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ORIGINAL PAPER

Repellents effectively disrupt the olfactory orientation of *Sitophilus granarius* to wheat kernels

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Abstract *Sitophilus granarius* (L.) (Coleoptera: Dryophthoridae) is one of the most important pests of stored cereals worldwide. Sustainable control means of this pest are urgently needed mainly owing to legislative limits to the commonly used fumigants and broad-spectrum contact insecticides. The effectiveness of one alcohol and seven aliphatic aldehydes, previously identified as repellents, to disrupt adult granary weevils orientation towards wheat grains were assessed in two-choice olfactometer bioassays. In the dose range tested, all compounds effectively reduced wheat grains attractiveness and inhibited the preferential orientation of adult weevils towards the host substrate. Moreover, at the highest doses the three aldehydes butanal, (*E*)-2-hexenal, and (*E,E*)-2,4-nonadienal, their binary (1:1) and the ternary (1:1:1) blends induced a significant preferential orientation of insects to the control, indicating actual repellence. Among all repellent stimuli, the ternary blend and the binary blends of butanal plus (*E*)-2-hexenal and (*E*)-2-hexenal plus (*E,E*)-2,4-nonadienal were the most effective. At certain doses, the observed insect response to these blends was more intense than that expected from individual compounds, demonstrating synergistic interactions between the blend components. Repellent aldehydes and their mixtures were effective in disrupting the olfactory orientation of adult granary weevil to a highly attractive oviposition and food substrate. Future development of proper formulations of these bioactive compounds is promising to set up semiochemical-based control means for this pest.

Keywords Dryophthoridae · Allomones · Insect-behavior-modifying compounds · Behavioral bioassay · Stored grains · Integrated pest management

Key message

- There is an urgent need to develop alternatives to chemical control of stored-product insect pests.
- In this study some plant volatile aldehydes and their combinations were shown to effectively disrupt the recognition process of the host substrate by the granary weevil.
- The bioactive compounds could be used to develop sustainable control strategies for this pest.

Introduction

The granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Dryophthoridae), is a major primary pest of stored grains but sometimes attacks processed foods (Dobie and Kilminster 1978; Schwartz and Burkolder 1991). Infestation by this pest leads to both severe quantitative and qualitative losses (Sauer et al. 1984; Rajendran 2002; Magan et al. 2003; Plarre 2010). Control of granary weevil is difficult due to the endophytic development of immature stages that are well protected within grains from pesticides, the increasing legislation

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limits to the use of some fumigants and broad-spectrum contact insecticides, including the worldwide withdrawal from routine use of methyl bromide as a fumigant in 2015 under the directive of the Montreal Protocol on ozone-depleting substances, and the increasing consumer demand for safe food (Phillips and Throne 2010). As a consequence, the identification of bioactive compounds to be used for the implementation of current integrated pest management strategies is necessary (Isman 2006; Germinara et al. 2007; Trematerra 2013; Li et al. 2013).

Among possible alternatives to synthetic insecticides the use of semiochemicals to manipulate the insect behavior has become a suitable tool for the management of a number of stored-product insect pests (Phillips and Throne 2010). Allelochemicals are chemicals mediating interactions between organisms from different species (Nordlund and Lewis 1976). Phytophagous insects rely on allelochemicals in the search for food, mate and egg-laying sites and to avoid suboptimal substrates (Visser 1986; Agelopulos et al. 1999). Allelochemicals able to repel insects (allomones) have the potential to provide direct control through deterring pests from food and oviposition sites (Agelopoulos et al. 1999; Cook et al. 2007). Possible applications of repellents to avoid infestation by stored-product insect pests are to treat empty stores in order to flush out hidden infestation before fresh grain is introduced, to create chemical barriers able to mask odors of grain bulks to insects, and to incorporate them into packaging materials to prevent insects from entering packaged foods (Cox 2004; Hou et al. 2004; Germinara et al. 2010, 2012a).

Repellent compounds in mixtures may act antagonistically, additively, or synergistically depending on whether the response to a mixture is less, equally or more intense than the sum of responses to individual components, respectively. From a practical viewpoint, identifying synergistic effects in complex mixtures are thought to be important in pest control since it may allow for the development of more effective control agents as well as the use of smaller absolute amounts in the mixture to achieve satisfactory levels of efficacy (Hummelbrunner and Isman 2001). Among natural products, some plant essential oils and their primary terpenoid constituents were found to exhibit repellent effects against granary weevil adults (Nerio et al. 2010; Conti et al. 2011; Benelli et al. 2012). In our previous studies, electroantennographic (EAG) tests showed the ability of the peripheral olfactory system of *S. granarius* males and females to perceive a wide range of cereal volatiles (Germinara et al. 2002). In behavioral pitfall bioassays testing different concentrations of individual EAG-active compounds, at specific doses, five compounds (1-butanol, 3-methyl-1-butanol, pentanal, maltol, and vanillin) acted as attractants, while twelve [1-hexanol, butanal, hexanal, heptanal, (*E*)-2-hexenal, (*E,E*)-2,4-nonadienal, (*E,E*)-2,4-decadienal, 2,3-butanedione, 2-pentanone, 2-hexanone, 2-heptanone, and furfural)] acted as repellents suggesting that host finding behavior by the granary weevil more likely depends on the balance of positive and negative stimuli (Germinara et al. 2008). Among previously identified repellent compounds, propionic acid and some short-chain aliphatic ketones were found to reduce wheat grain attractiveness to adult granary weevils (Germinara et al. 2007, 2012b). In the present study, hexanol and some volatile aldehydes, previously identified as granary weevil repellents, were assessed for their ability to disrupt the olfactory orientation of adult weevils to a feeding and oviposition substrate in two-choice olfactometer bioassays. Moreover, interactions between compounds which exhibited repellent activity even in the presence of the host substrate were evaluated.

Materials and methods

Insects

Sitophilus granarius were reared for seven generations on wheat (*Triticum durum* var. Simeto) grains in cylindrical glass containers (15 cm diameter × 15 cm height) closed by a nylon net (mesh size 0.5 mm). Colonies were maintained in the dark in a climatic chamber set at 25 ± 2 °C and 60 ± 5 % r.h. Approximately, four-week-old adults of mixed sex were used for the experiments.

Chemicals

Test compounds selected on the basis of their repellent activity towards granary weevil adults (Germinara et al. 2008) and purchased from Sigma-Aldrich (Milan, Italy) were: hexanol (99 %, Cat. No. 128570), butanal (99 %, Cat. No. 20710), hexanal (98 %, Cat. No. 115606) (*E*)-2-hexenal (98 %, Cat. No. 132659), heptanal (95 %, Cat. No. H2120), (*E,E*)-2,4-nonadienal (85 %, Cat. No. 180556), (*E,E*)-2,4-decadienal (85 %, Cat. No. 180513), furfural (99 %, Cat. No. 185914). Three binary (1:1) and one ternary (1:1:1) (w/w) blends of three compounds which showed actual repellence against granary weevil adults in the presence of the host substrate were also set up. For each compound and blend, decimal dilutions from 100 to 0.1 μg μL^{-1} in mineral oil (Cat. No. M8410) were prepared for use in behavioral tests. Solutions were stored at −20 °C until needed.

Behavioral tests

A two-choice pit-fall bioassay similar to that described in previous studies (Phillips et al. 1993; Germinara et al.

2008) was adopted to evaluate the ability of each test compound and blend to disrupt granary weevil orientation to odors of wheat grains. The test arena was a steel container (32 cm diameter × 7 cm height) with two diametrically opposed holes (3 cm diameter) located 3 cm from the side wall. A filter paper disc (0.7 cm diameter) was suspended at the center of each hole by a cotton wire taped to the lower surface of the arena. Glass flasks (500 mL) assigned to collect the responding insects were positioned under each hole. The inside necks of the collection flasks were coated with mineral oil to prevent insects from returning to the arena. The floor of the arena was covered in filter paper (Whatman No. 1) to facilitate insect movements. Twenty insects of mixed sex, left for at least 4 h without food, were placed under an inverted Petri dish (3 cm diameter × 1.2 cm height) at the center of the arena and allowed to acclimatize (30 min) prior to release. The arena was covered with a steel lid to prevent insects from escaping. Insects were presented with the odors emitted by wheat grains (200 g; 12.5 % moisture content) left in a collection flask, alone or plus a dose of a test compound or blend (10 μL of mineral oil solution) adsorbed onto the overlying filter paper disc and mineral oil (10 μL) adsorbed onto the opposed paper disc as control. Five doses (1, 10, 100, 500, and 1000 μg) of each compound were assessed. Tests lasted 3 h and were carried out in the dark at 25 ± 2 °C and 60 ± 5 % r.h. (Germinara et al. 2008). There were five replicates of each assay, and insects were only used once.

Data analysis

In each experiment, a response index (RI) was calculated using $RI = [(T-C)/Tot] \times 100$, where T is the number responding to the treatment, C is the number responding to the control and Tot is the total number of insects released (Phillips et al. 1993). For each bioassay, the mean numbers of insects in the treatment and control were compared by Student's t test for paired comparisons. For each compound, the mean numbers of insects found in the treatment and in the control and the mean RIs at different doses were subjected to analysis of variance (ANOVA), Levene's test of homogeneity of variance, and ranked according to Tukey's HSD test. Data were submitted to linear regression analysis in order to evaluate the effect of the dose on the response of the insects. ANOVA followed by Tukey's HSD test was also used to compare the mean RIs of blends with those of individual components at different doses and to rank the most effective stimuli in reducing granary weevil orientation to wheat grains. Synergism between compounds in a blend was analyzed by comparing the RI_o induced by a combination (0.5:0.5) of two compounds (observed effect) with the RIs induced by each compound (1 and 1, respectively) separately (expected effect). Hence, the expected RI was calculated using the formula: $RI_e = RI_a + RI_b/2$, where RI_a and RI_b were the observed RI caused by each compound alone (Gowing 1959). The same criterion was adopted to calculate the RI_e of the ternary blend. Negative RI_o–RI_e values were considered synergistic (Koppenhofer and Kaya 1998). Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS version 12.0.1).

Results

All compounds elicited significant reductions of insect orientation to odors of wheat kernels (WK) (Table 1). The RI was significantly lower than that to WK alone for six compounds starting from the 1 μg dose and for two compounds from the 10 μg dose.

With dose increase, for all compounds there were significant reductions in the number of insects in the treatment (df = 5, 24; $F = 12.57$–68.47; $P < 0.005$) and significant increases in the number of insects in the control (df = 5, 24; $F = 3.59$–10.64; $P < 0.015$). At the highest doses, seven compounds elicited negative RIs but only for butanal and (*E*)-2-hexenal at the 1000 μg dose and (*E,E*)-2,4-nonadienal starting from the 500 μg dose the number of the insects in the treatment was significantly lower (t-test, $P < 0.05$) than that in the control, indicating actual repellence (Table 1). Regression analyses showed a dose-dependent relationship for hexanol, (*E,E*)-2,4-decadienal, heptanal, (*E*)-2-hexenal, and hexanal ($R^2 = 0.965$–0.834; $P = 0.003$–0.030) but none for furfural, butanal, and (*E,E*)-2,4-nonadienal ($R^2 = 0.468$–0.677; $P = 0.203$–0.087).

In the range of dose tested, all blends of repellent compounds elicited mean RIs which were significantly lower than that to WK alone starting from the lowest dose (Table 2). With dose increase, all mixtures elicited a significant reduction of the insects orienting to the treatment (df = 5, 24; $F = 41.88$–64.80; $P < 0.001$) and a significant increase of those choosing the control (df = 5, 24; $F = 14.94$–19.95; $P < 0.010$), resulting in significant RI reductions. Negative and significant (t-test; $P < 0.05$) RIs were observed for the blend of butanal plus (*E,E*)-2,4-nonadienal at the highest dose and the other blends starting from the 500 μg dose (Table 2). Regression analyses showed a dose-dependent relationship with repellent properties for all blends ($R^2 = 0.953$–0.856; $P = 0.004$–0.022) except for butanal plus (*E*)-2-hexenal.

At different doses tested, the mean RIs elicited by the butanal plus (*E,E*)-2,4-nonadienal blend were not significantly different from those of individual components (Fig. 1). The mean RIs induced by the other binary blends, butanal plus (*E*)-2-hexenal, and (*E*)-2-hexenal plus (*E,E*)-

Table 1 Behavioral responses of *S. granarius* adults to odors emitted by 200 g of wheat kernels (WK) alone and in the presence of ascending doses (1, 10, 100, 500, 1000 μg) of individual volatile compounds in two-choice bioassays

Compounds	Stimulus	Treatment	Control	Student's *t*-test		Response index
				t value	*P* value	
Hexanol	WK	17.6 ± 0.7a	1.2 ± 0.4a	15.93	<0.001	82 ± 5.1a
	WK + 1 μg	10.8 ± 1.1bc	3.2 ± 0.9ab	3.77	0.020	38 ± 10.1b
	WK + 10 μg	11.0 ± 1.2b	2.8 ± 0.8ab	4.30	0.013	41 ± 9.5b
	WK + 100 μg	10.2 ± 1.5bc	3.0 ± 0.6ab	6.22	0.003	36 ± 5.8bc
	WK + 500 μg	9.2 ± 1.0bc	3.6 ± 0.4ab	4.63	0.010	28 ± 6.0bc
	WK + 1000 μg	6.4 ± 0.5c	5.4 ± 0.8b	1.12	0.326	5 ± 4.5c
		$F = 12.57$	$F = 3.89$			$F = 12,20$
		df = 5, 24	df = 5, 24			df = 5, 24
		$P < 0.001$	$P = 0.010$			$P < 0.001$
Butanal	WK	17.6 ± 0.7a	1.2 ± 0.4a	15.93	<0.001	82 ± 5.1a
	WK + 1 μg	10.2 ± 1.4b	4.2 ± 0.7ab	3.30	0.030	30 ± 9.1b
	WK + 10 μg	7.2 ± 0.8bc	6.4 ± 1.2bc	0.59	0.587	4 ± 6.8bc
	WK + 100 μg	7.4 ± 1.0bc	8.4 ± 0.7c	0.59	0.589	−5 ± 8.5c
	WK + 500 μg	6.0 ± 0.4cd	8.0 ± 1.1c	2.11	0.103	−10 ± 4.7c
	WK + 1000 μg	2.8 ± 0.6d	6.2 ± 0.4bc	4.54	0.010	−17 ± 3.7c
		$F = 33.66$	$F = 10.64$			$F = 31.30$
		df = 5, 24	df = 5, 24			df = 5, 24
		$P < 0.001$	$P < 0.001$			$P < 0.001$
Hexanal	WK	17.6 ± 0.7a	1.2 ± 0.4a	15.93	<0.001	82 ± 5.1a
	WK + 1 μg	10.2 ± 1.1b	5.4 ± 0.4c	3.64	0.022	24 ± 6.6b
	WK + 10 μg	8.6 ± 1.1bc	4.4 ± 0.5bc	2.94	0.042	21 ± 7.1b
	WK + 100 μg	5.8 ± 1.2bcd	3.6 ± 1.1bcd	1.19	0.301	11 ± 9.3b
	WK + 500 μg	3.0 ± 1.0d	1.8 ± 0.6ab	0.82	0.458	6 ± 7.3b
	WK + 1000 μg	5.0 ± 1.3cd	5.2 ± 0.6c	0.11	0.916	−1 ± 8.9b
		$F = 22.88$	$F = 7.72$			$F = 15.90$
		df = 5, 24	df = 5, 24			df = 5, 24
		$P < 0.001$	$P < 0.001$			$P < 0.001$
(*E*)-2-Hexenal	WK	17.6 ± 0.7a	1.2 ± 0.4a	15.93	<0.001	82 ± 5.1a
	WK + 1 μg	13.2 ± 0.7b	3.6 ± 0.2ab	14.15	<0.001	48 ± 3.4b
	WK + 10 μg	12.6 ± 1.0b	4.2 ± 1.1ab	4.07	0.015	42 ± 10.3bc
	WK + 100 μg	10.2 ± 1.4b	5.8 ± 0.7b	2.16	0.097	22 ± 10.2bc
	WK + 500 μg	5.8 ± 0.7c	3.0 ± 1.1ab	0.41	0.153	14 ± 8.0cd
	WK + 1000 μg	2.0 ± 0.6c	5.0 ± 0.3b	4.24	0.013	−15 ± 3.5d
		$F = 37.92$	$F = 4.77$			$F = 20.30$
		df = 5, 24	df = 5, 24			df = 5, 24
		$P < 0.001$	$P = 0.004$			$P < 0.001$
Heptanal	WK	17.6 ± 0.7a	1.2 ± 0.4a	15.93	<0.001	82 ± 5.1a
	WK + 1 μg	14.2 ± 0.6b	2.2 ± 0.7ab	10.52	<0.001	60 ± 5.7ab
	WK + 10 μg	13.6 ± 0.5b	3.0 ± 0.8ab	10.81	<0.001	53 ± 4.9b
	WK + 100 μg	13.0 ± 0.5b	4.6 ± 0.5bc	16.47	<0.001	42 ± 2.5b
	WK + 500 μg	6.8 ± 1.2c	4.4 ± 0.5bc	1.67	0.170	12 ± 7.2c
	WK + 1000 μg	5.2 ± 0.9c	6.2 ± 0.7c	0.66	0.546	−5 ± 7.6c
		$F = 39.28$	$F = 8.10$			$F = 31.09$
		df = 5, 24	df = 5, 24			df = 5, 24
		$P < 0.001$	$P < 0.001$			$P < 0.001$

Table 1 continued

Compounds	Stimulus	Treatment	Control	Student's *t*-test		Response index
				t value	*P* value	
(*E*,*E*)-2,4-Nonadienal	WK	17.6 ± 0.7a	1.2 ± 0.4a	15.93	<0.001	82 ± 5.1a
	WK + 1 μg	11.6 ± 0.5b	5.2 ± 0.4ab	7.34	0.002	32 ± 4.4b
	WK + 10 μg	8.6 ± 1.1bc	5.0 ± 1.0ab	1.99	0.116	18 ± 9.0b
	WK + 100 μg	5.8 ± 1.2c	9.0 ± 1.5b	1.45	0.219	−16 ± 11.0c
	WK + 500 μg	1.4 ± 0.5d	6.4 ± 1.3b	3.29	0.030	−25 ± 7.6c
	WK + 1000 μg	0.6 ± 0.2d	7.8 ± 0.9b	7.43	0.002	−36 ± 4.9c
		$F = 68.47$	$F = 7.38$			$F = 35.35$
		df = 5, 24	df = 5, 24			df = 5, 24
		$P < 0.005$	$P < 0.001$			$P < 0.001$
(*E*,*E*)-2,4-decadienal	WK	17.6 ± 0.7a	1.2 ± 0.4a	15.93	<0.001	82 ± 5.1a
	WK + 1 μg	7.0 ± 1.4b	3.0 ± 0.9ab	2.03	0.113	20 ± 9.9b
	WK + 10 μg	5.6 ± 0.6bc	2.4 ± 0.5ab	3.72	0.020	16 ± 4.3bc
	WK + 100 μg	4.6 ± 0.7bc	2.6 ± 0.9ab	1.41	0.230	10 ± 7.1bc
	WK + 500 μg	3.4 ± 0.7bc	3.0 ± 0.5ab	0.19	0.374	2 ± 2.0bc
	WK + 1000 μg	3.0 ± 0.8c	5.0 ± 0.4b	2.11	0.103	−10 ± 4.7c
		$F = 42.15$	$F = 3.59$			$F = 28.41$
		df = 5, 24	df = 5, 24			df = 5, 24
		$P < 0.001$	$P = 0.015$			$P < 0.001$
Furfural	WK	17.6 ± 0.7a	1.2 ± 0.4a	15.93	<0.001	82 ± 5.1a
	WK + 1 μg	12.6 ± 1.3b	2.0 ± 0.9a	5.02	0.007	53 ± 10.6a
	WK + 10 μg	8.8 ± 1.2bc	5.4 ± 0.7ab	1.88	0.133	17 ± 9.0b
	WK + 100 μg	4.6 ± 0.5d	5.0 ± 1.3ab	0.30	0.778	−2 ± 6.6b
	WK + 500 μg	5.6 ± 0.8cd	7.4 ± 1.4b	1.09	0.338	−9 ± 8.3b
	WK + 1000 μg	4.2 ± 0.8d	6.6 ± 0.9b	1.86	0.136	−12 ± 6.4b
		$F = 32.28$	$F = 6.28$			$F = 23.38$
		df = 5, 24	df = 5, 24			df = 5, 24
		$P < 0.001$	$P < 0.001$			$P < 0.001$

In a row, significant differences between treatment and control responses are indicated by Student's *t*-test ($P < 0.05$). For each compound tested, means in the same column were submitted to One-way ANOVA (Tukey's HSD test) for insects in the treatment ($F = 12.57$–68.47; df = 5, 24; $P < 0.005$), insects in the control ($F = 3.59$–16.64; df = 5, 24; $P < 0.015$), and RI values at different doses ($F = 12.20$–35.35; df = 5, 24; $P < 0.001$). Values with no letter in common are significantly different according to Tukey's HSD test

2,4-nonadienal, and the ternary one were often significantly lower than those of individual components (Tukey's test, $P < 0.05$). Synergistic interactions were found between (*E*)-2-hexenal and butanal and (*E*)-2-hexenal and (*E,E*)-2,4-nonadienal at all doses tested (Fig. 1). Butanal and (*E,E*)-2,4-nonadienal acted synergistically at 1 and 10 μg doses. Synergism among the three compounds was detected at the 500 and 1000 μg doses.

At the highest dose tested, there were significant differences (df = 6, 28; $F = 11.545$; $P < 0.001$) among repellent stimuli, with the ternary blend being the most effective (Table 3). At this dose, the mean RI to the ternary blend was statistically similar to those of butanal plus (*E*)-2-hexenal and (*E*)-2-hexenal plus (*E,E*)-2,4-nonadienal blends but significantly lower than those of individual compounds and butanal plus (*E,E*)-2,4-nonadienal (Tukey's test, $P < 0.05$).

Discussion

Some volatile aldehydes and hexanol, known to be emitted by grains of different cereal species (Maga 1978), showed repellent activity towards granary weevil adults when individually tested in the absence of a suitable host odor source (Germinara et al. 2008). In this study, hexanol, butanal, hexanal, (*E*)-2-hexenal, heptanal, (*E,E*)-2,4-nonadienal, (*E,E*)-2,4-decadienal, furfural, in the dose range previously tested (from 1 to 1000 μg), were assessed for their ability to disrupt olfactory orientation of insects to

Table 2 Behavioral responses of *S. granarius* adults to odors emitted by 200 g of wheat kernels (WK) alone and in the presence of ascending doses (1, 10, 100, 500, 1000 μg) of binary (1:1) and ternary (1:1:1) blends of repellent compounds in two-choice bioassays

Mixture	Stimulus	Treatment	Control	Student's *t*-test		Response index
				t value	*P* value	
Butanal + (*E*)-2-Hexenal	WK	17.6 ± 0.7a	1.2 ± 0.4a	15.93	<0.001	82 ± 5.1a
	WK + 1 μg	8.4 ± 1.0b	9.2 ± 0.5bc	0.57	0.596	−4 ± 7.0b
	WK + 10 μg	8.6 ± 1.1b	7.8 ± 1.1b	0.40	0.707	4 ± 9.9b
	WK + 100 μg	6.4 ± 1.1bc	9.8 ± 0.5bc	2.26	0.087	−17 ± 7.5bc
	WK + 500 μg	2.6 ± 1.2cd	11.6 ± 1.4c	5.69	0.005	−45 ± 7.9c
	WK + 1000 μg	1.2 ± 0.7d	9.6 ± 0.6bc	7.80	0.001	−42 ± 5.4c
		$F = 41.88$	$F = 19.02$			$F = 40.14$
		df = 5, 24	df = 5, 24			df = 5, 24
		$P < 0.001$	$P = 0.010$			$P < 0.001$
Butanal + (*E,E*)-2,4-Nonadienal	WK	17.6 ± 0.7a	1.2 ± 0.4a	15.93	<0.001	82 ± 5.1a
	WK + 1 μg	9.4 ± 1.0b	9.2 ± 1.2b	0.10	0.927	1 ± 10.3b
	WK + 10 μg	8.6 ± 0.9b	8.8 ± 0.5b	0.16	0.880	−1 ± 6.2b
	WK + 100 μg	6.2 ± 0.5bc	7.6 ± 0.7b	1.43	0.226	−7 ± 4.9bc
	WK + 500 μg	5.2 ± 0.9c	7.6 ± 1.0b	1.50	0.208	−12 ± 8.0bc
	WK + 1000 μg	1.6 ± 0.5d	8.6 ± 0.5b	9.90	<0.001	−35 ± 3.5c
		$F = 49.93$	$F = 14.94$			$F = 35.41$
		df = 5, 24	df = 5, 24			df = 5, 24
		$P < 0.001$	$P < 0.001$			$P < 0.001$
(*E*)-2-Hexenal + (*E,E*)-2,4-Nonadienal	WK	17.6 ± 0.7a	1.2 ± 0.4a	15.93	<0.001	82 ± 5.1a
	WK + 1 μg	9.4 ± 0.8b	7.6 ± 0.8b	1.45	0.221	9 ± 6.2b
	WK + 10 μg	10.2 ± 1.1b	7.8 ± 0.7b	1.37	0.242	12 ± 8.7b
	WK + 100 μg	7.4 ± 0.5bc	8.6 ± 0.6b	1.50	0.208	−6 ± 4.0b
	WK + 500 μg	0.8 ± 0.6c	11.0 ± 1.5bc	5.14	0.006	−51 ± 9.9c
	WK + 1000 μg	2.8 ± 0.6d	13.8 ± 1.2c	6.46	0.003	−57 ± 7.8c
		$F = 64.80$	$F = 19.95$			$F = 48.43$
		df = 5, 24	df = 5, 24			df = 5, 24
		$P < 0.001$	$P < 0.001$			$P < 0.001$
Butanal + (*E*)-2-Hexenal + (*E,E*)-2,4-Nonadienal	WK	17.6 ± 0.7a	1.2 ± 0.4a	15.93	<0.001	82 ± 5.1a
	WK + 1 μg	10.8 ± 1.4b	6.8 ± 1.0b	1.82	0.142	20 ± 11.0b
	WK + 10 μg	10.4 ± 1.2b	7.8 ± 1.2b	1.18	0.304	13 ± 11.0b
	WK + 100 μg	7.4 ± 0.7b	7.4 ± 1.2b	0.00	1.000	0 ± 8.2b
	WK + 500 μg	1.4 ± 0.4c	12.4 ± 1.2c	7.25	0.002	−55 ± 7.6c
	WK + 1000 μg	1.6 ± 0.7c	13.6 ± 0.8c	10.52	<0.001	−60 ± 5.7c
		$F = 46.25$	$F = 19.71$			$F = 39,25$
		df = 5, 24	df = 5, 24			df = 5, 24
		$P < 0.001$	$P = 0.004$			$P < 0.001$

In a row, significant differences between treatment and control responses are indicated by Student's *t*-test ($P < 0.05$). For each blend tested, means in the same column were submitted to One-way ANOVA (Tukey's HSD test) for insects in the treatment ($F = 41.88$–64.80; df = 5, 24; $P < 0.001$), insects in the control ($F = 14.94$–19.95; df = 5, 24; $P < 0.001$), and RI values at different doses ($F = 35.41$–48.43; df = 5, 24; $P < 0.001$). Values with no letter in common are significantly different according to Tukey's HSD test

WK, a highly attractive feeding and oviposition substrate of the granary weevil. At low doses, all compounds tested effectively reduced the attractiveness of WK to granary weevils. At the highest doses, hexanol, hexanal, heptanal, (*E,E*)-2,4-decadienal, and furfural achieved inhibition of preferential insect orientation towards WK as indicated by not significant RIs. However, the significant correlation observed for almost all of these compounds between concentration and reduction of insect orientation to the host substrate suggests that repellent effects could be induced by higher doses than were used. At the highest doses, the three aldehydes butanal, (*E*)-2-hexenal, and (*E,E*)-2,4-

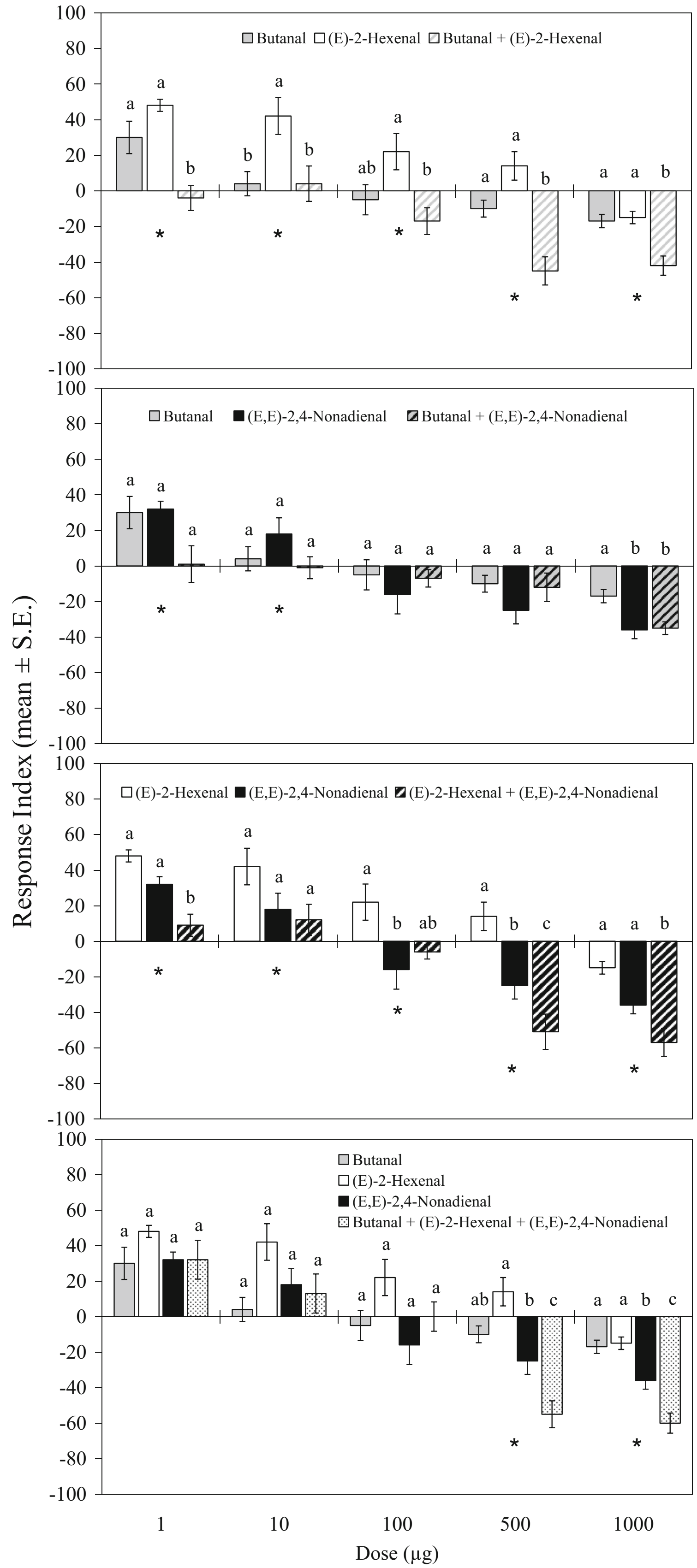

Fig. 1 Mean RIs of *S. granarius* adults to odors emitted by 200 g of WK in the presence of ascending doses (1, 10, 100, 500, 1000 µg) of butanal, (*E*)-2-hexenal, (*E,E*)-2,4-nonadienal, their binary, and the ternary blends in two-choice bioassays. Bars represent means (±SE), and statistical treatments were performed using One-way ANOVA (Tukey's HSD test) for binary blends of butanal plus (*E*)-2-hexenal ($F = 5.14–17.79$; df = 2, 12; $P = 0.001–0.024$), butanal plus (*E,E*)-2,4-nonadienal ($F = 0.47–6.70$; df = 2, 12; $P = 0.011–0.634$), and (*E*)-2-hexenal plus (*E,E*)-2,4-nonadienal ($F = 2.86–14.63$; df = 2, 12; $P = 0.001–0.097$) and the ternary blend ($F = 2.79–21.05$; df = 3, 16; $P = 0.001–0.116$). For each combination, bars with no letter in common are significantly different according to Tukey's HSD test. For a set test dose, the asterisk indicates synergism between compounds in the mixture (negative $RI_o–RI_e$)

Table 3 Comparison of the mean RIs of *S. granarius* adults to the highest dose of repellent stimuli

Stimulus (1000 μg)	Response index (Mean ± SE)[a]
(*E*)-2-Hexenal	−15,0 ± 3,5a
Butanal	−17,0 ± 3,7a
Butanal + (*E,E*)-2,4-Nonadienal	−35,0 ± 3,5ab
(*E,E*)-2,4-Nonadienal	−36,0 ± 4,9ab
Butanal + (*E*)-2-Hexenal	−42,0 ± 5,4bc
(*E*)-2-Hexenal + (*E,E*)-2,4-Nonadienal	−57,0 ± 7,8bc
Butanal + (*E*)-2-Hexenal + (*E,E*)-2,4-Nonadienal	−60,0 ± 5,7c

Values with no letter in common are significantly different according to Tukey's HSD test

[a] Values were submitted to One-way ANOVA (Tukey's HSD test) ($F = 11.55$; df = 6, 28; $P < 0.05$)

nonadienal elicited a preferential insect orientation towards the control indicating actual repellence. Based on these results, the behavioral activity of three binary blends and one ternary blend of the three repellent aldehydes was evaluated. All blends reduced WK attractiveness to insects at the lowest dose tested, inhibited the preferential orientation of insects towards the host substrate at the intermediate doses and repelled insects at the highest ones.

A significant reduction of the insects moving to the treatment and increase of those orienting to the control was elicited by increasing concentrations of all compounds and blends. From an ecological perspective, many factors can explain compound-mediated reduction of host substrate attractiveness and/or substrate avoidance by insects. Firstly, compounds tested may have altered the host odor profile which might no longer be recognized by insects as a relevant host odor signal. Many studies highlighted the importance of ratio and concentration of host plant volatiles in host location by herbivorous insects (Najar-Rodriguez et al. 2010; Webster et al. 2010; Cha et al. 2011). Secondly, high concentrations of the compounds tested may have dramatically altered the host odor substrate which was recognized as suboptimal by the insects and avoided. During cereal storage the oxidation process of grain lipids leads to an increasing production of some short-chain aliphatic aldehydes such as hexanal, heptanal, (*E,E*)-2,4-nonadienal, and (*E,E*)-2,4-decadienal which correlate with several sensory attributes including rancid flavor (Grosh and Schieberle 1991; Heiniö et al. 2002). Thirdly, the compounds were recognized as toxic and therefore avoided. Short-chain saturated and unsaturated aliphatic aldehydes are produced by plant tissues in response to mechanical and herbivory damage through the hydroperoxide lyase pathway of oxylipin metabolism (Matsui 2006) and they have been shown to possess fumigant and contact toxicity against various stored-product insect pests (Ferguson and Pirie 1948; Hammond et al. 2000; Hubert et al. 2008; Germinara et al. 2012a; Anfora et al. 2014).

Synergistic effects between butanal and (*E,E*)-2,4-nonadienal and the three aldehydes in the ternary blend occurred at specific doses suggesting concentration of individual components as a key factor to the nature of interactions. These results underlined the importance to elucidate interactions of repellents in mixtures in a wide range of doses to develop proper formulations. The ternary blend was the most effective among repellent stimuli indicating that the level of repellence was increased by the complexity of mixture. A simultaneous activation of different receptor sites may account for the high repellent effect of the ternary blend. Electrophysiological and molecular approaches, in fact, showed that repellents interact with olfactory and gustatory receptors in mosquito modulating their function through multiple molecular mechanisms (Paluck et al. 2010; Bohbot et al. 2011; Dickens and Bohbot 2013). Moreover, all three aldehydes tested in this study are perceived by the peripheral olfactory system of adult *S. granarius* males and females (Germinara et al. 2002).

The three repellent aldehydes occur widely in the aromas of natural and processed foods, including grains of different cereal species (Maga 1978; Maarse 1991; Zhou et al. 1999). They are listed in the FDA's official database on food additives (EAFUS, Everything Added to Food in the United States) and frequently used to produce natural flavor notes in processed foods (Whitehead et al. 1995; Oms-Oliu et al. 2010). Consequently, practical application of these aldehydes to protect stored products intended for human or animal consumption from attacks of insect pests appears to be safe.

Application of natural repellents is generally difficult due to their low stability and high evaporation rate. Therefore, they need to be embedded in specialized polymers in order to achieve a prolonged release of vapors, allowing increased repellent release time and exposure, smaller quantities of fumigants to be used, reduced loss of the active ingredients, and protection against environmental agents (high temperature, oxidation, UV light) (Kydonieus 1980; Chaskopoulou et al. 2009; Germinara et al. 2014; Rumbos et al. 2014; Ziaee et al. 2014). In conclusion, three plant volatile aldehydes were able to effectively disrupt the granary weevil orientation to a highly attractive host substrate with some notable synergistic interactions suggesting the feasibility of practical applications.

Author contribution

GSG, ADC, ad GR conceived, designed research and conducted experiments. GSG analyzed data and wrote the manuscript. All authors read and approved the manuscript.

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