. Ester and ketone components of the aggregation pheromone of *Drosophila hydei. J. Chem. Ecol.* 13:451–462.

SPENCE, G.E., HOFFMANN, A.A., and PARSONS, P.A. 1984. Habitat marking: Males attracted to residual odors of two *Drosophila* species. *Experientia* 40:763–765.

SPIETH, H.T. 1974. Courtship behavior in *Drosophila. Annu. Rev. Entomol.* 19:385–405.

YATES, F. 1940. The recovery of interblock information in balanced incomplete block designs. *Ann. Eugen.* 10:317–325.