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ATTRACTION OF *Oryzaephilus surinamensis* (L.) AND *Oryzaephilus mercator* (FAUVEL) (COLEOPTERA: CUCUJIDAE) TO SOME COMMON VOLATILES OF FOOD[1]

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Abstract—Responses by adult *Oryzaephilus surinamensis* (L.) and *Oryzaephilus mercator* (Fauvel) to various food volatiles were assessed by means of a two-choice, pitfall olfactometer. The individual experimental stimuli, all potential products of lipid oxidation, had a range of attractive doses of $\leq$ 1000-fold over the test dose ranges of 0.001–100 μg, or 0.01–1000 μg. Of 13 aliphatic C_3–C_{14} aldehydes and benzaldehyde tested for *Oryzaephilus* spp., 10 C_3–C_{10} aliphatic aldehydes and benzaldehyde showed some attractiveness for both species. For *O. mercator*, nonanal had the lowest lower threshold for positive response at 0.01 μg. The addition of small amounts of nonanal or of a 1 : 1 : 1 mixture of hexanal, octanal, and nonanal to small amounts of cucujolide aggregation pheromones enhanced response by mixed-sex *O. mercator* to the pheromones. Eleven aliphatic C_2–C_9 free fatty acids showed some attractiveness for both *Oryzaephilus* spp. Isovaleric acid and valeric acid had the lowest lower thresholds for positive response at 0.1 μg for *O. mercator* and *O. surinamensis*, respectively. Four olefinic oat volatiles were found to possess various degrees of attractiveness for both *Oryzaephilus* spp. The data suggest that food volatiles in this study might be used by *Oryzaephilus* spp. as host-finding kairomones in nature.

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Key Words—*Oryzaephilus surinamensis* (L.), *Oryzaephilus mercator* (Fauvel), Coleoptera, Cucujidae, attractants, aliphatic C_3–C_{14} aldehydes, benzaldehyde, aliphatic C_2–C_9 free fatty acids, 2(*E*),4(*E*)-heptadienal, 2(*E*),4(*E*)-nonadienal, 3(*E*),5(*E*)-octadien-2-ol, 3(*E*),5(*E*)-octadien-2-one, oat volatiles.

INTRODUCTION

The sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), and the merchant grain beetle, *O. mercator* (Fauvel), are cosmopolitan pests of stored products. *O. surinamensis* is one of the most common pests of stored grain and processed cereals in Canada, United States, Britain, Australia, Asia, Africa, and South America (Sinha and Watters, 1985). *O. mercator* has become firmly established as a household pest in North America, especially on cereal products (Loschiavo and Sabourin, 1982).

Identification of attractive semiochemicals for *Oryzaephilus* spp. could contribute to the development of integrated control programs (Levinson and Levinson, 1979; Burkholder, 1981). Feeding males of both *Oryzaephilus* spp. produce macrolide aggregation pheromones, a class of pheromones given the trivial name cucujolide because of its prevalence in the family Cucujidae (Oehlschlager et al., 1988). These cucujolides have been identified as 3(*Z*),11(*R*)-dodecen-11-olide [(*R*)-II] and 3(*Z*),6(*Z*),11(*R*)-dodecadien-11-olide [(*R*)-IV] for *O. mercator*, and (*R*)-IV, 3(*Z*),6(*Z*)-dodecadienolide (IX), and 5(*Z*),8(*Z*),13(*R*)-tetradecadien-13-olide [(*R*)-V] for *O. surinamensis* (Pierce et al., 1985, 1987). In addition, males and females of both *Oryzaephilus* spp. maintained at low population densities produce another aggregation pheromone, (*R*)-(−)-1-octen-3-ol (Pierce et al., 1989).

Pierce et al. (1981) reported that *Oryzaephilus* spp. were attracted to Porapak Q-trapped volatiles from rolled oats or brewer's yeast. Unpublished analyses of these volatiles by coupled gas chromatography–mass spectroscopy (GC-MS) revealed the presence of hexanal, heptanal, octanal, nonanal, decanal, dodecanal, and benzaldehyde in the rolled oat volatiles, while benzaldehyde and isobutyric, 2-methylbutyric, isovaleric, and valeric acids were detected in brewer's yeast volatiles. Hexanal and nonanal comprised ~10% and ~14%, respectively, of the Porapak Q-trapped volatiles from rolled oats. Mikolajczak et al. (1984) have identified a number of volatile attractants from rolled oats for *O. surinamensis*. Additionally, some attractants from carobs also have been identified for *O. surinamensis* by O'Donnell et al. (1983) and Stubbs et al. (1985).

Our current objective was to screen various host-produced volatiles (primarily aldehydes and free fatty acids) as potential attractants for both *Oryzae-*

philus spp. Such attractants could be candidates for the formulation of a more cost-effective field bait containing host volatiles as well as cucujolides.

METHODS AND MATERIALS

Experimental Insects. O. mercator and *O. surinamensis* were reared on large-flake rolled oats and brewer's yeast (95:5, w/w) in 3.8-liter glass jars in an environmental chamber maintained at 28–30°C and 40–60% relative humidity in darkness.

Experimental Stimuli for Bioassay. Fatty acids, aldehydes, 2(*E*),4(*E*)-heptadienal, and 2(*E*),4(*E*)-nonadienal were purchased from commercial supply houses, and all but the fatty acids were distilled before use. Compounds were checked by gas chromatography (GC) before use and found to be ≥99% pure. Macrocyclic lactones for bioassay were racemic cucujolide II (Oehlschlager et al., 1983) and racemic cucujolide IV (Millar and Oehlschlager, 1984). Addition of methyllithium in ether to an etheral solution of freshly distilled 2(*E*),4(*E*)-heptadienal (Aldrich Chemical Company, Inc., Milwaukee, Wisconsin) gave, after aqueous work-up and bulb-to-bulb distillation, 3(*E*),5(*E*)-octadien-2-ol in 88% yield, which was 95% pure by GC analysis. Oxidation of 3(*E*),5(*E*)-octadien-2-ol with active MnO_2 in pentane gave, after removal of MnO_2 by filtration, evaporation of solvent, and bulb-to-bulb distillation, 3(*E*),5(*E*)-octadien-2-one in 81% yield, which was 95% pure by GC analysis. Analyses of the dienol and dienone by GC-MS indicated that the impurity in each was an isomer of unknown double-bond configuration.

Instrumental Methods. A Hewlett-Packard 5830A gas chromatograph equipped with a 18835B capillary inlet system and a flame-ionization detector was employed for analyses by GC. Helium was the carrier gas, and the injection port and detector temperatures were 260°C and 270°C, respectively. Samples were analyzed on an open-tubular glass column (40 m × 0.5 mm ID) coated with SP-1000 (Supelco Canada Ltd., Oakville, Ontario, Canada). The temperature program for analytical GC was 70°C for 2 min, then 4°C/min to 180°C, holding for 30 min or less.

GC-MS was performed on a Hewlett-Packard 5895B GC-MS-data system fitted with a fused silica column (30 m × 0.25 mm ID) coated with Carbowax 20 M (J&W Scientific, Folsom, California) with helium as the carrier gas.

Bioassay Procedures. Bioassays were conducted at 23°C and 60% relative humidity using mixed-sex beetles 5–12 weeks posteclosion. To ensure a uniform state of preconditioning for *O. mercator*, each replicate of 12 beetles was held in a 60-ml glass vial without food for 20 hr at 23°C darkness prior to a bioassay (Borden et al., 1979). To obtain maximum responsiveness from *O.*

surinamensis, up to 1500 beetles were preconditioned without food for 48 hr at 23°C in darkness in a 6-liter Erlenmeyer flask, through which charcoal-filtered, humidified air was drawn at 1.5 liters/min (Pierce et al., 1985). Since response by *O. surinamensis* to olfactory stimuli is extremely sensitive to population density (Pierce et al., 1983), test beetles were maintained at a low density of 1000 beetles/kg medium for at least one week before bioassays commenced.

A two-choice, pitfall olfactometer (Pierce et al., 1981) was utilized to test attractiveness of experimental stimuli. Filter paper disks, treated with a 10-μl aliquot of either an experimental stimulus in purified pentane or purified pentane as a control, were put singly into the bottoms of two glass vials suspended from holes in the bottom of a plastic Petri dish arena. (All test compounds dissolved completely in pentane at the concentrations used.) Twelve beetles were released into the dish, and the lid was replaced. Bioassays for each test solution were replicated 12 times (i.e., 12 olfactometers), using 12 fresh beetles in each replicate. After 2 hr in darkness, the numbers of beetles in experimental and control vials were recorded. For the compounds in Tables 1, 3, and 4 (below), all doses for a particular test compound were done in the same bioassay session, beginning with 12 replicates of the lowest dose and proceeding in sequence to the highest dose. The responsiveness of the test beetles was checked by bioassaying a standard low dose of cucujolides at the start of a bioassay session.

The untransformed data were analyzed by the paired-sample t test (Zar, 1984). For some experiments, the data also were analyzed by ANOVA followed by the Newman-Keuls test. Results were expressed as mean percent response = 100 (experimental − control)/N where experimental and control were the number of beetles in the vials containing the experimental and control disks, respectively, and N was the total number of insects released into the dishes. For each test solution, the number of responding insects was $\geq 90\%$ of those released.

RESULTS

For *O. mercator*, nonanal was the aldehyde having the lowest threshold for positive response, with significant positive responses demonstrated at experimental doses ranging from 0.01 to 10 μg (Table 1). Hexanal, octanal, 2-methylpropanal, and benzaldehyde had the next lowest thresholds for positive response at 0.1 μg. Over the test dose range examined, nonanal, hexanal, and 2-methylpropanal had the greatest attractive ranges of 1000-fold each.

For *O. surinamensis*, hexanal, pentanal, 2-methylpropanal, and nonanal were the aldehydes having the lowest thresholds for positive response at the

TABLE 1. RESPONSE BY *O. mercator* AND *O. surinamensis* IN TWO-CHOICE, PITFALL BIOASSAY TO HOST-PRODUCED ALDEHYDES

Test species	Aldehyde tested	$\bar{X}$% response[a]					
		0.001 μg	0.01 μg	0.1 μg	1 μg	10 μg	100 μg
O. mercator	Propanal	8.3	1.4	5.6	45.9***	63.9***	5.5
	2-Methylpropanal	11.1	−5.6	19.4*	37.5**	36.0***	44.4***
	Butanal	12.5	−8.3	−6.9	12.5	23.6*	43.0***
	3-Methylbutanal	−1.2	3.1	2.8	15.3	38.9***	62.5***
	Pentanal	8.3	1.4	12.5	41.7***	55.6***	50.0***
	Hexanal	9.0	18.1	27.6**	40.3***	46.6***	33.3**
	Heptanal	−1.4	15.3	17.4	34.5***	39.6***	2.8
	Octanal	7.6	7.6	29.8**	43.1***	39.6***	−2.0
	Nonanal	6.2	30.6**	43.8***	56.8***	38.9***	11.1
	Decanal	−3.4	5.5	2.8	21.5**	50.0***	−15.3
	Benzaldehyde	6.2	13.9	22.8*	33.3***	41.7***	−7.0
O. surinamensis	Propanal	1.9	−2.1	−7.7	0.7	13.5	20.9*
	2-Methylpropanal	−5.6	15.9	23.9*	24.3**	29.7**	58.3***
	Butanal	−3.0	13.2	4.3	3.6	12.1	33.8***
	3-Methylbutanal	4.2	2.8	6.8	24.4*	26.7**	44.0***
	Pentanal	7.8	−0.8	25.6*	27.1**	58.2***	48.9***
	Hexanal	12.5	15.9	27.4**	47.6***	46.2***	−9.7
	Heptanal	11.4	14.5	20.3	43.7***	45.7***	24.8*
	Octanal	4.2	9.7	16.0	22.9*	33.8**	12.7
	Nonanal	6.9	18.1	24.4*	31.0**	27.1**	9.2
	Decanal	−8.4	16.7	20.9	25.7*	33.3***	17.7
	Benzaldehyde	4.2	0.7	5.8	17.2*	35.2**	11.3

[a] Significant response (t test) to experimental stimulus indicated by: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. $N = 12$ replicates per treatment, 12 adults per replicate.

0.1-μg dose (Table 1). Over the test dose range examined, pentanal and 2-methylpropanal had the greatest attractive ranges of 1000-fold each. Neither species responded to undecanal, dodecanal, or tetradecanal over an experimental dose range of 0.001–100 μg (data not shown).

When combined in a 1 : 1 : 1 mixture, three of the more attractive aldehydes for *O. mercator*, hexanal, octanal, and nonanal, induced attractive responses over a 0.01- to 100-μg dose range (Table 2). When low amounts (0.002 or 0.02 μg) of cucujolides II and IV, response to the aggregation pheromones by mixed-sex *O. mercator* was enhanced.

For both species, thresholds for positive response to free fatty acids were relatively high (Table 3). For *O. mercator*, isovaleric acid had the lowest threshold at 0.1 μg, and isovaleric and heptanoic acids gave the greatest attrac-

TABLE 2. RESPONSE BY *O. mercator* IN TWO-CHOICE, PITFALL BIOASSAY TO RACEMIC CUCUJOLIDES II + IV, ALDEHYDES, AND MIXTURES OF II + IV AND ALDEHYDES

	Dosage (μg)			
Exp.[a]	II + IV (1:1)	Nonanal	Hexanal + octanal + nonanal (1:1:1)	$\bar{X}$% response[b]
1			0.001	10.1
			0.01	24.8*
			0.1	49.9***
			1	66.9***
			10	54.2***
			100	30.6**
2	0.002			22.5*a
		0.01		25.5**a
	0.002	0.01		56.5***b
			0.01	21.9*a
	0.002		0.01	45.3***b
3	0.02			36.1**a
		0.1		41.1***ab
	0.02	0.1		49.0***b
			0.1	45.6***ab
	0.02		0.1	66.8***c

[a] Each experiment was completed in a separate 2-hr session.
[b] Significant response (t test) to experimental stimulus indicated by: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. $N = 12$ replicates per treatment, 12 adults per replicate. Means within experiments 2 or 3 followed by the same letter not significantly different (Newman-Keuls test, $P < 0.05$).

tive ranges of 1000-fold each over the test dose range examined. Valeric acid induced the lowest threshold for positive response by *O. surinamensis* at 0.1 μg; and acetic, octanoic, and nonanoic acids gave the greatest attractive ranges, each of 1000-fold, over the test dose range examined. All but heptanoic, octanoic, and nonanoic acids were repulsive or elicited no significant response at the highest dose for *O. mercator* (Table 3). For *O. surinamensis*, all fatty acids in high amounts were repulsive except for acetic, heptanoic, octanoic, and nonanoic acids, which were attractive at the 1000-μg dose.

Of four olefinic oat volatiles tested (Table 4), 2(*E*),4(*E*)-heptadienal, 3(*E*),5(*E*)-octadien-2-ol, and 3(*E*),5(*E*)-octadien-2-one had attractive ranges of 0.1–10 μg for *O. mercator*. 2(*E*),4(*E*)-Heptadienal and 3(*E*),5(*E*)-octadien-2-ol were attractive at 0.1–10 μg for *O. surinamensis*. Over the dose range examined for both *Oryzaephilus* spp., 2(*E*),4(*E*)-nonadienal was attractive at 1–100 μg. 3(*E*),5(*E*)-Octadien-2-one was attractive only at the 10-μg dose for *O. surinamensis*.

TABLE 3. RESPONSE BY *O. mercator* AND *O. surinamensis* IN TWO-CHOICE, PITFALL BIOASSAY TO FREE FATTY ACIDS

Test species	Fatty acid[a]	$\bar{X}$% response[b]					
		0.01 μg	0.1 μg	1 μg	10 μg	100 μg	1000 μg
O. mercator	Acetic		9.6	17.4*	47.9***	47.9***	−52.1***
	Propionic		−13.2	7.6	46.2***	32.4*	−44.4***
	Isobutyric		−2.1	19.4*	37.5***	56.3***	−22.2**
	Butyric		7.0	7.6	54.2***	52.4***	−36.1***
	2-Methylbutyric		2.1	27.8*	41.0***	40.3**	−6.9
	Isovaleric	4.5	20.7*	24.3**	57.6***	48.6***	−34.7**
	Valeric		6.9	18.7	53.4***	47.9***	−36.1***
	Hexanoic		−8.4	5.6	57.6***	47.9***	9.7
	Heptanoic		4.9	17.4*	41.6***	41.7**	25.7*
	Octanoic		2.1	11.1	36.8***	36.1**	33.3**
	Nonanoic		−0.7	−3.5	37.5**	42.5***	38.2**
O. surinamensis	Acetic		−2.2	23.0**	42.3***	46.5***	42.9***
	Propionic		14.0	25.8*	26.8**	53.1***	−64.1***
	Isobutyric		−0.7	31.9**	63.6***	5.6	−47.0***
	Butyric		10.0	41.0***	68.0***	31.3**	−52.4***
	2-Methylbutyric		12.8	36.1***	69.8***	−56.0***	
	Isovaleric		9.8	29.1**	39.7***	−52.9***	
	Valeric	−13.2	30.0**	34.2***	85.2***	−9.2	−70.4***
	Hexanoic		12.5	42.3***	−8.0	−50.0***	
	Heptanoic		−7.0	16.0	39.0***	29.5***	26.2*
	Octanoic		−19.7	36.1**	48.2***	64.5***	71.3***
	Nonanoic		3.6	26.0**	48.1***	53.1***	66.2***

[a] Ranked in order of increasing boiling point.
[b] Significant response (*t* test) to experimental stimulus indicated by: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. $N = 12$ replicates per treatment, 12 adults per replicate.

TABLE 4. RESPONSE BY *O. mercator* AND *O. surinamensis* IN TWO-CHOICE, PITFALL BIOASSAY TO SOME OLEFINIC OAT VOLATILES

Test species	Experimental stimulus	$\bar{X}$% response[a]				
		0.01 μg	0.1 μg	1 μg	10 μg	100 μg
O. mercator	2(*E*),4(*E*)-Heptadienal	19.5	39.1***	80.3***	82.4***	13.0
	2(*E*),4(*E*)-Nonadienal	−0.7	16.6	48.6***	48.6***	36.6***
	3(*E*),5(*E*)-Octadien-2-ol	18.4	24.6*	54.0***	71.8***	−8.7
	3(*E*),5(*E*)-Octadien-2-one	−7.6	24.8*	42.9***	74.6***	−10.7
O. surinamensis	2(*E*),4(*E*)-Heptadienal	14.0	31.8**	36.0***	44.7***	−34.2**
	2(*E*),4(*E*)-Nonadienal	16.2	10.6	39.8***	57.3***	56.9***
	3(*E*),5(*E*)-Octadien-2-ol	0.7	25.8*	51.1***	36.1***	−15.7
	3(*E*),5(*E*)-Octadien-2-one	19.4	16.1	0.7	30.3**	3.5

[a] Significant response (*t* test) to experimental stimulus indicated by: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. $N = 12$ replicates per treatment, 12 adults per replicate.

DISCUSSION

Biological Implications. The positive responses to host volatiles in this study suggest that some of these compounds might be used by *Oryzaephilus* spp. as host-finding kairomones in nature. Compared to the cucujolide pheromones and 1-octen-3-ol (A.M. Pierce et al., 1985, 1987, 1989), however, the potential kairomones had relatively higher thresholds for positive response.

The aldehydes and free fatty acids listed in Tables 1 and 3 are ubiquitous natural products in cereals (Maga, 1978) and are common products of lipid oxidation. An increase in levels of free fatty acids is often used as an indicator of deteriorating grain stores (Zeleny, 1954), since grain-infesting fungi (Zeleny, 1954) and bacteria (Kaminski et al., 1980) are well-known producers of free fatty acids. Infestation by *O. surinamensis* in wheat plus dockage resulted in high levels of fat acidity and in infestation by *Penicillium* spp. and bacteria (Sinha, 1983). Because *Oryzaephilus* spp. are commonly associated with a wide variety of stored products of high oil content, it is evidently of adaptive advantage to utilize products of lipid oxidation as host-finding kairomones.

2(*E*),4(*E*)-Heptadienal, 2(*E*),4(*E*)-nonadienal, and 3(*E*),5(*E*)-octadien-2-one have been identified in rancid oat groats (Heydanek and McGorrin, 1981b), while 2(*E*),4(*E*)-nonadienal and 3(*E*),5(*E*)-octadien-2-one have been found in rolled oats (Mikolajczak et al., 1984) and dried oat groats (Heydanek and McGorrin, 1981a), respectively. The above three compounds are probable lipid-autooxidation products (Heydanek and McGorrin, 1981b); 3(*E*),5(*E*)-octadien-2-ol is a likely precursor of 3(*E*),5(*E*)-octadien-2-one.

In their study of oat volatile attractants for *O. surinamensis*, Mikolajczak et al. (1984) found that 2(*E*),4(*E*)-nonadienal was attractive to *O. surinamensis* at only two doses, 0.1 and 1 μg, with repulsion at 100 μg. In the same study, heptanal, hexanal, octanal, and benzaldehyde were moderately attractive, and nonanal was not attractive. Our results (Table 1) indicate lower threshold concentrations and greater active dose ranges than found by Mikolajczak et al. (1984), with the exception of the opposite trend for propanal.

At one test dosage for each compound, propanal, hexanal, hexanoic acid, and nonanoic acid were attractive to *O. surinamensis*, while nonanal and propionic acid were not attractive (O'Donnell et al., 1983). The authors noted that it is unwise to make conclusions about the relative attractancy of such compounds without testing them over a range of doses. In a study of volatile attractants for *O. surinamensis* from carobs, hexanoic acid was more attractive than acetic, isobutyric, butyric, or 2-methylbutyric acids over a range of stimulus dilutions (Stubbs et al., 1985), whereas we found little attraction of *O. surinamensis* by hexanoic acid (Table 3). However, the use of a bioassay based on an insect activity detector in the studies by O'Donnell et al. (1983) and Stubbs et al. (1985) makes comparisons with our results difficult.

Short- and medium-chain aldehydes and fatty acids have been implicated as kairomones for other stored-product insects. Octanal and octanoic acid induced aggregation of larval *Trogoderma glabrum* (Herbst) (Nara et al., 1981). Adults of *Tribolium castaneum* (Herbst) and *Trogoderma granarium* Everts were repelled by C_5–C_9 and C_5–C_8 fatty acids, respectively; valeric acid was a phagostimulant for larvae of *T. granarium* and *Dermestes maculatus* De Geer, while octanoic acid and nonanoic acid were feeding deterrents (Cohen et al., 1974). In comparison with our experiments, these assays were conducted over a very narrow dose range, and further experimentation could well alter the above conclusions.

Practical Implications. The data in Table 2 suggest that, for *O. mercator*, adding nonanal or a mixture of hexanal, octanal, and nonanal to field traps containing cucujolides might be an economical way of lowering the response threshold for the less volatile cucujolides. Adding the highly volatile (*R*,*S*)-1-octen-3-ol to cucujolide mixtures also enhanced the response to the cucujolides in laboratory bioassays for *O. mercator* and *O. surinamensis* (Pierce et al., 1989) and in field traps for *O. surinamensis* (Pierce et al., 1990). Hexanal, octanal, and nonanal in mixture did not interfere with response by *O. mercator* to the individual aldehydes since the response to the three-part aldehyde mixture extended over a greater range, 0.01–100 μg, than response to any of the three individual aldehydes (Tables 1 and 2). Thus, in the absence of pheromones, the three-part aldehyde mixture would be a better trap bait for *O. mercator* than the individual aldehydes.

Other attractive compounds in this study might be more effective as field baits when presented as mixtures. The more volatile, shorter-chain aldehydes such as 2-methylpropanal and pentanal might function over longer distances as useful attractants, while the less volatile, longer-chain compounds such as heptanoic, octanoic, and nonanoic acids might be effective at short range. Because of the limited attractive ranges of some of the potential kairomones, however, release rates would have to be carefully controlled.

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