

Response of Mated Insects of Both Sexes of Granary Weevil to Blends of Volatiles – Short Communication

ANNA WENDA-PIESIK¹, DARIUSZ PIESIK^{2*} and ANITA KRASIŃSKA^{2,3}

¹ Department of Plant Growth Principles and Experimental Methodology and Department of Entomology and Molecular Phytopathology, ²UTP University of Science and Technology, Bydgoszcz, Poland; ³Group of Economic and Administration Schools, Bydgoszcz, Poland

*Corresponding author: piesik@utp.edu.pl

Abstract

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We report the behavioural responses of mated granary weevils to blends of cereal volatiles. Four doses were applied on filter paper (1, 10, 100, and 1000 ng/min in 50 µl of hexane applied on filter paper). A Y-tube experiment revealed that mated females of *Sitophilus granarius* were attracted to blend 1, 4, and 5 at concentrations of 1000, 100, 1, and 10 ng/min, respectively. Mated males were attracted only to blend 5 at a concentration of 10 ng/minute. Yet, the mated weevil females and males were repelled by the highest concentration (1000 ng/min) for all tested blends. Moreover, both mated sexes were repelled by 100 ng/min in blends 2, 3, 5, and 6. Additionally, females were repelled by 10 ng/min in blend 6. We claim that the blend of volatiles and their concentration can induce the insect behaviour.

Keywords: cereal insects; cereal volatiles; attractants; repellents

Abbreviation list of chemical compounds

VOCs = volatile organic compounds; blend 1 – aliphatic alcohols (1-butanol = 1-BUT, 1-pentanol = 1-PEN, 1-hexanol = 1-HEX, 3-methyl-1-butanol = 3-MET); blend 2 – aliphatic aldehydes (butanal = BUT, pentanal = PEN, hexanal = HEX, heptanal = HEP, (*E*)-2-hexenal = (*E*)-2-HEX, (*E,E*)-2,4-peptadienal = (*E,E*)-2,4-HEP, (*E,E*)-2,4-nonadienal = (*E,E*)-2,4-NON, (*E,E*)-2,4-decadienal = (*E,E*)-2,4-DEC); blend 3 – aliphatic ketones (2-pentanone = 2-PEN, 2-hexanone = 2-HEX, 2-heptanone = 2-HEP, 2,3-butanedione = 2,3-BUT); blend 4 – aromatics (3-methoxy-2-methyl-4-pyrone (maltol) = MAL, 132 furfural = FUR, phenylacetaldehyde = PHE, 3-methoxy-4-hydroxy-benzaldehyde (vanillin) = VAN); blend 5 – aliphatic alcohols, aliphatic aldehydes, aliphatic ketones, aromatics; blend 6 – plant VOCs ([(*Z*)-ocimene = (*Z*)-OCI, linalool = LIN, benzyl acetate = BAC, methyl salicylate = MAT, β-caryophyllene = β-CAR, (*E*)-β-farnesene = (*E*)-β-FAR])

Stored product pests can cause direct losses in product weight and also indirect losses. Pests develop large populations, consume food commodities, and contaminate the goods with shed skins, faeces, hairs, webbing, cadavers, and toxins (BELDA & RIUDAVETS 2010; GRETHE *et al.* 2011; NIEDERMAYER & STEIDLE 2013). The degree of damage is directly related to the

infestation rate which, in turn, is determined by factors such as the number of laid eggs, and the survival and fecundity of offspring (NAWROT *et al.* 2010; ABAY *et al.* 2012). In developed countries, these insects can cause losses of up to 9%, but in developing countries the losses can be more than 50% (GERMINARA *et al.* 2010; PIASECKA-KWIATKOWSKA *et al.* 2014).

The granary weevil *S. granarius* is one of the most harmful pests of stored grain that causes severe losses throughout the world (GERMINARA *et al.* 2010). Chemical treatment with insecticides has been the method of choice for many years. However the number of available active substances is shrinking (CLARKE *et al.* 2011; OPIT *et al.* 2012).

Intensive use of insecticides leads to resistance in insects (WHALON *et al.* 2012). For such reasons many researchers have been searching for environmentally friendly and low toxicity new natural products to provide alternatives (HOLOPAINEN & GERSHENZON 2010; BOCZEK *et al.* 2013; GANTNER & NAJDA 2013).

The orientation cues for adults are volatile blends emitted by the grain of several cereal species (RIETDORF & STEIDLE 2002), and the volatile compounds are crucial in the progress of infestation by this pest. In the present study, we examined the behavioural response of mated granary weevil adults of both sexes to blends of VOCs.

MATERIAL AND METHODS

Insects. Experiments were performed in 2014/2015 at the UTP University of Science and Technology, Bydgoszcz, Poland, at the Department of Entomology and Molecular Phytopathology. *S. granarius* individuals were reared on whole wheat kernels in continuous dark at $22 \pm 2^\circ\text{C}$ and relative humidity of $60 \pm 5\%$.

Synthetic chemicals. Synthetic volatiles were obtained from Sigma-Aldrich (Chemical Co. Inc., Poznań, Poland), purity between 85 and 99%. Cereal compounds were selected based on their presence in cereal grains (GERMINARA *et al.* 2010; PIESIK *et al.* 2011, 2014; DELANEY *et al.* 2013; PIESIK & WENDA-PIESIK 2015). To screen behavioural activity of the pest volatile compounds were tested in six blends at four concentrations (1, 10, 100, 1000 ng/min) compared to the absence of the compound (0). In the Y-tube, each of the five VOC concentrations in hexane was tested against the hexane solvent alone. Each of individual VOCs was present in a blend at the specified concentration. Thus, for instance for blend 1 (aliphatic alcohols) 1 ng/min means that 1 ng 1-BUT + 1 ng 1-PEN + 1 ng 1-HEX + 1 ng 3-MET were added to 50 μl hexane. A dose of a blend was placed in one arm of the Y-tube and tested against 50 μl hexane without the blend (0 ng/min).

Y-tube. Insects of both sexes were exposed to each other for 72 h in a cage where also food was supplied. After 3 days they were separated (different cages) and following another 24 h the experiments began. Beetles tended to walk along the Y-tube (this system has been previously tested on various insect species). Twenty *S. granarius* adults of either sex were tested (20♀ and 20♂ for each blend) at each concentration for six blends of VOCs. Adults were observed for 5 minutes.

Data analysis. Chi-square goodness of fit tests (χ^2 -test), with the Yates correction for small samples (1×2), were conducted to indicate whether the choice of Y-tube arms was influenced by a preference to odour source (synthetic blend vs. hexane solvent) at each exposure concentration \times sex \times exposure duration combination. Non-significant tests indicated that the observed beetle counts did not significantly deviate from an expected ratio of 10:10 (the arm with hexane solvent only and synthetic blend). Significant tests indicated attraction (more individuals chose the Y-tube arm with a synthetic blend) or repellency (more individuals chose the Y-tube arm with hexane solvent only).

RESULTS AND DISCUSSION

The highest activity against stored product insects has been reported for methanol extracts, ethyl acetate extracts, and hexane extracts (ABAY *et al.* 2012). We have found in the behavioural responses of mated granary weevils to blends of cereal volatiles that mated females of *S. granarius* were attracted to blend 1, 4, and 5 at concentrations of 100, 100, 1, and 10 ng/min, respectively. In contrast, mated males were attracted only to blend 5 at a concentration of 10 ng/minute. Both sexes were repelled by the highest concentration (1000 ng/min) for all tested blends. Additionally, both mated sexes were repelled by 100 ng/min in blends 2, 3, 5, and 6.

The above results are in good agreement with those of ZIAEE *et al.* (2014) and ZOUBIRI and BAALIOUAMER (2012), who studied essential oils against *Tribolium confusum* Jacquelin du Val. and *S. granarius* adults. GERMINARA *et al.* (2010) demonstrated that at the end of the aging period, the percentage of *S. granarius* adults found in cartons coated with propionic acid-loaded mono and multilayer PCL and zein was only 13.1, 11.3, 18.0 and 10.7% of the total number of insects used in the bioassay.

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Table 1. Effect of synthetic blend 1 (4 aliphatic alcohols), blend 2 (8 aliphatic aldehydes), blend 3 (4 aliphatic ketones), blend 4 (4 aromatics), blend 5 (aliphatic alcohols, aliphatic aldehydes, aliphatic ketones, and aromatics), and blend 6 (6 plant VOCs) on the number of *Sitophilus granarius* adult females and males choosing (after mating) to enter the Y-tube arm containing the blend odour or the Y-tube arm containing purified, humidified air and hexane solvent (no odour)

Blend	Mixed compounds	Dose (ng/min)	No. of females			No. of males		
			+	–	χ^2	+	–	χ^2
1		control (0.0)	9	11	0.05 ^{ns}	10	10	0.05 ^{ns}
	1-BUT	1 (1)	12	8	0.45 ^{ns}	10	10	0.05 ^{ns}
	+ 1-PEN	2 (10)	13	7	1.25 ^{ns}	8	12	0.45 ^{ns}
	+ 1-HEX	3 (100)	15	5	4.05* (a)	7	13	1.25 ^{ns}
	+ 3-MET	4 (1000)	3	17	8.45** (r)	2	18	11.3*** (r)
2	BUT							
	+ PEN	control (0.0)	11	9	0.05 ^{ns}	11	9	0.05 ^{ns}
	+ HEX	1 (1)	10	10	0.05 ^{ns}	7	13	1.25 ^{ns}
	+ HEP	2 (10)	14	6	2.45 ^{ns}	12	8	0.45 ^{ns}
	+ (E)-2-HEX	3 (100)	3	17	8.45** (r)	4	16	6.05* (r)
	+ (E,E)-2,4-HEP	4 (1000)	3	17	8.45** (r)	3	17	8.45** (r)
	+ (E,E)-2,4-NON							
3		control (0.0)	12	8	0.45 ^{ns}	7	13	1.25 ^{ns}
	2-PEN	1 (1)	8	12	0.45 ^{ns}	11	9	0.05 ^{ns}
	+ 2-HEX	2 (10)	6	14	2.45 ^{ns}	6	14	2.45 ^{ns}
	+ 2-HEP	3 (100)	4	16	6.05* (r)	5	15	4.05* (r)
	+ 2,3-BUT	4 (1000)	3	17	8.45** (r)	3	17	8.45** (r)
4		control (0.0)	11	9	0.05 ^{ns}	7	13	1.25 ^{ns}
	MAL	1 (1)	14	6	2.45 ^{ns}	8	12	0.45 ^{ns}
	+ FUR	2 (10)	6	14	2.45 ^{ns}	6	14	2.45 ^{ns}
	+ PHE	3 (100)	17	3	8.45** (a)	13	7	1.25 ^{ns}
	+ VAN	4 (1000)	2	18	11.3*** (r)	4	16	6.05* (r)
5		control (0.0)	7	13	1.25 ^{ns}	11	9	0.05 ^{ns}
	aliphatic alcohols	1 (1)	15	5	4.05* (a)	9	11	0.05 ^{ns}
	+ aliphatic aldehydes	2 (10)	16	4	6.05* (a)	15	5	4.05* (a)
	+ aliphatic ketones	3 (100)	2	18	11.3*** (r)	4	16	6.05* (r)
	+ aromatics	4 (1000)	0	20	18.5*** (r)	1	19	14.45*** (r)
6	(Z)-OCI	control (0.0)	13	7	1.25 ^{ns}	7	13	1.25 ^{ns}
	+ LIN	1 (1)	9	11	0.05 ^{ns}	10	10	0.05 ^{ns}
	+ BAC	2 (10)	5	15	4.05* (r)	12	8	0.45 ^{ns}
	+ MAT	3 (100)	3	17	8.45** (r)	3	17	8.45** (r)
	+ β -CAR	4 (1000)	1	19	14.45*** (r) ²	2	18	11.3*** (r)
	+ (E)- β -FAR							

Level of significance: ^{ns}not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; r – repellent; a – attractant, + Y-tube arm with the tested amount of a compound, volatile diluted in hexane emitted from filter paper; – Y-tube arm only with hexane emitted from filter paper

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