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Short Communication

Do Mated *Tribolium confusum* Adults Respond to Blends of Odors?

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Abstract

In this paper we report the behavioral responses of mated *Tribolium confusum* to blends of volatiles using a Y-tube olfactometer. Four doses of volatiles (1 ng·min^{-1}, 10 ng·min^{-1}, 100 ng·min^{-1}, and 1,000 ng·min^{-1} in 50 µl of hexane applied on filter paper) were tested. A Y-tube experiment revealed that both mated females and males of *Tribolium confusum* were attracted to blends 4 and 5 at concentrations of 100 ng·min^{-1} and 10 ng·min^{-1}, respectively. Moreover, mated females of tested insects were also attracted to blends 1 and 5. Yet the weevil females and males were repelled by the highest concentrations (1,000 ng·min^{-1}) for all tested blends. Additionally, a 100 ng·min^{-1} concentration also repelled both sexes in blends 2, 3, 5, and 6.

Keywords: *Tribolium confusum,* confused flour beetle, cereal volatiles, attractants, repellents

Introduction

Stored product pests can cause direct losses in product weight. They can affect stock, damage products by reducing their weight, or contaminate them [1]. Large quantities of these crops (during storage) – accounting for 5-10% in temperate and 20-30% or more in tropical regions – are lost annually due to different insect pests [2]. As calculated by Grethe et al. [3], an increase of 48% in food production would be sufficient when post-harvest losses are seriously reduced [4].

Tribolium confusum Jacquelin du Val (Coleoptera: Tenebrionidae) is one of the major foodstuff pests responsible for important economic losses and is still central to the world food supply since cereals continue to be the main source of food for people [5-6]. The confused flour beetle is a cosmopolitan pest of stored grains, cereal products, fruit, and nut products, and they may infest mills, food warehouses, or retail stores. Moreover, they can contaminate processing plants and warehouses by whole insects, eggs, insect fragments, and frass [7].

For years, chemical treatment with insecticides has been the method of choice. However, the number of available active substances has been shrinking due to many reasons [8-9]. Pest management programs for stored-product insects inside mills and warehouses may include applications with insecticides of different liquid formulations, including aerosols [10]. Therefore, aerosol

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insecticide applications are predicted to cause direct mortality only to individuals dispersing between refugia and out in open areas because they can either be directly exposed to settling droplets or encounter the residual insecticide on treated surfaces [11]. The control of these insects relies heavily on the use of cost-effective synthetic insecticides such as fumigants (mainly phosphine and methyl bromide) [12], but their massive use has triggered problems such as resistant behavior and environmental pollution with negative side effects on human health [13-15]. Possible strategies aimed at reducing the use of synthetic insecticides include natural insecticide fumigants produced by plants [16].

Eco-friendly strategies to prevent such insect attacks on final packaged products are therefore highly required, e.g., essential oils (extracted from aromatic plants) could represent an alternative to chemical treatments due to their repellent properties [17-21].

Plants are vulnerable to attack by organisms, but even being immobile are not merely passive victims [22]. To protect themselves, they have evolved an arsenal of physical and chemical defences [23-25], e.g., there are many types of volatile organic compounds (VOCs) released by plants in response to insect attack (terpenes, fatty acid derivatives, benzenoids, phenyl propanoids, and amino acid-derived metabolites) [26]. Plant-induced VOC defensive functions include directly deterring herbivores [27-28], indirectly attracting natural enemies of attackers [29], and priming defences of uninjured organs on the same plant [30-32]. An insect dose response to an individual plant VOC can reveal the range of concentrations over which herbivore or parasitoid attraction or repellence occurs [33-34].

In the present study, we examined the behavioral response of mated adults of *T. confusum* of both sexes to blends of VOCs.

Experimental Procedures

Insects

Experiments were performed in 2014-15 at the UTP University of Science and Technology, Bydgoszcz, Poland at the Department of Entomology and Molecular Phytopathology. *T. confusum* were reared on whole wheat kernels in continuous dark at 22±2ºC and relative humidity of 60±5%.

Synthetic Chemicals

Synthetic volatiles were obtained from Sigma-Aldrich (Chemical Co. Inc., Poznań, Poland) and their purity was 85-99%. Cereal compounds were selected based on their presence in cereal grains [35], but plant VOCs were chosen based on their presence in various cereal green plants as a result of biotic stress [36-38]. To screen behavioral activity of the volatile compounds we tested six blends at four concentrations (1, 10, 100, 1,000 ng•min-197) compared to the absence of the compound (0). In the Y-tube, each of the five VOC concentrations in hexane was tested against hexane solvent alone. Each of the 98 individual VOCs was present in a blend at the specified concentration. Thus for blend 1 (aliphatic alcohols), 1 ng•min^{-1} means that 1 ng 1-BUT + 1 ng 1-PEN + 1 ng 1-HEX + 1 ng 3-MET was 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^{-1}).

Y-tube

Insects of both sexes were exposed to each other for 72h in a cage where food was also supplied. After three days they were separated (different cages) and following another 24h the experiment were begun. Beetles tended to walk along the Y-tube (this system has been previously tested on various insect species) [21]. Twenty adult *T. confusum* of each sex were tested (20♀ and 20♂ for each blend) at each concentration for six blended VOCs. Adults were observed for 5 min.

Data Analysis

Chi-square goodness of fit tests (X2-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 for odor source (synthetic blend vs. hexane solvent) at each exposure concentration × sex × exposure duration combination. Non-significant tests indicated that the observed beetle counts did not significantly deviate from an expected ratio of 10:10 (the hexane solvent only arm 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 only hexane solvent).

Discussion of Results

We found in our behavioral responses of mated **c**onfused flour beetles that mated females of *T. confusum* were attracted to blends 1, 4, and 5 at concentrations of 100 ng·min^{-1}, 100 ng·min^{-1}, 1 and 10 ng·min^{-1}, respectively (Tables 1, 4, 5). In contrast, mated males were attracted only to blend 5 at a concentration of 10 ng·min^{-1}. Both sexes were repelled by the highest concentration (1,000 ng·min^{-1}) for all tested blends. Additionally, both mated sexes were repelled by 100 ng·min^{-1} in blends 2, 3, 5, and 6 (Tables 2, 3, 5, 6)..

The above results are in good agreement with those of Zoubiri and Baaliouamer [39], who tested the bioactivity of the essential oil extracted by hydrodistillation from *Lantana camara* leaves and found that the composition of *L. camara* essential oil included large amounts of sesquiterpene – mainly β-caryophyllene and caryophyllene oxide. Ziaee et al. [40] found that essential oil had strong fumigant toxicity against adult insects; however, toxicity diminished in the presence of wheat commodity. Plant

Table 1. Effect of synthetic blend No. 1 of four aliphatic alcohols [1-BUT + 1-PEN + 1-HEX + 3-MET] on the number of mated *Tribolium confusum* adult females and males choosing to enter a Y-tube arm containing the blend odor or the Y-tube arm containing purified humidified air and hexane solvent (no odor).

Name of mixed compounds	Dose	ng·min^{-1}	No. of females			No. of males		
			+ [4]	– [5]	χ^2 (1)	+ [4]	– [5]	χ^2 (1)
	Control	0.0	7	13	1.25 ns	11	9	0.05 ns
1-BUT	1	1	8	12	0.45 ns	10	10	0.05 ns
+ 1-PEN	2	10	9	11	0.05 ns	13	7	1.25 ns
+ 1-HEX	3	100	16	4	6.05* (a) [3]	14	6	2.45 ns
+ 3-MET	4	1000	3	17	8.45** (r) [2]	4	16	6.05* (r) [2]

Legend:
(1) level of significance (ns: non-significant), (*p<0.05), (**p<0.01), (***p<0.001)
2 r: repellent
3 a: attractant
4 + Y: tube arm with the tested amount of the compound, volatile diluted in hexane emitted from filter paper
5 – Y: tube arm only with hexane emitted from filter paper

Table 2. Effect of synthetic blend No. 2 of eight aliphatic aldehydes [BUT + PEN + HEX + HEP + (*E*)-2-HEX + (*E*,*E*)-2,4-HEP + (*E*,*E*)-2,4-NON + (*E*,*E*)-2,4-DEC] on the number of mated *Tribolium confusum* adult females and males choosing to enter a Y-tube arm containing the blend odor or the Y-tube arm containing purified humidified air and hexane solvent (no odor).

Name of mixed compounds	Dose	ng·min^{-1}	No. of females			No. of males		
			+ [4]	– [5]	χ^2 (1)	+ [4]	– [5]	χ^2 (1)
BUT + PEN	Control	0.0	10	10	0.05 ns	13	7	1.25 ns
+ HEX	1	1	11	9	0.05 ns	8	12	0.45 ns
+ HEP	2	10	14	6	2.45 ns	11	9	0.05 ns
+ (*E*)-2-HEX	3	100	4	16	6.05* (r) [2]	4	16	6.05 * (r) [2]
+ (*E*,*E*)-2,4-HEP + (*E*,*E*)-2,4-NON + *E*,*E*)-2,4-DEC	4	1000	2	18	11.3*** (r) [2]	3	17	8.45** (r) [2]

Table 3. Effect of synthetic blend No. 3 of four aliphatic ketones [2-PEN + 2-HEX + 2-HEP + 2,3-BUT] on the number of mated *Tribolium confusum* adult females and males choosing to enter a Y-tube arm containing the blend odor or the Y-tube arm containing purified humidified air and hexane solvent (no odor).

Name of mixed compounds	Dose	ng·min^{-1}	No. of females			No. of males		
			+ [4]	– [5]	χ^2 (1)	+ [4]	– [5]	χ^2 (1)
	Control	0.0	14	6	2.45 ns	13	7	1.25 ns
2-PEN	1	1	9	11	0.05 ns	7	13	1.25 ns
+ 2-HEX	2	10	11	9	0.05 ns	11	9	0.05 ns
+ 2-HEP	3	100	3	17	8.45** (r) [2]	5	15	4.05 * (r) [2]
+ 2,3-BUT	4	1000	3	17	8.45** (r) [2]	4	16	6.05* (r) [2]

essential oil synergized the performance of diatomaceous earth (DE) samples such that they generally became more insecticidal than DE alone. Nenaah [2] found that essential oils of *Achillea biebersteinii*, *A. santolina,* and *A. mellifolium* showed considerable toxic and growth inhibitory activities against the red flour beetle, *Tribolium castaneum* (Herbst). Germinara et al. [35] 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, respectively. Piesik et al.

Table 4. Effect of synthetic blend No. 4 of four aromatics [MAL, FUR, PHE, VAN] on the number of mated *Tribolium confusum* adult females and males choosing to enter a Y-tube arm containing the blend odor or the Y-tube arm containing purified humidified air and hexane solvent (no odor).

Name of mixed compounds	Dose	ng·min^{-1}	No. of females			No. of males		
			+ [4]	– [5]	$\chi^{2\,(1)}$	+ [4]	– [5]	$\chi^{2\,(1)}$
	Control	0.0	8	12	0.45 ns	10	10	0.05 ns
MAL	1	1	12	8	0.45 ns	10	10	0.05 ns
+ FUR	2	10	7	13	1.25 ns	13	7	1.25 ns
+ PHE	3	100	16	4	6.05 * (a) [3]	15	5	4.05 * (r) [2]
+ VAN	4	1000	2	18	11.3*** (r) [2]	3	17	8.45** (r) [2]

Table 5. Effect of synthetic blend No. 5 of aliphatic alcohols, aliphatic aldehydes, aliphatic ketones, and aromatics on the number of mated *Tribolium confusum* adult females and males choosing to enter a Y-tube arm containing the blend odor or the Y-tube arm containing purified humidified air and hexane solvent (no odor).

Name of mixed compounds	Dose	ng·min^{-1}	No. of females			No. of males		
			+ [4]	– [5]	$\chi^{2\,(1)}$	+ [4]	– [5]	$\chi^{2\,(1)}$
	Control	0.0	7	13	1.25 ns	11	9	0.05 ns
aliphatic alcohols	1	1	16	4	6.05 * (a) [3]	10	10	0.05 ns
+ aliphatic aldehydes	2	10	16	4	6.05 * (a) [3]	16	4	6.05 * (a) [3]
+ aliphatic ketones	3	100	3	17	8.45** (r) [2]	5	15	4.05 * (r) [2]
+ aromatics	4	1000	2	18	11.3*** (r) [2]	3	17	8.45** (r) [2]

Table 6. Effect of synthetic blend No. 6 of six plant VOCs [(Z)-OCI, LIN, BAC, MAT, **β**-CAR, (E)-**β**-FAR] on the number of mated *Tribolium confusum* adult females and males choosing to enter a Y-tube arm containing the blend odor or the Y-tube arm containing purified humidified air and hexane solvent (no odor).

Name of mixed compounds	Dose	ng·min^{-1}	No. of females			No. of males		
			+ [4]	– [5]	$\chi^{2\,(1)}$	+ [4]	– [5]	$\chi^{2\,(1)}$
(Z)-OCI	Control	0.0	10	10	0.05 ns	12	8	0.05 ns
+ LIN	1	1	8	12	0.45 ns	14	6	2.45 ns
+ BAC	2	10	7	13	1.25 ns	10	10	0.05 ns
+ MAT	3	100	2	18	11.3*** (r)	4	16	6.05 * (r) [2]
+ *β*-CAR + (E)-*β*-FAR	4	1000	2	18	11.3*** (r) [2]	3	17	8.45** (r) [2]

Legend:
(1) level of significance (ns: non-significant), (*p<0.05), (**p<0.01), (***p<0.001)
2 r: repellent
3 a: attractant
4 + Y: tube arm with the tested amount of the compound, volatile diluted in hexane emitted from filter paper
5 – Y: tube arm only with hexane emitted from filter paper

[41] found attraction to synthetic components for adult cereal leaf beetles *Oulema melanopus* L. (Coleoptera: Chrysomelidae) at 7,500 ng·h^{-1} for two GLVs ((Z)-3-hexenal, (Z)-3-hexen-1-yl acetate) and two terpenes (linalool and β-caryophyllene), and attraction at lower doses of 60 ng·h^{-1} for ((Z)-3-hexenal, (Z)-3-hexen-1-yl acetate, and 300 ng·h^{-1} for linalool.

Chemical Compounds and Abbreviation List

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-Heptadienal = (*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-hydroxybenzaldehyde (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).

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