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AGGREGATION PHEROMONE CHARACTERIZATION AND COMPARISON IN *Drosophila ananassae* and *Drosophila bipectinata*

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Abstract—(*Z*)-11-Octadecenyl acetate (Z11-18:Ac) and (*Z*)-11-eicosenyl acetate (Z11-20:Ac) were identified as the aggregation pheromones of *Drosophila ananassae*, and Z11-20:Ac was identified as the aggregation pheromone of *Drosophila bipectinata*. Z11-18:Ac and Z11-20:Ac were not attractive alone; however, in combination with fermenting food odors, the acetates attracted flies of both sexes in a wind-tunnel olfactometer. The pheromones were present in the ejaculatory bulb of sexually mature male flies and transferred to the female during mating. Male *D. bipectinata* released little if any Z11-20:Ac to the food; however, recently mated females released Z11-20:Ac to the surrounding surfaces in just a few hours after mating. *D. ananassae* males, on the other hand, appeared to release more Z11-18:Ac and Z11-20:Ac to the surroundings than mated females. Although *D. bipectinata* males had no Z11-18:Ac, flies were as attracted to Z11-18:Ac as to an equal quantity of Z11-20:Ac. *D. ananassae* were attracted to Z11-18:Ac but not to Z11-16:Ac or Z11-20:Ac. However, Z11-20:Ac in combination with Z11-18:Ac was significantly more attractive than Z11-18:Ac alone.

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

INTRODUCTION

In all *Drosophila* species studied to date, aggregation pheromones exist that are produced exclusively by the sexually mature males, attractive to both sexes,

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and most active in combination with food odors (Bartelt and Jackson, 1984; Bartelt et al., 1985a,b, 1986, 1988; Moats et al., 1987; Schaner et al., 1987, 1989) In the *virilis* species group, the aggregation pheromones are a blend of hydrocarbons and esters of tiglic and hexanoic acids (Bartelt and Jackson, 1984; Bartelt et al., 1985a, 1986, 1988). The hydrocarbons are transferred by the male to the food media (unpublished). In *D. hydei*, a member of the *repleta* group, the aggregation pheromone is a mixture of ketones along with the esters of tiglic acid (Moats et al., 1987). The esters of tiglic acid are a component of the pheromone blend common to both groups, yet each required compounds with different chemical functional groups in combination with the esters for optimal aggregation response. In the *melanogaster* subgroup of the *melanogaster* group, *D. melanogaster* and *D. simulans* sexually mature males produce (*Z*)-11-octadecenyl acetate (Z11-18 : Ac) (Bartelt et al., 1985b; Schaner et al., 1987); however, in the *ananassae* subgroup, *D. malerkotliana* males produce (*Z*)-11-eicosenyl acetate (Schaner et al., 1989). In both subgroups the pheromone is stored in the ejaculatory bulb, transferred to the females during mating, emitted by the female to the food within a few hours after mating, and functions as the aggregation pheromone.

We report here the identification, transfer, and bioassay characteristics of the aggregation pheromones in *D. ananassae* and *D. bipectinata*, also of the *ananassae* subgroup.

METHODS AND MATERIALS

Flies. D. ananassae (strain 14024-0371) and *D. bipectinata* (strain 14024-0381) were obtained from the National Drosophila Species Resource Center at Bowling Green, Ohio. Flies were reared on Instant Drosophila Medium Formula 4-24 (Carolina Biological Supply Co., Burlington, North Carolina) in 1-liter jars or in 3.5 × 10-cm vials under a light–dark cycle of 16 : 8 hr and 22–24°C.

The method of extraction, chromatography, identification, bioassay, and pheromone transfer have been reported previously (Bartelt and Jackson, 1984; Bartelt et al., 1985b; Schaner et al., 1987, 1989). Briefly, flies were separated by sex at 0–6 hr old and extracted at 6–7 days of age by soaking the flies in hexane at room temperature for 24 hr. Hexane extracts were fractionated on open columns of silicic acid eluted with: hexane; 10% ether in hexane; 50% ether in hexane; and 10% methanol in methylene chloride.

Bioassays were conducted in a wind-tunnel olfactometer containing ca. 1000 (0- to 2-day-old) flies that had been without food or water for approximately 2 hr. An extract, fraction, synthetic compound, or control solvent was applied to a filter paper inserted around the lip of a glass vial. Each bioassay

test consisted of placing two differently treated vials to be compared into the olfactometer for 3 min. The bioassay data were transformed to the log $(X + 1)$ scale before analysis to stabilize variance, and analysis was done by the method of Yates (1940).

Production, Transfer, and Dispersal of Pheromone. The methods have previously been described (Schaner et al., 1989). Briefly, ejaculatory bulbs were removed from adult male flies at various ages, extracted with hexane, and analyzed by gas chromatography (GC). The transfer of pheromone during mating was quantitated by removing the reproductive tract of females and the ejaculatory bulb and ducts of males immediately after mating, crushing the organs with the head of a dissecting pin, extracting with hexane, and analyzing by GC relative to an internal standard.

RESULTS AND DISCUSSION

Identification of Pheromone Components. Crude hexane extracts of either male or female flies were not active in aggregation assays (2.6 and 2.1 mean catch, respectively, for *D. ananassae* and *D. bipectinata* male extracts alone). Other *Drosophila* frequently require food or food odors in combination with the pheromone to demonstrate or enhance aggregation activity (Bartelt et al., 1985b, 1986, 1988; Schaner et al., 1989). Both *D. bipectinata* and *D. ananassae* crude hexane extract of males were synergistic with fermenting food (Table 1, A). For convenience, solvents such as acetone (Bartelt et al., 1985b; Schaner et al., 1989) and 0.1% acetic acid were tested to mimic the coattraction observed with fermenting food (Table 1, B). Both acetone and acetic acid were effective coattractants. Acetic acid in combination with the crude extracts attracted more flies, so it was used throughout this investigation.

The crude hexane extracts of mature male flies along with acetic acid were clearly active in bioassay for both species (Table 2), whereas the extracts from female flies were inactive. After chromatography of the extracts of male flies, the most active fraction in both species was the 10% ether–hexane fraction, which contains the acetate esters (Bartelt et al., 1985b; Schaner et al., 1987, 1989). Gas chromatography and gas chromatography–mass spectrometry (GC-MS) of the 10% ether–hexane fraction revealed that in *D. ananassae* the fraction was 29% (*Z*)-11-octadecenyl acetate (*Z*11–18:Ac) and although no (*Z*)-11-eicosenyl acetate (*Z*11–20:Ac) was detected in this fraction, a small amount of *Z*11–20:Ac was later discovered in the ejaculatory bulb. In *D. bipectinata* the fraction was 50% *Z*11–20:Ac with no detectable *Z*11–18:Ac. The position of the unsaturation was determined by GC-MS of the dimethyl disulfide derivative (Nichols et al., 1986).

In bioassay, the 10% ether–hexane fraction accounted for more than all of

TABLE 1. FIVE SERIES OF BIOASSAY EXPERIMENTS WITH EXTRACTS OF *D. ananassae* AND *D. bipectinata* TO ISOLATE AND CHARACTERIZE AGGREGATION PHEROMONE.

Treatment[a]	Mean bioassay catch	
	D. ananassae	*D. bipectinata*
A. Synergism of crude extracts of males with fermenting food	(N = 12)	
Control	0.4 A[b]	1.6 A
Crude extract of males	2.6 B	2.1 A
Food[c]	10.3 C	10.3 B
Crude extract + food	27.8 D	20.3 C
B. Synergism of crude extract of males with food odors	(N = 12)	
Crude extract of males	2.5 A	2.2 A
Crude extract + acetone	7.4 B	6.0 B
Crude extract + acetic acid[d]	11.4 C	34.9 C
C. Synergism of synthetic pheromone with fermenting food	($N \geq$ 12)	
Z11-18:Ac	0.9 B	ND[e]
Z11-20:Ac	ND	0.7 A
Food	6.4 C	19.5 B
Z11-18:Ac + food	24.5 D	ND
Z11-20:Ac + food	ND	57.3 C
Hexane control	0.0 A	0.6 A
D. Comparative bioassay response to synthetic acetates	(N = 18)	
Control (acetic acid)	1.7 A	1.2 A
Z11-16:Ac + acetic acid	2.4 A	1.9 A
Z11-18:Ac + acetic acid	11.3 B	11.4 B
Z11-20:Ac + acetic acid	3.0 A	9.2 B
E. Synergism of synthetic Z11-18:Ac and Z11-20:Ac in *D. ananassae*	(N = 12)	
Control (acetic acid)	6.9 A	ND
Z11-18:Ac + acetic acid	16.4 B	ND
Z11-20:Ac + acetic acid	6.7 A	ND
Z11-18:Ac + Z11-20:Ac + acetic acid	24.6 C	ND

[a] All fly-derived fractions and their synthetic counterparts were used at 300 ng acetate ester for *D. bipectinata* and 12 ng acetate ester for *D. ananassae*.
[b] Means followed by the same letter were not significantly different at the 5% level (LSD).
[c] Fermenting food was Formula 4-24 Instant Drosophila Medium (Carolina Biological Supply, Burlington, North Carolina) to which active yeast had been added at least 24 hr prior to testing.
[d] Acetic acid is a 0.1% solution.
[e] Not determined.

TABLE 2. AGGREGATION RESPONSE IN A WIND-TUNNEL OLFACTOMETER TO HEXANE EXTRACTS OF *D. ananassae* AND *D. bipectinata* AND FRACTIONS OF THESE EXTRACTS.

Fraction or extract from respective species	Relative response[a] ($N = 8$)	
	D. ananassae	*D. bipectinata*
Crude extract of males	100***[b]	100***
Hexane fraction	−2	−8
	(2.1, 1.9, 10.5)[c]	(3.4, 2.5, 14.3)
10% ether fraction	142***	224***
	(1.2, 22.4, 16.1)	(2.4, 11.6, 6.5)
50% ether fraction	44***	−51
	(1.4, 6.5, 12.9)	(3.5, 0.8, 8.8)
10% MEOH/CH_2Cl_2 fxn.	9**	−23
	(1.2, 2.9, 19.5)	(2.7, 1.0, 10.1)
Crude extract of females	7	−16
	(1.9, 2.9, 16.6)	(2.6, 1.5, 9.6)

[a]Relative response = [(fraction − control)/(male crude extract − control)] × 100.
[b]** and *** denote significance of *t* tests vs. controls at the 0.01 and 0.001 level, respectively.
[c]The numbers in parenthesis are mean catch of flies to 0.1% acetic acid control, fraction + 0.1% acetic acid, male crude extract + 0.1% acetic acid.

the activity of the male crude extract (Tables 2 and 3). This suggests that the active compounds were in the 10% ether–hexane fraction, but the crude extract may have had compounds that diminish the aggregation activity. Synthetic Z11-18:Ac and Z11-20:Ac accounted for all the aggregation activity of the male 10% ether–hexane fraction for *D. ananassae* and *D. bipectinata*, respectively.

Since the crude hexane extracts of males were synergistic with fermented food, synthetic Z11-18:Ac and Z11-20:Ac were bioassayed with *D. ananassae* and *D. bipectinata*, respectively, to determine whether they also were synergistic with the aggregation activity of food. The synthetic acetate esters were synergistic with fermenting food (Table 1, C).

Aggregation Activity Specificity. The specificity of the flies' response to a certain chain length Z11-acetate was tested, and *D. ananassae* showed high specificity for Z11-18:Ac and no attraction toward Z11-20:Ac (Table 1, D). Because males possess both Z11-18:Ac and Z11-20:Ac, the two were combined to test for synergism. Z11-18:Ac and Z11-20:Ac together were significantly more attractive than Z11-18:Ac alone (Table 1, E). *D. bipectinata* responded equally to either Z11-18:Ac or Z11-20:Ac even though males produce only Z11-20:Ac. Neither species was attracted to Z11-16:Ac. In previous studies (Schaner et al., 1989), *D. melanogaster* and *D. simulans* were

TABLE 3. COMPARATIVE AGGREGATION ACTIVITY OF EXTRACTS, FRACTIONS, AND SYNTHETIC COMPOUNDS

Treatment	Mean bioassy catch (N= 8)	
	D. ananassae	*D.bipectinata*
Control (acetic acid)[a]	2.2 A[b]	5.8 A
Male crude + acetic acid	10.0 B	16.7 B
−10% Ether fxn. + acetic acid	15.6 C	23.7 C
Syn. Z11-18:Ac[c] + acetic acid	14.7 BC	ND[e]
Syn. Z11-20:Ac[d] + acetic acid	ND[e]	25.1 C

[a] Acetic acid is a 0.1% solution.
[b] Means followed by the same letter were not significantly different at the 5% level (LSD).
[c] 12 ng matching contents in 10% ether fxn. and crude from *D. ananassae* males.
[d] 160 ng matching content in 10% ether fxn. and crude from *D. bipectinata* males.
[e] not determined.

attracted only to Z11-18:Ac, but *D. malerkotliana* responded to both Z11-18:Ac and Z11-20:Ac. *D. melanogaster* preferred Z11-16:Ac slightly over controls, but neither of the other species were attracted to Z11-16:Ac. Of the five species so far tested against these three acetate esters, there was a range of specificity that does not follow taxonomic lines, but varies within each species group. A more complete rationale for the specificity variation awaits further studies of other species.

Dose Response. For *D. ananassae*, when a dose of Z11-18:Ac was increased from 1.2 ng to 12 ng, there was a dramatic increase in the aggregation activity (Figure 1). Activity increased only slightly when the dose increased to 1200 ng. The dose of 12 ng of Z11-18:Ac was the lowest dosage to result in a maximum dose plateau so far observed for this type of aggregation pheromone. For *D. bipectinata* (Figure 1), the same trend was observed. There was also a dramatic increase in response when the dose of Z11-20:Ac was increased from 20 ng to 200 ng. From 200 ng to 2000 ng, and from 2000 ng to 20,000 ng, the response of the flies increased slightly, but not significantly.

Location, Production, Transfer, and Dispersal of Aggregation Pheromones. As previously observed in *D. melanogaster*, *D. simulans*, and *D. malerkotliana* (Brieger and Butterworth, 1970; Schaner et al., 1987, 1989), the ejaculatory bulb of *D. ananassae* and *D. bipectinata* mature male flies was the site of storage of the aggregation pheromone. Analysis of *D. ananassae* ejaculatory bulb contents (Figure 2) showed an increase in Z11-18:Ac with age from 0 ng/fly at day 1 up to a plateau of ca. 52 ng/fly on day 7. The ejaculatory bulbs of male *D. ananassae* also contained Z11-20:Ac, which increased after day 1 to a plateau of about 15–17 ng/fly. *D. ananassae* was the only species

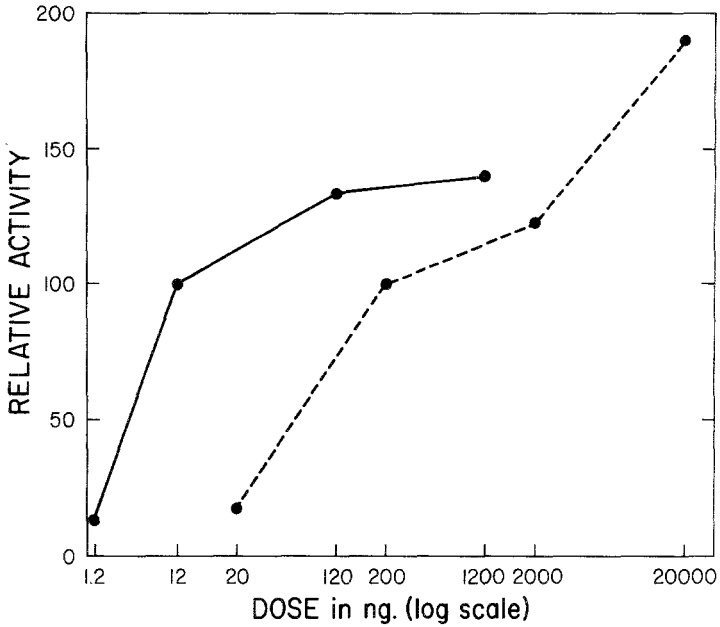

FIG. 1. Dose-response curves relative to the amount of pheromone that was hexane extractable from content of one mature male fly (100%). Solid line was the response for *D. ananassae* to Z11-18:Ac. Dashed line was the response for *D. bipectinata* to Z11-20:Ac.

found to possess both the aggregation pheromone common to the *melanogaster* subgroup (Z11-18:Ac) and that of the *ananassae* subgroup (Z11-20:Ac).

D. bipectinata male flies produce ca. 30 ng of Z11-20:Ac at 1 day of age (Figure 2) and ca. 300 ng/fly by 4 days of age, where they reach a plateau. Z11-18:Ac was not detected in ejaculatory bulb extracts of *D. bipectinata*, even though they repsonded to Z11-18:Ac in aggregation assays (Table 1, D). The amount of Z11-20:Ac produced in the ejaculatory bulb and the bioassay response towards both Z11-18:Ac and Z11-20:Ac was the same as that observed for *D. malerkotliana* (Schaner et al., 1989).

As was observed in *D. melanogaster* (Brieger and Butterworth, 1970; Butterworth, 1969; Jallon et al., 1981; Bartelt et al., 1985b), *D. simulans* (Schaner et al., 1987), and *D. malerkotliana* (Schaner et al., 1989), a portion of the aggregation pheromone in the ejaculatory bulb of *D. ananassae* and *D. bipectinata* was transferred to the female fly during mating (Table 4). *D. bipectinata* male flies transferred about two thirds of their Z11-20:Ac to the female during mating and nearly half of the Z11-20:Ac was released by the female flies to the vial within 6 hr. Neither mated nor virgin males of *D. bipectinata* released

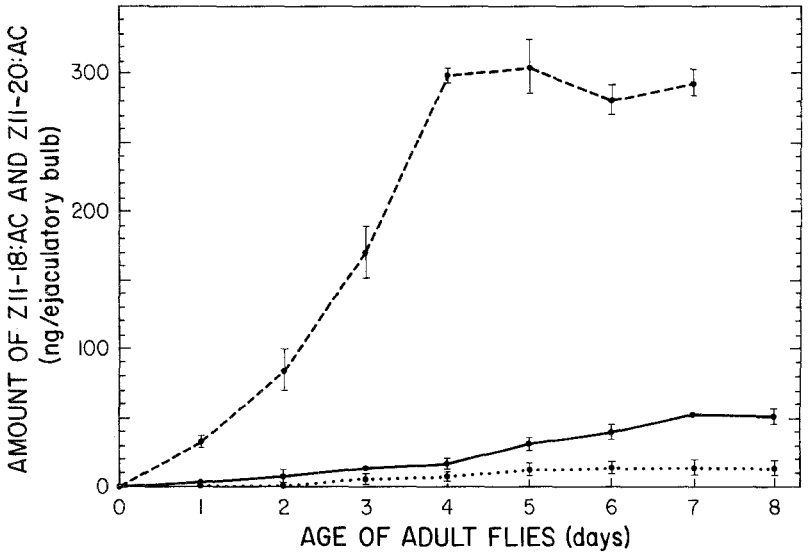

FIG. 2. Pheromone content in the ejaculatory bulb of male flies as they age. Dashed line for Z11-20:Ac in *D. bipectinata*. Solid line for Z11-18:Ac and dotted line for Z11-20:Ac in *D. ananassae*. The bars indicate standard deviation.

Z11-20:Ac to their surroundings in 6 hr. *D. ananassae* male flies, on the other hand, transferred both Z11-18:Ac (ca. 14 ng/fly) and Z11-20:Ac (ca. 5 ng/fly) to the female reproductive tract. Within 6 hr after mating, female flies released only a trace of both compounds to the vial and had retained most of the transferred compounds in their reproductive tracts. Both mated and virgin *D. ananassae* males release Z11-18:Ac to their surroundings in 6 hr (Table 4). This was the first time that the release by males, either mated or virgin, was greater than the release by mated female flies. By 12 hr after mating, the mated females had released approximately half of the Z11-18:Ac that was transferred. The release by mated males during 12 hr after mating was ca. 35 ng of Z11-18:Ac and ca. 2 ng of Z11-20:Ac per fly, which was about seven times more than was released by the mated females. Virgin males also released similar amounts. This suggests that there was not only release, but also synthesis of at least Z11-18:Ac. Unlike the *Drosophila* species observed so far, *D. ananassae* males were the primary releasers of the aggregation pheromone rather than mated females, as observed here for *D. bipectinata* and previously for *D. melanogaster* (Bartelt et al., 1985b), *D. simulans* (Schaner et al., 1987), and *D. malerkotliana* (Schaner et al., 1989).

In nature, mating, feeding, and oviposition all occur at the same location, and flies are attracted to these locations by odors (Spieth, 1974). We believe this attraction is caused not only by food odors but often by a combination of

TABLE 4. TRANSFER OF AGGREGATION PHEROMONES FROM MALES TO FEMALES DURING MATING AND RELEASE OF PHEROMONES BY MATED MALES AND FEMALES

	Pheromone (ng/fly)		
	D. ananassae[b]		*D. bipectinata*[c]
Source[a]	Z11-18:Ac	Z11-20:Ac	Z11-20:Ac
Virgin male	56	18	297
Mated male	15	6	110
Virgin female	0	0	0
Mated female	14	5	200
6 hrs (12 hr) after mating[d]			
Mated male	19 (21)	8 (6)	117
Vial	6 (35)	0 (2)	0
Mated female	9 (7)	4 (0)	126
Vial	tr (5)	tr (tr)	96
Virigin male	47 (45)	16 (16)	300
Vial	8 (38)	0 (3)	0

[a] Ejaculatory bulb for males and reproductive tract for females.
[b] $N \geq 2$ groups of 10.
[c] $N \geq 3$ groups of 5.
[d] Flies were stored in vials for 6 hr after mating and then the ejaculatory bulb for males and reproductive tract for females were extracted. The vials were also extracted. The remainder of the fly was extracted but the content of acetates was always below detectable levels.

food odors and fly-derived compounds. *D. ananassae* and *D. bipectinata* males produce an aggregation pheromone that is synergistic with food odors. In the case of *D. bipectinata*, males cannot release the pheromone, but a recently mated female is capable of emitting levels of Z11-20:Ac that are attractive with a food source in our olfactometer. On the other hand, a *D. ananassae* male or recently mated female can release levels of Z11-18:Ac that are also attractive with a food source. These results suggest that in nature, flies are not only attracted to feeding sites by food odors, but they may demonstrate an even stronger attraction to food sources that are already occupied by others of the same species.

In summary, aggregation pheromones in *D. ananassae* and *D. bipectinata* have been identified and characterized, and attraction towards them has been demonstrated. The pheromones are (*Z*)-11-octadecenyl acetate and (*Z*)-11-eicosenyl acetate in *D. ananassae* and (*Z*)-11-eicosenyl acetate in *D. bipectinata*. In both species the pheromones are produced exclusively by sexually mature male flies, stored in the ejaculatory bulb, transferred to the female during mating, synergistic with food odors, and attracted flies of both sexes in a wind-tunnel olfactometer.

The *ananassae* species group is divided into two species complexes based on the structure of the male genitalia (reviewed by Lemeunier et al., 1986). *D. malerkotliana* and *D. bipectinata* of the *bipectinata* complex both produce Z11–20 : Ac, which is released by mated female flies, and they respond to either Z11–18 : Ac or Z11–20 : Ac. *D. ananassae* in the *ananassae* complex produces both Z11–18 : Ac and Z11–20 : Ac, which is released by mature male flies, and they respond to Z11–18 : Ac and a combination of Z11–18 : Ac with Z11–20 : Ac, but not to Z11–20 : Ac alone. Whether the production and response specificity is present in other members of the *ananassae* and *bipectinata* complexes remains to be determined.

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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 aggregation pheromone of *Drosophila virilis* (Diptera: Drosophilidae). *J. Chem. Ecol.* 11:1197–1208.

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

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

BARTELT, R.J., SCHANER, A.M., and JACKSON, L.L. 1988. Aggregation pheromones in *Drosophila borealis* and *Drosophila littoralis*. *J. Chem. Ecol.* 14:1319–1327.

BARTELT, R.J., SCHANER, A.M., and JACKSON, L.L. 1989. Aggregation pheromone components in *Drosophila mulleri*: A chiral ester and an unsaturated ketone. *J. Chem. Ecol.* 15:399–411.

BRIEGER, G., and BUTTERWORTH, G.M. 1970. *Drosophila melanogaster*: Identity of male lipid in reproductive system. *Science* 67:1262.

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

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

LEMEUNIER, F. DAVID, J.R., TSACAS, L., and ASHBURNER, M. 1986. The *melanogaster* species group, pp. 147–256, *in* M. Ashburner, H.L. Carson and J. N. Tompson (eds.). The Genetics and Biology of *Drosophila*. Vol. 3e. Academic Press, New York.

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

NICHOLS, P.D., GUCKERT, J.B., and WHITE, D.C. 1986. Determination of monounsaturated fatty acid double-bond positions and geometry for microbial monocultures and complex consortia by capillary GC/MS of their dimethyl disulfide adducts. *Microbial Methods* 5:49–55.

SCHANER, A.M., BARTELT, R.J., and JACKSON, L.L. 1987. (Z)-11-Octadecenyl acetate, and aggregation pheromone in *Drosophila simulans*. *J. Chem. Ecol.* 13:1777–1786.

SCHANER, A.M., JACKSON, L.L., GRAHAM, K.J., and LEU, R.D. 1989. (*Z*)-11 Eicosenyl acetate, an aggregation pheromone in *Drosophila malerkotliana*. *J. Chem. Ecol.* 15:265–273.

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. Engen.* 10:317–325.