Pergamon

Journal of Insect Physiology 48 (2002) 945–950

Journal of Insect Physiology

www.elsevier.com/locate/jinsphys

Semiochemicals from ovaries of gravid females attract ovipositing female houseflies, *Musca domestica*

Yong Jiang [a,b], Chao-liang Lei [b], Chang-ying Niu [b], Yu-ling Fang [a], Chun Xiao [a], Zhong-ning Zhang [a,*]

[a] *Chinese Academy of Sciences, Institute of Zoology, State Key Laboratory of Integrated Management of Insects and Rodents, No. 19, Zhong-guan-cun Road, 100080 Beijing, China*

[b] *Huazhong Agricultural University, Department of Plant Protection, No. 1, Section Wuchang, Shi-zi Moutain Road, 430070 Wuhan, China*

Received 28 March 2002; received in revised form 11 July 2002; accepted 15 July 2002

Abstract

Chemical signals originating from the ovaries of gravid females of *Musca domestica* (Diptera: Cyclorrhapha: Muscidae) attract ovipositing females to common egg-laying sites. Behavioral experiments indicated that females preferred to oviposit in fermented wheat bran containing ovaries from reproductively mature houseflies. Females preferred to oviposit in fermented wheat bran than wet wheat bran. This effect was additive with the attraction to housefly ovaries. Solvent extracts from housefly ovaries were attractive to gravid females. Extracts obtained with hexane were most attractive to gravid females for egg laying, and extracts obtained with ethyl acetate attracted more egg laying than extracts obtained by dichloromethane. Gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC–MS) analysis showed that tricosane and (*Z*)-9-tricosene were the main components of the hexane extracts. Both tricosane and (*Z*)-9-tricosene were shown to elicit dose-dependent aggregation of gravid females in oviposition bioassays, but high doses of either chemical were not attractive.

Keywords: *Musca domestica*; Houseflies; Semiochemicals; Ovaries; Gravid female; Egg laying; Tricosane; (Z)-9-tricosene

1. Introduction

The pheromone of the house fly, *Musca domestica*, consists of (*Z*)-9-tricosene (Carlson et al., 1971), *cis*-9,10-epoxytricosane, (*Z*)-14-tricosene-10-one (Uebel et al., 1978) and a complex mixture of methylalkanes (Uebel et al., 1976; Nelson et al., 1981), which function as short-range attractants, sex-recognition factors, and arrestants (Adams and Holt, 1987). Oviposition pheromones that affect egg laying by gravid female insects have been identified; for example, acetophenone and veratrole for *Schistocerca gregaria* (Rai et al., 1997), and an unidentified mix of chemicals for *Simulium damnosum* complex (McCall, 1995; McCall et al., 1997). Interestingly, those semiochemicals can be obtained from egg masses, freshly deposited larvae, eggs, or ovaries. Experimental populations of houseflies can aggregate and lay their eggs in common sites (Jiang et al., 1999). We identified materials from the ovaries of mature houseflies in order to find semiochemicals that can regulate egg-laying behavior, possibly for the industrial production of houseflies, as well as to support the control of wild houseflies.

2. Methods and materials

2.1. Insects

Housefly, *Musca domestica* L., were obtained from the colony at the Institute of Insect Resources of Huazhong Agricultural University (China). Insects were reared under crowded conditions using aluminum cages (50 × 50 × 50 cm). Cages containing adults were kept at 27±1 °C under an illumination regime of 12 h light: 12 h dark. The uncontrolled relative humidity varied

* Tel.: +86-10-62612241; fax: +86-10-62565689.
E-mail address: zhangzn@panda.ioz.ac.cn (Z.-n. Zhang).

0022-1910/02/$ - see front matter.
PII: S0022-1910(02)00162-2

from 70 to 85%. Adult flies had access to unlimited water on cotton wool, cube sugar and powdered full cream milk, and, for oviposition, wheat bran mixed with water (1:1) placed into a culture dish, changed daily. Three times weekly, on Mondays, Wednesdays, and Fridays, a measured volume of eggs and young larvae produced during the preceding 24 h was floated off the milk-soaked cotton wool and inoculated onto 150 g of a bran-based larval medium (made up from 750 g wheat bran, 160 g powdered full cream milk, 120 g dried yeast, and 800 ml water) contained in a polyethylene bag lining a one-liter mason jar. After one week, during which the larval culture was stirred daily, the bag containing pupae was transferred into another empty cage for a week until eclosion was complete.

2.2. Experimental cages

Four-choice behavioral bioassays were undertaken in aluminum mesh cages (50 × 50 × 50 or 20 × 20 × 20 cm). All sides of the cage were mesh except for the front, which had a sliding cloth door. The ceiling had an electric bulb at the rear end. Houseflies were provided with four oviposition dishes (10.0 cm dia. × 2.5 cm h.) at the front of the false floor and close to the sliding door. Two of these dishes were filled with wheat bran (control) with moisture content of 50% (50 ml water to 50 g wheat bran) while the other two were filled with moist wheat bran containing a mass of freshly deposited eggs (0.05 g) or one pair of ovaries from a gravid female fly. The false floor in the middle had the four dishes placed at distances of 8.0 cm from each other and 12 cm diagonally. For two-choice experiments, two of the diagonally placed dishes were removed.

2.3. Solvent extraction of ovaries

Laboratory-raised gravid housefly females were anesthetized at –20 °C. Ovaries were removed from females and were allowed to dry at ambient temperature (25–27 °C) for 30 min. The ovaries were then placed in a tube (5 ml) and solvent added. After 10 min, the extract was removed. Extractions were carried out with hexane, dichloromethane, and ethyl acetate individually, each pair of ovaries using 5 μl of the solvents. The extracts were stored at –20 °C until use. Amounts of extracts corresponding to two or four ovary equivalents were tested individually or in combination in two- or four-choice bioassays.

2.4. Behavioral bioassays

Egg-laying responses of ovipositing *M. domestica* were determined in three types of experiments:

1. Gravid females were offered a choice among wet wheat bran, wheat bran containing housefly eggs, fermented wheat bran, and fermented wheat bran that contained two housefly ovaries. The dimensions of the experimental cages in this and all other choice experiments were 20 × 20 × 20 cm. Ten houseflies (5 ♀ and 5 ♂) were used for each of the 20 replicates. Male flies were included to insure that the females were mated. This experiment was replicated using larger cages (50 × 50 × 50 cm). The results (not shown) were very similar.
2. Gravid females were given a choice between (i) wet wheat bran (control), (ii) fermented wheat bran, (iii) wet wheat bran with two ovaries (placed under wheat bran for 3 hrs) that were then removed and (iv) fermented wheat bran with two ovaries (placed under wheat bran for 3 hrs) that were then removed. Ten houseflies (5 ♀ and 5 ♂) were used for each of the 20 replicates.
3. Gravid females were offered a choice between wet wheat bran, fermented wheat bran, and wet wheat bran or fermented wheat bran treated with various solvent extracts of ovaries. In these experiments, the extracts were delivered on strips of filter paper (10 × 2.5 cm) placed into the dishes about 0.5 cm below the surface of the moistened or fermented wheat bran. Filter paper strips treated with similar amounts of respective solvents were used as control stimuli. Ten houseflies (5 ♀ and 5 ♂) were used for each of the 20 replicates.

In all the experiments, each replicate consisted of 10 insects tested for four days. The dishes were removed daily and then replaced by freshly prepared dishes with their respective treatments. The resulting larvae were counted 3 days later. In all four-choice experiments (avoid any position effect), the position of the dishes was rotated in a 4 × 4 Latin square design. In two-choice experiments, the dishes were rotated diagonally. All the bioassays were conducted in the laboratory at 27±1 °C, relative humidity of 80–85% and 12:12, L:D photoperiod. The tested insects were fed with unlimited cube sugar and powdered full cream milk.

The results were analysed using Wilcoxon's T test (Wilcoxon, 1945) and Friedman's test (Friedman, 1940).

2.5. Analysis of extracts

Aliquots of the extracts of housefly ovaries were analyzed by gas chromatography (GC) and by gas chromatography–mass spectrometry (GC–MS). GC analyses were performed on a Hewlett-Packard (HP) 5890 Series gas chromatograph equipped with a flame ionization detector (FID) and an HP capillary column (BP-20, 50 m × 0.2 mm ID; BP-5, 50 m × 0.22 mm ID), using nitrogen as carrier gas at a flow rate of 0.8 ml/min. Analyses of extracts were conducted in splitless mode.

GC conditions were optimized to allow adequate resolution of all candidate peaks. The oven temperature was initially isothermal at 80 °C (1 min), programmed to 200 °C at 5 °C/min, and held for 30 min (BP-20), or initially isothermal 80 °C (1 min), programmed to 230°C at 10°C/min, and held for 40 minutes (BP-5). Chromatographic peaks were integrated by an HP 3392A integrator. GS–MS analyses were carried out on a Finnigan Voyager mass spectrometer (EI, 70eV) coupled to a Trace 2000 gas chromatograph using a HP capillary column BP-20 (25 m × 0.2 mm ID) that was programmed from 80 °C to 200 °C at a rate of 10 °C/min.

2.6. Responses of gravid famales to major components of semiochemicals from ovaries

Tricosane and (*Z*)-9-tricosene were the major two components of the semiochemicals released from mature ovaries of houseflies. Responses of gravid females to authentic samples of the two components were determined by a two-choice test. Tricosane (>98%, ACROS ORGANICS, Belgium) and (*Z*)-9-tricosene (>98%, Tokyo Chemical Industry Co. Ltd., Japan) were dissolved in HPLC-grade hexane (Fisher Chemicals, USA). Various doses of tricosane and (*Z*)-9-tricosene (6, 12, 24, 48, 96, 192, and 384 μg) were prepared. Each dose was tested individually, applied on filter paper strips (2.5 × 2.5 cm) which were air-dried and placed into the oviposition container, 0.5 cm below the surface of the fermented wheat bran. Similar amounts of hexane were used in the other dish as a control. In all experiments, each of 6–10 replicates per treatment consisted of 10 (5♀and 5♂) matured adults tested for 4 days.

3. Results

3.1. Choice tests

Initial four-choice experiments undertaken in cages in which gravid females were given choice among wet wheat bran, wheat bran with ovaries, fermented wheat bran, and fermented wheat bran plus matured ovaries, indicated a significant preference for fermented wheat bran plus ovaries. Wet wheat bran plus ovaries was less attractive, but was significantly preferred compared to fermented wheat bran or wet wheat bran alone (Table 1).

Results in which females were given four choices indicated that ovipositing females preferred to oviposit in fermented wheat bran contaminated with ovaries significantly more than in wet wheat bran contaminated with matured ovaries, and much more than in fermented wheat bran or in wet wheat bran alone (Table 2). Ovary extracts also elicited significant attraction of gravid females (Table 2). Fermented wheat bran with hexane extract was the most preferred, followed by ethyl acetate extract. The dichloromethane extract was least preferred although it was significantly more attractive than the control. These results clearly indicated that chemicals emanating from matured ovaries caused ovipositing females to aggregate at a common egg-laying site.

Table 1
Response of ovipositing *Musca domestica* females to wheat bran and fermented wheat bran mixed with housefly ovaries; 20 replicates for each test

Treatments	Eggs deposited	
	No. ±SE	%±SE
Wet wheat bran (WWB)	36±11.9	6.5±1.9^{d}
WWB + 1 pair of ovary	115±22.3	30.8±3.8^{b}
Fermented wheat bran (FWB)	97±18.0	11.6±2.5^{cd}
FWB + 1 pair of ovary	282±43.8	51.1±5.2^{a}

Means within an experiment followed by the same letter are not significantly different ($P < 0.05$). N = 5 pairs of insects per test (5 ♀ and 5 ♂).

3.2. Analysis of extracts

The GC profile of the hexane extracts of matured ovaries showed two major peaks (Fig. 1). GC–MS analysis of the extracts allowed the characterization of the major peaks (peak a and peak b) as tricosane (peak a), m/z (rel. intensity) at 43 (56), 57 (100), 71 (65), 85 (51), 99 (16), 113 (9), 141 (5), 211 (1), m^{+}325 (1); and (*Z*)-9-tricosene (peak b), m/z (rel. intensity) at 41 (44), 43 (78), 55 (91), 57 (99), 83 (100), 97 (96), 111 (48), 125 (25), 153 (10), 181 (2), m^{+}323 (6). These identities were confirmed by coinjection studies with authentic samples. These two peaks appeared in BP-20, respectively, at 18.15 and 18.40 min, or at 39.15 and 37.87 min in BP-5. One pair of ovaries was calculated to contain 103.827 and 120.560 ng of tricosane and (*Z*)-9-tricosene, respectively.

3.3. Oviposition response of gravid houseflies to tricosane and (Z)-9-tricosene

Table 3 shows the response of houseflies to the major components identified from the ovary extracts as compared to the control. In the case of tricosane, oviposition (60–90%) was significantly enhanced in the trays impregnated with 48–192 μg of the chemical. Responses at lower or higher doses were not significantly different from controls. Egg-laying houseflies showed significant responses at 48-, 96-, and 192-μg doses of (*Z*)-9-tricosene. At lower dose of this chemical (6, 12, and 24 μg), oviposition responses were not significantly different from controls. At the highest dose (384 μg), there was no significant difference between the treated and control trays.

Table 2
Response of ovipositing *Musca domestica* females to a choice of wet wheat bran or fermented wheat bran treated with housefly eggs or solvent extracts of housefly ovaries (20 replicates for each test)

Treatments	Eggs deposited	
	No.	%±SE
Wet wheat bran (WWB)	82±18.9	11.5±4.2[d]
Fermented wheat bran (FWB)	131±33.8	18.5±4.1[c]
WWB treated by matured ovaries[*1]	215±69.1	30.2±3.9[b]
FWB treated by matured ovaries[*2]	283±81.2	39.8±5.6[a]
Fermented wheat bran (FWB)	67±27.7	11.8±5.1[d]
FWB + hexane extract	204±78.3	36.0±9.9[a]
FWB + dichloromethane extract	116±28.6	20.5±5.4[c]
FWB + ethyl acetate extract	179±68.4	31.6±7.7[b]

Means within an experiment followed by the same letter are not significantly different ($P < 0.05$). [*1] Moist wheat bran from which ovaries had been removed, [*2]Fermented wheat bran from which ovaries had been removed.

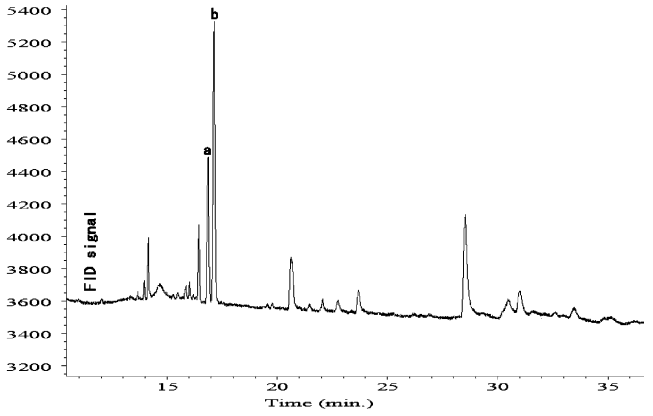

Fig. 1. Gas chromatogram of hexane extracts of matured ovaries injected into a 50-m BP-20 capillary colum. Peak a: Tricosane Peak b: (Z)-9-tricosene

4. Discussion

Bryant and Hall (1975) found "contagious oviposition behavior" in gravid houseflies. Initially, we found that the adults were more likely to aggregate in the corner or edge of the cage. Furthermore, aggregations of wild houseflies can also be found in poultry houses or animal corrals. However, the eggs in the substrate were always accumulated in clusters that can contain more than one thousand eggs (Lei et al., 1992). It is clear that gravid female houseflies prefer to lay their eggs in a common site. This study shows that the semiochemicals originating from matured housefly ovaries can attract gravid females to lay their eggs. Whereas we are not sure the two compounds, n-tricosane and (*Z*)-9-tricosene, are the main components assisting oviposition, apparently they assist aggregation in the housefly. The study of the freshly deposited eggs is under way.

Table 3
Response of ovipositing houseflies to various doses of tricosane and (Z)-9-tricosene

Treatment	Dose (μg)	Replicates	Average No. of eggs	Response (%)	
				Control±SE	Treated±SE
Tricosane	6	9	456±82.1	49.2±9.6a	50.8±9.6a
	12	8	573±77.2	46.0±10.3a	54.0±10.3a
	24	8	526±54.5	41.3±7.3a	58.7±7.3a
	48	9	617±98.6	30.6±8.7a	69.4±8.7b
	96	10	498±68.4	12.7±5.9a	87.3±5.9b
	192	8	541±73.7	26.5±6.4a	73.5±6.4b
	384	7	724±105.5	48.7±3.2a	51.3±3.2a
(Z)-9-tricosene	6	6	652±111.3	45.9±6.3a	54.1±6.3a
	12	6	487±54.9	45.6±7.9a	54.4±7.9a
	24	8	601±97.2	41.7±8.2a	58.3±8.2a
	48	9	643±88.6	38.5±7.5a	61.5±7.5a
	96	9	539±73.8	20.2±4.6a	79.8±4.6b
	192	7	496±47.1	31.9±9.4a	68.1±9.4b
	384	6	582±68.8	57.3±9.7a	42.7±9.7a

Means within an experiment followed by the same letter are not significantly different ($P < 0.05$).

According to Mpuru et al. (2001), the amount of internal (*Z*)-9-tricosene present in a 4-day-old female housefly is ten times that present in a 1-day-old female (25.0: 0.23 μg /mg body weight), During the same period, the amount of external (*Z*)-9-tricosene increases nearly 600 times (11.9: 0.02 μg/mg body weight). The amount of *n*-tricosane changes in the same way (21.0: 4.02; 12.5: 1.02 μg/mg body weight). In male houseflies, the amounts of these change little from 1 day to 4 days. The role of the sex pheromone, (*Z*)-9-tricosene is clear (Carlson et al., 1971) but the function of tricosane was previously unknown. Our study indicates that both of these compounds are semiochemical attractants, and that the production of both increases during ovary development. It also reveals that the pheromonal activity of (*Z*)-9-tricosene in houseflies is not limited to sex recognition by males.

Generally, individual components of insect pheromones can play different roles in different conditions. In bark beetles, certain chemicals that are the components of sex pheromones can also be the components of aggregation pheromones (Vite and Pitman, 1969; Vite and Renwick, 1971). Furthermore, in *Schistocerca gregaria*, the same semiochemical plays a dual role in different stages of insects: nymphal volatiles as nymphal aggregants and adult maturation retardants, while the adult volatiles are known to act as adult aggregants and maturation accelerants (Assad et al., 1997; Mahamat et al., 1993). Although this work has not shown that n-tricosane and (*Z*)-9-tricosene are the sole components of housefly oviposition aggregation, it is clear that (*Z*)-9-tricosene interacts additively with wheat bran volatiles to promote oviposition. Thus, (*Z*)-9-tricosene is not only a sex recognition factor.

The change in the chain length of the alkenes from predominantly 27 carbons and longer to predominantly 23 carbons as the female becomes vitellogenic is primarily due to regulation by ovarian-produced ecdysteroids (Adams et al., 1984; Blomquist et al., 1998), and (*Z*)-9-tricosene becomes a major component (Adams et al., 1984). However, the biosynthetic pathway of tricosane is still unknown.

The role of hemolymph in transporting cuticular hydrocarbons and hydrocarbon pheromones in insects has recently become fully appreciated (Schal et al., 1998a). Old models of hydrocarbon formation showed epidermal cells (oenocytes) synthesizing and transporting hydrocarbons directly to the surface (Hadley, 1984). However, ovary is also a target tissue for the transport of hydrocarbons (Schal et al., 1998b). In the *Simulium damnosum* complex, the main chemical composition of extracts from ovaries with oviposition aggregation pheromone activity was identical to volatile emissions from freshly laid eggs (McCall et al., 1997). We have previously shown that the extracts from freshly deposited eggs, ovaries, and oviducts of houseflies all attract gravid females (Jiang et al., 1999).

Compounds involved in aggregation of gravid females may be useful in determining the location of oviposition sites (for example, they could be used to concentrate houseflies in areas where pathogens or other biocontrol agents could cause high mortality). The semiochemicals might also be used in traps for monitoring populations as (*Z*)-9-tricosene is already used worldwide for this purpose.

Acknowledgements

This work was supported by grants from the NKBRD (G20000162) and NSF Hubei (2000ABB128). We thank Professor Liu Xun for his advice. We also thank Professor Reynolds (University of Bath, UK) for his help in English writing.

References

Adams, T.S., Holt, G.G., 1987. Effect of pheromone components when applied to different models on male sexual behavior in the housefly *Musca domestica*. J Insect Physiol 33, 9–18.

Adams, T.S., Holt, G.G., Blomquist, G.J., 1984. The role of 20-hydroxyecdysone in housefly sex pheromone biosynthesis. J Insect Physiol 30, 287–294.

Assad, Y.O.H., Hassanali, A., Torto, B., Mahamat, H., Bashir, N.H.H., El Bashir, S., 1997. Effects of fifth-instar volatiles on sexual maturation of adult desert locust *Schistocerca gregaria*. J Chem Ecol 23 (5), 1373–1388.

Blomquist, G.J., Tillman, J.A., Mpuru, S., 1998. The cuticle and cuticular hydrocarbons of insects: Structure, function, and biosynthesis in the housefly. In: Vander Meer, R.K., Breed, M.D., Espelie, K.E., Winston, M.L. (Eds.), Pheromone Communication in Social Insects: Ants, Wasps, Bees, and Termites. Westview Press, Boulder, CO, pp. 34–54.

Bryant, E.H., Hall, A.E., 1975. The role of medium conditioning in the population dynamics of the housefly. Res Population Ecology 16, 188–197.

Carlson, D.A., Mayer, M.S., Sillhacek, D.L., James, J.D., Beroza, M., Bierl, B.A., 1971. Sex attractant pheromone of the housefly: Isolation, identification and synthesis. Science 174, 76–78.

Friedman, M., 1940. A comparison of alternate tests of significance for the problem of m ranking. Ann Math Statist 11, 86–92.

Hadley, N.F., 1984. Cuticle: biochemistry. In: Bereiter-Hahn, J., Matoltsy, A.G., Richards, K.S. (Eds.), Biology of the Integument. Springer-Verlag, Berlin, pp. 685–702.

Jiang, Y., Lei, C.L., Zong, L.B., Zhong, C.Z., 1999. Initial studies on the oviposition aggregation pheromone of housefly, Musca domestica. Journal of Huazhong Agricultural University (supplement) 29, 38–42 In Chinese.

Lei, C.L., Zhong, C.Z., Zong, L.B., Liu, T.Y., 1992. A preliminary study on the ovipositional rhythm of housefly. Zoology Research 13, 116–117 In Chinese.

Mahamat, H., Hassanali, A., Odongo, H., Torto, B., El Bashir, E.S., 1993. Studies on the maturation accelerating pheromone of the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae). Chemoecology 4 (34), 159–164.

McCall, P.J., 1995. Oviposition pheromone in the *Simulium damnosum* complex. Medical and Veterinary Entomology 9, 101–108.

McCall, P.J., Heath, R.R., Dueben, B.D., Wilson, M.D., 1997. Oviposition pheromone in the *Simulium damnosum* complex: biological activity of chemical fractions from gravid ovaries. Physiol Ent 22 (3), 224–230.

Mpuru, S., Blomquist, G.J., Schal, C., Roux, M., Kuenzli, M., Dusticier, G., Clément, J.L., Bagnères, A.G., 2001. Effect of age and sex on the production of internal and external hydrocarbons and pheromones in the housefly, *Musca domestica*. Insect Biochem Molec Biol 31, 139–155.

Nelson, D.R., Dillwith, J.W., Blomquist, G.J., 1981. Cuticular hydrocarbons of the house fly *Musca domestica*. Insect Biochem 11, 187–197.

Rai, M.M., Hassanali, A., Saini, R.K., Odongo, H., Kahoro, H., 1997. Identification of components of the oviposition aggregation pheromone of the gregarious desert locust, *Schistocerca gregaria* (Forskal). J Insect Physiol 43 (1), 83–87.

Schal, C., Sevala, V.L., Carde, R.T., 1998a. Novel and highly speciic transport of a volatile sex pheromone by hemolymph lipophorin in moths. Naturwissenschaften 85, 339–342.

Schal, C., Sevala, V.L., Young, H., Bachmann, J.A.S., 1998b. Synthesis and transport of hydrocarbons: Cuticle and ovary as target tissues. Amer Zool 38, 382–393.

Uebel, E.C., Schwarz, M., Lusby, W.R., Miller, R.W., Sonnet, P.E., 1978. Cuticular non-hydrocarbons of the female housefly and their evaluation as mating simulants. Lloydia 41, 63–67.

Uebel, E.C., Sonnet, P.E., Miller, R.W., 1976. House fly sex pheromone: Enhancement of mating strike activity by combination of (Z)-9-tricosene with branched saturated hydrocarbons. Environ Entomol 5, 905–908.

Vite, J.P., Pitman, G.B., 1969. Insect and host odors in the aggregation of the western pine beetle. Can Ent 101, 113–117.

Vite, J.P., Renwick, J.A.A., 1971. Population aggregating pheromone in the bark beetle, *Ips grandicollis*. J Insect Physiol 17, 1699–1704.

Wilcoxon, F., 1945. Individual comparisons by ranking methods. Biomet Bull 1, 80–83.