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Brief Report

Repellent Effect of Volatile Fatty Acids on Lesser Mealworm (*Alphitobius diaperinus*)

Bartosz Baran[1]*, Michał Krzyżowski[1], Mikołaj Cup[2], Jakub Janiec[3], Mateusz Grabowski[4], Jacek Francikowski[1]

[1] Department of Animal Physiology and Ecotoxicology, Faculty of Biology and Environmental Protection, University of Silesia; bartosz.m.baran@gmail.com, michal.krzyzowski@o2.pl, jacek.francikowski@us.edu.pl
[2] College of Inter-Faculty Individual Studies in Mathematics and Natural Science, University of Warsaw; mkicup@interia.eu
[3] Faculty of Physics, University of Warsaw; jakub.janiec@me.com
[4] Medical University of Silesia; mateusz.grabowski@med.sum.edu.pl
* Correspondence: bartosz.m.baran@gmail.com; Tel.: +48-32 359 1174

Abstract: Volatile fatty acids (VFAs) are a group of common metabolites with a potential of universal infochemicals dedicated to transferring of information between higher organisms and bacteria either from microbiome or external environment. VFAs are common substances among various insect orders, there are numerous studies exploring their influence on the behavior of different insect species. In relation to papers published by J. E. McFarlane, we assess the effects of formic, acetic, propionic, butyric, valeric acids on spatial preference of common stored food grain products, and poultry industry pest – lesser mealworm (*Alphitobius diaperinus*). We present novel method of continuous, simultaneous assessment of site preference as well as travelled distance in constant-flow olfactometer. All tested VFAs except valeric had a significant repellent effect with formic acid bing effective in the lowest concentration. Additionally, VFAs significantly altered distance travelled by insects. Obtained results indicate a potential role of VFAs in the olfactory guided behavior of *A. diaperinus*, we speculate that reaction to the presence if VFAs may deviate form specificity of species' original habitat.

Keywords: Volatile fatty acids; *Alphitobius diaperinus*; locomotor activity; repellency.

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1. Introduction

Volatile fatty acids (VFAs) are carboxylic acids with an aliphatic chain consisting of up to six carbon atoms and are produced by the microorganisms, which are either present in the environment or inhabiting the animal's digestive tracts [1]. Over the last decades, VFAs have been extensively investigated due to the potential role as a group of universal infochemicals dedicated to transferring of information between higher organisms, native microbiome and their environment. The composition of VFAs among different insect orders is fairly similar, therefore studying role of VFAs may greatly contribute to general understanding of spatial behaviour and chemical ecology of various species [2]. Papers published by McFarlane [2–5], are essential in this field and as such directly inspired presented study. For the purposes of this paper, we developed flexible, cost effective system and a new method of analysing the influence of volatile compounds on spatial preference and behavioural activity of insects.

Results published by McFarlane [2,6] showed the presence of VFAs in various habitats, body surfaces and faeces of many insects species. Over time, speculation grew about their potential role in information transfer. Given their characteristics, including high volatility and wide availability in the environment, those compounds present a high potential for information transfer, such as an indication of individuals density in a given space.

However, VFAs should not be considered as pheromones strict sense, as those are in principle considered to be deliberately produced and secreted by an animal, whereas in VFA are byproducts of digestive processes in the insect's gut. Therefore, they should be considered in the broader category as – infochemicals.

Here we would like to investigate the effects of formic, acetic, propionic, butyric and valeric acid on cosmopolitan pest *Alphitobius diaperinus*. It is well known for its rapidly increasing resistance to pesticides and because of being the cause of major economic losses in the poultry industry [7]. Selection of the compounds was guided by their reported efficiency against the insect related to *A. diaperinus* - *Tenebrio molitor* described by Weaver [8].

2. Materials and Methods

2.1 VFAs.

Carboxylic acids (**Fig. 1**) of increasing carbon chain length (analytical grade purity) used in presented research - formic, acetic, propionic, butyric, valeric; were obtained from POCH S.A.

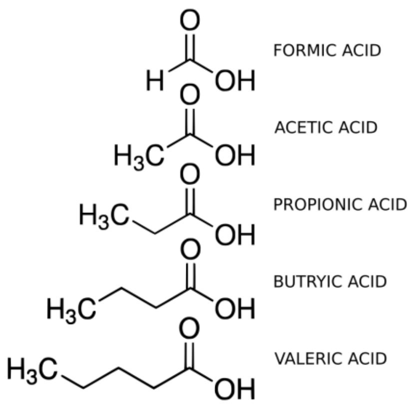

Fig. 1 Structural formulas of carboxylic acids used in presented study.

2.2 Insects.

In the experiment specimens of both sexes of *Alphitobius diaperinus* (Panzer) were used. Insects were acquired from infested oatmeal storage, subsequently reared in constant conditions of 30±1 °C, 50% relative humidity and photoperiodic regime of 12:12 light to dark. The culture was maintained on water *ad libitum* and standard dog food pellets.

2.3 Behavioral Tests.

Paradigm applied in presented research is an iteration of classic T-maze 1in which insect can choose between two arms of the maze. Each insect was placed separately in a rectangular chamber (3mm height, 15mm width, 160mm long) made of clear Lucite, with silicon tubes attached to both ends. The tubes were providing a constant flow of clear, humidified air from one end and humidified air with the tested odour from the other. Airflow was kept at 10 l/h. Inlet air was

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pumped through a bubbler containing mineral oil (to capture possible contaminants from the pump) and then was separated into either a water bubbler or a bubbler with an aqueous solution of carboxylic acid. Constant homogeneous background light was provided by a red transilluminator placed below the setup. Insects were able to explore the chambers freely. Recordings of experimental procedure were captured by Microsoft LifeCam 500 webcam and VirtualDub 1.10.4 software as .avi files. For assessing effects of each concentration 48 insects were used. Each insect was put in chamber separately and left undisturbed for 10 minute accommodation, thereafter recording started. Recordings last for 10 minutes at frame rate 15fps and resolution of 640x860px. The whole set-up was placed in an enclosed, ventilated test chamber, providing isolation from external visual and acoustic stimuli. For each VFA, a series of dilutions was prepared - consequently: 0.0001, 0.001, 0.01, 0.1, 1, 10 [Mol] in ultrapure, deionised water. Bubbler providing control airstream was filled with equal volume of ultrapure water.

2.4 Data Analysis.

Analysis of insects' movement was performed with SwissTrack® software [9]. Acquired trajectories were analyzed in the R using *adehabitat* package [10]. For analysis, the chamber was divided into six fictive compartments (**Fig. 2**).

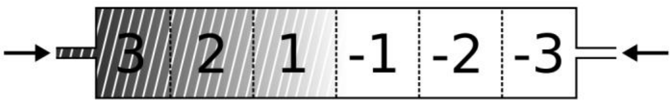

Fig. 2 Test chamber divided into compartments, coloration indicates the odor end and the gradient of odor concentration (assessed prior to study with NH4Cl deposition).

For each compartment, a value (I_i) from -3 (control end) to 3 (odour end) was then assigned. Preference index (PI) was calculated by multiplying compartment value by the time (t) spent (frames) by the insect in each specific compartment.

$$PI = \sum_{i=1}^{6} t * I_i \qquad I = \{-3, -2, -1, 1, 2, 3\}$$

Results for all compartments were summed up (min -27000, max 27000). If the value of PI was positive, then the tested odour was considered attractant. Consequently, if negative – repellent. In addition, overall characteristics describing movement dynamics were calculated, including total distance travelled by every insect during test and total resting time.

Statistical analysis was conducted using Statistica® software v10. Groups were compared using non-parametric Kruskal-Wallis test (Kruskal-Wallis ANOVA) with median test, significance level of 0.05 was applied. For multiple comparisons, Bonferroni correction was used.

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3. Results

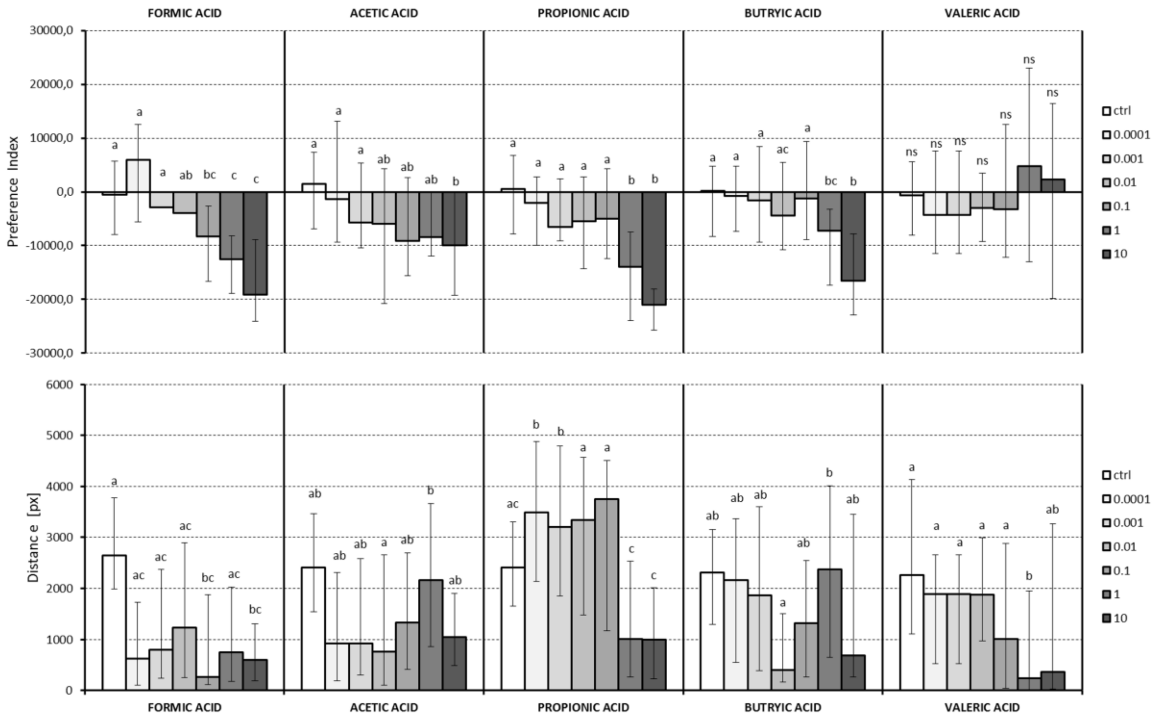

Fig. 3 Preference index (PI) and distance travelled by insects exposed to VFAs in different concentrations and control group. Letters indicate statistically different groups, Kruskal-Wallis test p<0,05.

Analysis of the PI (**Fig. 3**) indicated that all of tested VFAs, which caused statistically significant alteration of behavior, exhibited repellent properties. Formic acid, the shortest carboxylic acid, was the most effective. Just 0.1M concentration showed a repellent effect, additionally, significantly reducing the insect's distance and movement time. For acetic acid, the repellent effect was only observed at the highest concentration of 10M. It slightly affected the distance travelled by the insects excluding 1M concentration. Propionic acid had a repellent effect at 1M and 10M. The increased distance travelled, with respect to other groups, was observed in the 0.0001M and 0.001M groups. It is also possible to observe a reduction of the travelled distance, in relation to the other concentrations, in 1M and 10M. The butyric acid showed a repellent effect at 1M and 10M concentrations. We have not proven statistically significant changes in the distance travelled by insects, excluding 1M concentration. Valeric acid did not show any repellent effect at all concentrations used. The change in distance travelled was observed only at a concentration of 1M.

4. Discussion

The setup developed for the presented research is an iteration of T-maze olfactometer. Despite several reports presenting similar systems [11,12], the presented invention is based on the open access software and is assembled from easily accessible parts. In addition, its construction allows for easy adaptation to the conducted experiments. The results obtained in this study indicate the high usefulness of the developed system as a tool for assessing the effects of volatile substances on the behavioural parameters of insect activity.

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The findings show statistically significant differences in the studied parameters between different acids and their concentrations. The results are coherent and demonstrate the relation of acid chain length to its repellent properties, which decreased with increasing length. The obtained results are different from the ones acquired by Weaver [8] in the study on *Tenebrio molitor*, where acetic acid was reported to be the most effective. In our research, formic and propionic acids were the most effective as repellents. However, we consider that the fatty acids may play a potential role in the spatial preference behaviour of *A. diaperinus* and colony population dynamics. In the case of overcrowding, the concentration of the volatile compounds correlates with the quantity increases along with number of individuals. The elevated concentration of these substances has a strong repellent effect that, by promoting dispersion of insects, prevents further overcrowding, therefore, modulating population density by means of negative feedback loop.

In the natural habitat, *A. diaperinus* occurs at the bottom of the caves inhabited by bats, which guano contains significant amounts of urea [13] that are metabolised by microorganisms into VFAs [14]. Consequently, VFAs are compounds commonly found in the natural environment of *A. diaperinus*. Microbiological guano transformations progress in stages [15], so it is possible that VFAs indicate important information about the quality of the potential habitat. In addition, in secondary habitats inhabited by *A. diaperinus* (hen houses), VFAs also occur (due to bacterial fermentation of uric acid) and may be one of the factors predisposing *A. diaperinus* to such a quick and efficient colonisation of this new niches.

VFAs are compounds closely related to the activity of microorganisms and the communication of organisms to microbes. However, McFarlane research, as well as the present work, points to the possible wider role of VFAs as universal information factors, including the transmission of information also in the external environment. The reputed properties of some VFAs provide hope for the development of novel, information-based, methods of fighting and deterring *A. diaperinus*, which may help, in the future, to significantly reduce overuse of synthetic pesticides.

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