

## IS DIMETHYLDECANAL A COMMON AGGREGATION PHEROMONE OF *Tribolium* FLOUR BEETLES?

LUDOVIC ARNAUD,<sup>1</sup> GEORGES LOGNAY,<sup>2</sup> MARJOLAINE VERSCHEURE,<sup>2</sup> LIONEL LEENAERS,<sup>1</sup> CHARLES GASPARD,<sup>1</sup> and ERIC HAUBRUGE<sup>1</sup>

<sup>1</sup>Department of Pure and Applied Zoology Gembloux Agricultural University Passage des déportés 2, 5030 Gembloux, Belgium

<sup>2</sup>Department of General and Organic Chemistry Gembloux Agricultural University Passage des déportés 2, 5030 Gembloux, Belgium

### Abstract

Flour beetles are cosmopolitan and common pests in grain stores and flour mills. Their ability to exploit a wide variety of stored products has contributed to their status as major pests of stored food. Although it was previously reported that the same aggregation pheromone, 4,8-dimethyldecanal (DMD), is shared by three flour beetle species (*Tribolium castaneum*, *T. confusum*, and *T. freemani*), the volatiles released by the other *Tribolium* species associated with stored products have not yet been examined. In the present study, the volatiles produced by males and females of eight *Tribolium* species were examined by solid phase microextraction (SPME). SPME samples were analyzed by gas chromatography coupled to mass spectrometry (GC-MS). Experiments were conducted to identify volatiles emitted by the adults of different *Tribolium* species and to determine whether DMD is a common aggregation pheromone. We observed that DMD is not a common pheromone of the eight species tested, but is common to *T. castaneum*, *T. confusum*, *T. freemani*, and *T. madens*. Two other volatiles were detected, 1-pentadecene, which is shown here to be a common semiochemical of flour beetles, and 1,6-pentadecadiene, which was detected in five species (*T. audax*, *T. brevicornis*, *T. destructor*, *T. freemani*, and *T. madens*).

**Key Words**—Tenebrionidae, *Tribolium*, aggregation pheromone, epideictic pheromone, 4,8-dimethyldecanal, 1-pentadecene, SPME, GC-MS.

Worldwide losses of stored products due to postharvest insect attack are estimated to be 15% annually, and tremendous costs are involved in protecting commodities against insect infestations (Reichmuth et al., 1997). Tenebrionid flour beetles (*Tribolium* spp.) are common pests of numerous stored products. Their occurrence results in contamination and substantial economic damage due to loss of the product and a decrease in nutritional value (Sokoloff, 1974). Pesticides have been widely used to control pest infestations. However, many stored product pests are now resistant to many insecticides (Georghiou and Lagunes-Tejeda, 1991), and for some insecticide registrations have not been renewed due to safety hazards. Therefore, new methods of pest management are needed. The application of insect semiochemicals might represent a way to reduce the level of damages caused by stored product insects to acceptable levels.

Although many studies have examined chemical communication in flour beetles, only a few have identified the volatiles released by the adults and investigated their role as pheromones (Keville and Kownowski, 1975; Suzuki et al., 1975, 1984, 1987; Suzuki, 1980; Faustini and Burkholder, 1987). An aggregation pheromone, 4,8-dimethyldecanal (DMD), which is used in pheromone trapping systems (Hussain et al., 1994), has been identified in *T. castaneum* and *T. confusum* (Suzuki, 1980) and later also in *T. freemani* (Suzuki et al., 1987). Although other semiochemicals produced by flour beetles have been identified, currently only DMD is used to monitor flour beetles in stored product facilities.

The *Tribolium* genus can be subdivided in five species groups (Hinton, 1948; Sokoloff, 1974; Howard, 1987). Beetles of more than one species may occur in sympatry (Sinha and Watters, 1985). Interspecific attraction was observed between species belonging to the same or a different species group. However, cross-attraction was not reciprocal for all species tested (Suzuki et al., 1987, 1988a; Faustini et al., 1982). This phenomenon could lead to imperfect pre-reproductive isolation between some species. Moreover, within the *castaneum* species group, sterile hybrids are observed between *T. castaneum* and *T. freemani* (Nakakita et al., 1981). Hybrids are even observed between *T. audax* and *T. madens* (Sokoloff, 1972), two species belonging to distinct species groups (sensu Howard, 1987). Although *T. confusum* belongs to a different species group from *T. castaneum* and *T. freemani*, evidence indicates that *T. castaneum*, *T. confusum*, and *T. freemani* males produce the same aggregation pheromone (4,8-dimethyldecanal, DMD) (Suzuki, 1980; Suzuki et al., 1987). However, the response to the pheromone of the different species, and also of populations of the same species, depends upon the chirality

of the molecules (Levinson and Mori, 1983; Suzuki and Mori, 1983; Barak and Burkholder, 1984/85; Boake and Wade, 1984; Suzuki et al., 1987). In addition, *Tribolium* species have other semiochemicals, such as quinones and cuticular hydrocarbons, in common (Lockey, 1978; Markarian et al., 1978; Wirtz et al., 1978; Howard, 1987). Until now the role of these compounds and their potential utilization to monitor flour beetles has not been examined.

In the present study, we examined the production of volatile compounds by males and females of eight *Tribolium* species belonging to three species groups (sensu Howard, 1987): the *brevicornis* group: *T. audax* and *T. brevicornis*; the *castaneum* group: *T. castaneum*, *T. freemani*, and *T. madens*; and the *confusum* group: *T. anaphe*, *T. confusum*, and *T. destructor*. Our aims were to identify the volatile semiochemicals emitted by adults of the different species and to observe whether DMD was a common aggregation pheromone of flour beetles.

## METHODS AND MATERIALS

**Insects.** Eight *Tribolium* species originating from different geographic areas were used (Table 1). Beetles were cultured in a dark incubator at  $28 \pm 3^\circ\text{C}$  and  $65 \pm 5\%$  relative humidity with wheat flour and brewer's yeast (10/1, w/w) as rearing medium. Beetles were sexed as pupae by morphological characters (Ho, 1969) and maintained individually (to ensure their virginity) in small vials ( $5.5\text{cm}^3$ ) with 0.5 g of the medium. One-month-old adults were used in the experiments.

**Volatile Collection: Sample Technique.** To identify the volatiles emitted by virgin male and female *Tribolium*, we used a glass vial ( $10\text{cm}^3$ ) where one adult was placed with 0.2 g of wheat bran as feed. Vials were sealed with a rubber septum and kept in an incubator at  $28 \pm 3^\circ\text{C}$  and  $65 \pm 5\%$  relative humidity. Volatile semiochemicals secreted by the adult were sampled after four days. For that purpose, glass vials were maintained at  $35^\circ\text{C}$ , and volatiles were sampled for 30 min by solid phase microextraction (SPME) with a polydimethylsiloxane (PDMS) fiber (100  $\mu\text{m}$ , Supelco, Sigma-Aldrich, Belgium) previously conditioned for 1 hr at  $250^\circ\text{C}$ . Moreover, the SPME fiber was systematically reconditioned before each analysis. Since a preliminary set of 10 SPME samplings performed with males of *T. castaneum* revealed no qualitative differences, only three males and three

TABLE 1. *Tribolium* MCLAY (COLEOPTERA, TENEBRIONIDAE) SPECIES USED AND THEIR ORIGIN

Species	Common name	Origin, Year
<i>T. anaphe</i> (Hinton)	—	Nigeria, 1956
<i>T. audax</i> Halstead	American flour beetle	Canada, 1969
<i>T. brevicornis</i> (LeConte)	Giant flour beetle	USA, unknown
<i>T. castaneum</i> (Herbst)	Red flour beetle	USA, 1960
<i>T. confusum</i> DuVal	Confused flour beetle	Germany, 1967
<i>T. destructor</i> Uyttenboogaart	Dark flour beetle	Ethiopia, 1968
<i>T. freemani</i> (Hinton)	Kashmir flour beetle	Japan, 1980
<i>T. madens</i> (Charpentier)	Black flour beetle	Yugoslavia, 1959

females of each species were investigated. A vial with 0.2 g of wheat bran was used as control.

**SPME and GC-MS: Analytical Method.** GC-MS analyses were performed on a mass spectrometer Hewlett-Packard 5972 coupled to a Hewlett-Packard 5890 Series II gas chromatograph (splitless mode at  $250^\circ\text{C}$ ) fitted with a Hewlett-Packard HP-5 MS column (30 m  $\times$  0.25 mm; 1  $\mu\text{m}$ ). The temperature program was from 40 to  $200^\circ\text{C}$  at  $8^\circ\text{C}/\text{min}$  and from 200 to  $280^\circ\text{C}$  at  $20^\circ\text{C}/\text{min}$ . Helium was used as carrier gas at 1.0 ml/min. Mass spectra were recorded in the EI mode at 70 eV (scanned mass range: 35–400 amu). The interface and the source were maintained at  $280^\circ\text{C}$  and  $250^\circ\text{C}$ , respectively. Identifications were performed by comparing the recorded mass spectra with those of the Wiley 275.L computed data base and by determination of the retention times and mass spectra interpretations of pure DMD (Biosyste`mes, France) and 1-pentadecene (Sigma-Aldrich, Belgium).

Quantification of DMD was carried out by integration of the peak areas by using an external standard method. For calibrations, solutions of known amounts of DMD in *n*-hexane were injected in analytical conditions identical to that of SPME analyses.

TABLE 2. PRODUCTION OF 4,8-DIMETHYLDECANAL (DMD) BY *Tribolium* MALES AND FEMALES<sup>a</sup>

Species	DMD (ng/beetle)	
	Male	Female
<i>T. anaphe</i>	nd <sup>b</sup>	nd
<i>T. audax</i>	nd	nd
<i>T. brevicornis</i>	nd	nd
<i>T. castaneum</i>	0.71 ± 0.12 (N = 3) a	nd
<i>T. confusum</i>	0.18 ± 0.01 (N = 3) b	nd
<i>T. destructor</i>	nd	nd
<i>T. freemani</i>	0.12 ± 0.02 (N = 3) b	nd
<i>T. madens</i>	0.22 ± 0.17 (N = 3) b	nd

<sup>a</sup> Three males and three females were sampled for each species. Volatiles were trapped with a SPME fiber during 30 min.

<sup>b</sup> nd = not detected. Values followed by the same letter are not significantly different (Tukey's test,  $P > 0.05$ ).

TABLE 3. PRODUCTION OF 1-PENTADECENE AND 1,6-PENTADECADIENE BY *Tribolium* MALES AND FEMALES<sup>a</sup>

Species	1-Pentadecene			1,6-pentadecadiene	
	Male (N)	Female (N)	Estimated quantity (ng)	Male (N)	Female (N)
<i>T. anaphe</i>	2	3	0.73 ± 0.35	nd	nd
<i>T. audax</i>	3	2	0.46 ± 0.26	3	2
<i>T. brevicornis</i>	3	2	1.93 ± 0.28	3	2
<i>T. castaneum</i>	1	nd	0.21	nd	nd
<i>T. confusum</i>	1	1	0.49 ± 0.41	nd	nd
<i>T. destructor</i>	1	1	0.09 ± 0.05	nd	1
<i>T. freemani</i>	3	3	0.21 ± 0.07	1	3
<i>T. madens</i>	3	2	0.12 ± 0.01	3	2

<sup>a</sup> Three males and three females were sampled for each species. Volatiles were trapped with a

## RESULTS

DMD is not a common semiochemical of the genus *Tribolium*; it is produced in *T. castaneum*, *T. confusum*, *T. freemani*, and *T. madens* (Table 2). Two other volatiles were detected, 1-pentadecene, which is shown here to be a common semiochemical of flour beetles, and 1,6-pentadecadiene, which was detected in five species (*T. audax*,

*T. brevicornis*, *T. destructor*, *T. freemani*, and *T. madens*) (Table 3). SPME fiber during 30 min. *N* = number of adults that released the compound, nd = not detected.

Typical chromatograms (total ion current) of the SPME sampling of the volatiles released by flour beetles are illustrated in Figure 1. The peaks at 16.15 min (retention time,  $t_R$ ) and 18.70 min correspond to DMD and 1-pentadecene, respectively. Both the recorded mass spectra and retention times were identical to those of the authentic compounds. In addition, their fragmentation patterns were indistinguishable from those in the Wiley 275.L data base. Although the molecular ion ( $M^+$  184) of DMD has never been observed, a characteristic ion was obtained at  $m/z$  140. It corresponds to a fragmentation resulting from the McLafferty rearrangement due to the  $\beta$ -cleavage of the aldehyde group.

An additional constituent (RRT = 1.016; 1-Pentadecene as reference) detected mainly in *T. brevicornis* and in lower proportions in *T. audax*, *T. destructor*, *T. freemani*, and *T. madens* showed a mass spectrum identical to that of *cis*-cyclododecene from the Wiley 275.L computed library. Nevertheless, the injection of a reference mixture of *cis*- and *trans*-cyclododecene (Fluka, Belgium) did not corroborate this interpretation. To establish the identity of the molecule, 250 flour beetles (*T. brevicornis*) were extracted with 50 ml peroxide-free diethyl-ether. After concentration to 500  $\mu$ l under a gentle stream of nitrogen, the ethereal extract was submitted to GC-MS analysis. A well-resolved peak with both RRT (1.012) and fragmentation pattern similar to that observed during SPME analysis was observed. The molecular peak  $M^+$  = 208 indicated a pentadecadiene iso-mer. The peak was attributed to 1,6-pentadecadiene (Suzuki et al., 1975; Wirtz et al., 1978; Howard, 1987). Although it was observed here in only five *Tribolium* species, it was previously observed as a common chemical of the same eight species examined here (Howard, 1987).

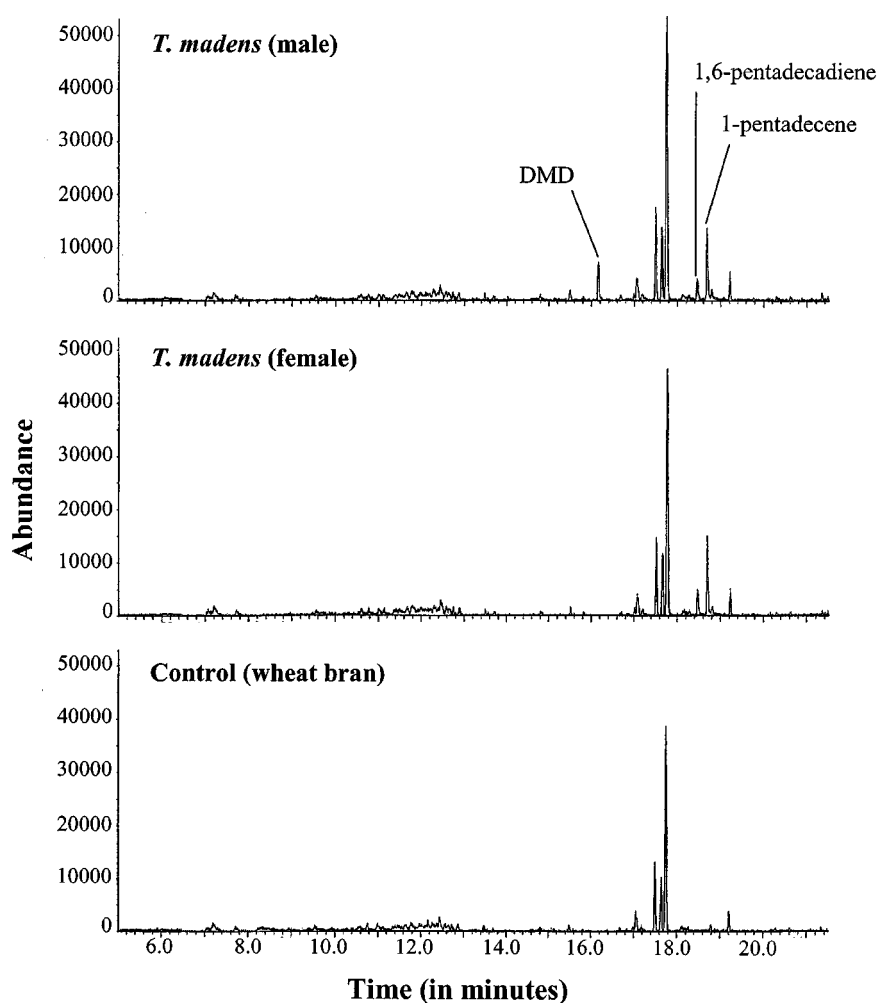


FIG. 1. Typical chromatogram (total ion current) of the PDMS SPME sampling of the volatiles released by male and female *Tribolium madens*. 4,8-Dimethyldecanal ( $t_R$  = 16.15 min), 1,6-pentadecadiene ( $t_R$  = 18.48 min), 1-pentadecene ( $t_R$  = 18.68 min). The other unidentified compounds are generated by the wheat bran.

The volatiles released by males and females of the eight species are shown in Tables 2 and 3. The values presented in the tables are an estimation of the quantities of the volatiles released by the adults by comparison of the area of the peak in insect volatiles to that of a known amount of DMD and of 1-pentadecene. Not all of the three semiochemicals were released by adults of each species. Furthermore, the species differed in the quantity of compounds they released.

DMD has been identified previously in *T. castaneum*, *T. confusum*, and *T. freemani* and is now reported for the first time in *T. madens* males. However, it was not secreted by female beetles. One-way ANOVA showed that males of the three species differ in the quantity of DMD secreted ( $F_{3,8} = 6.80$ ,  $P = 0.014$ ). However, there were no significant differences among *T. confusum*, *T. freemani*, and *T. madens* (Tukey's test,  $P > 0.05$ ).

We observed that 1-pentadecene was released from all species (Table 3). However, it appeared that this compound was not secreted by all individuals of a species and that the production was highly variable among individuals. The 1,6-pentadecadiene ( $t_R = 18.48$  min) was detected in adults of *T. audax*, *T. brevicornis*, *T. destructor*, *T. freemani*, and *T. madens*, but was not found systematically in each replicate of each species.

## DISCUSSION

Since the identification of the same aggregation pheromone for three *Tribolium* species (Suzuki, 1980; Suzuki et al., 1987), DMD has been considered a common volatile in flour beetles. A synthetic pheromone mostly composed of the (4*R*,8*R*) isomer was, thus, commonly used to trap the confused and the red flour beetles in stored product commodities. Indeed, we observed that DMD is not a common volatile of *Tribolium* flour beetles. It was produced only by males of four of the eight species tested.

Hussain (1993) observed that, in *T. castaneum*, DMD was released also by young females (<2 days old) and by larvae and pupae. We used 1-month-old insects in our experiments. This could explain the absence of DMD production by females. The quantities of DMD detected with our sampling method are much smaller than those reported by Hussain (1993) and Bloch Qazi et al. (1998). Working with a Super-Q column, these authors had observed that, on average, a single *T. castaneum* male produces 633 ng/24 hr and 163 ng/48 hr, respectively. In high-density conditions (2000 males in a 500-ml Erlenmeyer flask for 40 days), Suzuki and Sugawara (1979) observed that about 3.17 ng of DMD was secreted by one adult per day. Hussain (1993) observed that DMD production decreases while population density increases.

Ethyl- and methyl-benzoquinones are secreted by adult flour beetles (Alexander and Barton, 1943; Markarian et al., 1978; Howard and Mueller, 1987; Hussain, 1993; Pappas and Wardrop, 1996). Quinones were trapped with a Super-Q column connected to an aeration chamber containing one adult red flour beetle by Hussain (1993). We did not trap any quinones in our experiments. Quinones are released by adult flour beetles in stressful (Tschinkel, 1975) or overcrowded conditions (Faustini and Burkholder, 1987). Either the PDMS fiber (apolar phase) was not suitable to trap quinones (polar molecules), or our sampling conditions were not stressful to the insects. In order to differentiate between these hypotheses, we used a Carboxen-PDMS SPME fiber, which is suitable for polar compounds. Moreover, vials containing one *T. castaneum* male (unstressful conditions) or 25 unsexed *T. castaneum* adults (stressful conditions) were sampled. Sampling was conducted as described above. By using the PDMS fiber, DMD was detected in both kind of samples, but quinones were not. Ethyl- and methyl-benzoquinones were easily detected with the Carboxen-PDMS fiber in the stressful conditions (25 adults), but no detectable amounts of quinones were observed in the unstressful conditions (1 male). DMD was not detected with that fiber. Both hypotheses can, thus, explain our results: PDMS fiber is not suitable to detect quinones, and stressful conditions are necessary to bring about the release of quinoid compounds. An interesting observation is that, contrary to Hussain (1993), DMD was detected even in stressful conditions.

The unsaturated hydrocarbon 1-pentadecene has been observed in studies examining cuticular lipids and defensive secretions of flour beetles (Keville and Kannotski, 1975; Tschinkel, 1975; Suzuki et al., 1975, 1988b; Locky, 1978; Markarian et al., 1978; Wirtz et al., 1978; Hebanowska et al., 1990). In *T. confusum*, 1-pentadecene was believed to bring the sexes together and induce copulation because, although it was produced by males and females, only males appeared to be affected (Keville and Kannotski, 1975). However, it was first hypothesized that 1-pentadecene was used to facilitate the absorption of quinones by *Tribolium* enemies (Endt and Wheeler, 1971). Suzuki et al. (1975) observed that 1-pentadecene was repellent to *T. castaneum* and *T. confusum* adults at very low concentrations (0.1–10  $\mu\text{g}/21\text{-mm}$  disk). Considering the amount of 1-pentadecene used by Keville and Kannotski (1975) (2–2.5 mg/6 mm disk) and the quantity of 1-pentadecene secreted by adults of the confused flour beetle (3  $\mu\text{g}/\text{beetle}/3$  hr) (Endt and Wheeler, 1971), the repellent hypothesis seems to be more likely. We, thus, hypothesized that 1-pentadecene may function as an epideictic (spacing) pheromone to flour beetles and a

defensive secretion against *Tribolium* enemies. However, its exact function is not yet established. In this study, we observed that 1-pentadecene was produced by males and females of all species tested, except *T. castaneum* males. However, this volatile has been previously observed in the male red flour beetle (Howard, 1987; Hussain, 1993). Therefore, 1-pentadecene could be considered a common volatile of *Tribolium* flour beetles.

Our findings increase knowledge on chemical communication in *Tribolium* flour beetles and have implications in integrated pest management of these stored-products insects. Hitherto, DMD has been considered a common aggregation pheromone of flour beetles and is used in pheromone traps (Barak and Burkholder, 1984/85; Hussain et al., 1994). However, in flour beetles, the efficiency of the pheromone trap depends on the isomeric composition of the synthetic compound, on the target species (Levinson and Mori, 1983; Suzuki and Mori, 1983; Barak and Burkholder, 1984/85; Suzuki et al., 1984, 1987), and even on the population of the species (Boake and Wade, 1984). Although it was not produced by every adult, we observed that 1-pentadecene was a common volatile to flour beetles. Therefore, it could be used as an alternative semiochemical to DMD, which is restricted to some species. Behavioral experiments are still needed to determine its precise role in *Tribolium* chemical communication and to determine its potential use in monitoring and/or controlling flour beetles in stored products. If its repellent role is confirmed, 1-pentadecene could instead be used as a protectant of stored products against *Tribolium* flour beetles.

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