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Sitophilus granarius responses to blends of five groups of cereal kernels and one group of plant volatiles

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ABSTRACT

In this paper we report the behavioral responses of granary weevil to 6 blends of cereal volatiles. Four doses (1 ng min^{-1}, 10 ng min^{-1}, 100 ng min^{-1}, and 1000 ng min^{-1} in 50 μl of hexane applied on filter paper) were tested. A Y-tube experiment revealed that females and males of Sitophilus granaries were attracted to the blend 1, 4, and 5 at concentrations of 10 and 100 ng min^{-1}, 10 ng min^{-1}, 1 and 10 ng min^{-1}, respectively. Moreover, females and males of granary weevil were not attracted to any concentration of blend 2 and 3. Yet, the weevil females and males were repelled by the highest concentrations (100 and 1000 ng min^{-1}) for all tested blends, except of blend 1, where concentration 100 ng min^{-1} was an attractant for both sexes. Moreover, females and males were repelled by three tested concentrations of blend 6 (10, 100 and 1000 ng min^{-1}).

1. Introduction

Stored product pests can be very damaging to grain and grain based commodities for a variety of reasons. They can cause direct losses in product weight and also indirect losses. They can affect stock, damage products by reducing their weight or by contaminating them (Belda and Riudavets, 2010). As calculated by Grethe et al. (2011), an increase of 48% in food production would be sufficient when post-harvest losses are reduced seriously. One step in reducing post-harvest losses is the reduction of damage caused by insect pests (Niedermayer and Steidle, 2013).

The granary weevil *Sitophilus granarius* (L.) is one of the most harmful pests of stored grain that cause severe losses throughout the world (Germinara et al., 2010, 2008). The degree of damage is directly related to the infestation rate which, in turn, is determined by factors such as the number of eggs laid, and the survival and fecundity of offspring (Nawrot et al., 2010).

For years, chemical treatment with insecticides has been the method of choice, however the number of available active substances is shrinking due to many reasons, e.g. consumer demands for residue-free products (Clarke et al., 2011; Opit et al., 2012; Pimentel et al., 2008). Furthermore treatments with inert gases as well as physical methods such as the use of heat or cold are expensive and may not even be available for small scale farmers in developing countries (Niedermayer and Steidle, 2013).

It's well known that plants are vulnerable to attack by organisms during their life, but even being immobile are not merely passive victims (Dicke et al., 2009), and to protect themselves, they have evolved an arsenal of physical and chemical defences (Boczek et al., 2013; Dicke et al., 2009; Gantner and Najda, 2013; Holopainen and Gershenzon, 2010; Vickers et al., 2009). There are many types of VOC released by plants in response of insect attack (terpenes, fatty acid derivatives, benzenoids, phenyl propanoids and amino acid-derived metabolites) (Dudareva and Pichersky, 2008). Plant-induced VOC defensive functions include directly deterring herbivores (Laothawornkitkul et al., 2008; Unsicker et al., 2009), indirectly attracting natural enemies of attackers (Mumm et al., 2008) and priming defences of uninjured organs on the same plant (Rodriguez-Saona et al., 2009). Yet, a caveat of caution needs to be pointed out concerning ecological and evolutionary aspects of VOC induction (Kessler and Heil, 2011). An insect dose response to an individual plant VOC can reveal the range of concentrations over which herbivore or parasitoid attraction or repellence occurs (Delaney et al., 2013; Piesik et al., 2011a, 2014).

In the present study, we examined the behavioral response of granary weevil adults of both sexes to six blends of VOC (to examine attraction/avoidance to those volatiles).

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2. Material and methods

2.1. Insects

Experiments were performed in 2014 at the UTP University of Science and Technology, Bydgoszcz, Poland at the Department of Entomology and Molecular Phytopathology. *S. granarius* were reared on whole wheat kernels in continuous dark at 22 ± 2 °C and relative humidity of 60 ± 5%. Only newly-emerged adults of both sexes were used for the experiments.

2.2. Synthetic chemicals

Synthetic volatiles were obtained from Sigma–Aldrich (Chemical Co. Inc., Poznań, Poland) and their purity was between 85 and 99%. Cereal compounds were selected based on their presence in cereal grains (Germinara et al., 2008), but plant VOC were chosen based on their presence in various cereal green plants as the result of biotic stress (Delaney et al., 2013; Piesik et al., 2011a, 2014). To screen behavioral activity of the volatile compounds were tested at six blends at four concentrations (1, 10, 100, 1000 ng min^{-1}) 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 individual VOC was present in a blend at the specified concentration. Thus, for instance for the 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}).

2.3. Y-tube

Beetles tended to walk along the Y-tube (this system has been previously tested on various insect species) (Piesik et al., 2009, 2011a,b; Piesik et al., 2014). The tubes were washed and rotated to limit the effects of chemical residues from previous bioassay subjects on the current test subject. Twenty adult *S. granarius* of each sex were tested at each concentration for six blend VOC. One adult was tested at a time (5 min test duration).

2.4. Data analysis

Chi-square goodness of fit tests (X2-test), with the Yates correction for small samples (1 × 2), were conducted to indicate whether 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 (hexane solvent only arm and synthetic blend). Significant tests indicated attraction (more individuals chose Y-tube arm with a synthetic blend) or repellency (more individuals chose the Y-tube arm with only hexane solvent).

2.5. Chemical compounds and abbreviation list

VOC = 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, Furfural = FUR, Phenylacetaldehyde = PHE, 3-Methoxy-4-hydroxy-benzaldehyde (vanillin) = VAN); blend 5 - aliphatic alcohols, aliphatic aldehydes, aliphatic ketones, aromatics, blend 6 – plant VOC ((Z)-ocimene = (Z)-OCI, linalool = LIN, benzyl acetate = BAC, methyl salicylate = MAT, β-caryophyllene = β-CAR, (E)-β-farnesene = (E)-β-FAR).

3. Results

Weevils chose one arm of the Y-tube usually within 4–5 min. Chi-square tests revealed that female *S. granaries* were significantly attracted at 10 and 100 ng min^{-1} of blend 1 (Table 1). Males seemed to be less sensitive and were attracted only to concentration 100 ng min^{-1}. The highest tested concentration (1000 ng min^{-1}) significantly repelled both sexes. No attractive concentration for blends 2 and 3 was observed in respond of both sexes. Adults of both sexes were repelled by the concentrations of 100 and 1000 ng min^{-1} (Tables 2 and 3). Similar activity of insects was recorded for blend 4 and blend 5. Adults of both sexes were repelled by the concentrations of 100 and 1000 ng min^{-1} and attracted to concentration 10 ng min^{-1} (Tables 4 and 5). Moreover, 1 ng min^{-1} of blend 5 also attracted females of *S. granaries* (Table 5). No attractive concentration for blend 6 was observed in respond of females and males. Insects were repelled by the concentrations of 10, 100, and 1000 ng min^{-1} (Table 6).

4. Discussion

In our experiment Y-tube behavioral experiment, we chose to use equal amounts of each VOC in the blends for a given test dose. Under Y-tube conditions, females and males of *S. granaries* responded positively to the concentrations from 1 to 100 ng min^{-1} of blend 1, 4, and 5. However, females and males were significantly repelled by the highest concentrations of all tested blends1 (100 and 1000 ng min^{-1}, except of blend 1 and concentration 100 ng min^{-1}, where males where attracted). The above results are in good agreements to those from the congener *Gastrophysa viridula* with *Rumex confertus* (Piesik et al., 2012) and *Gastrophysa polygoni* with *R. confertus* (Piesik et al., 2011a). Piesik et al. (2008) found that only female *Cephus cinctus* Norton (*Hynenoptera*: *Cephidae*) were attracted to some concentrations of (Z)-3-hexen-1-yl acetate and (Z)-3-hexenol, and the terpene β-ocimene. Yet, *C. cinctus* females were repelled by the highest tested concentration (8400 ng h^{-1}) of (Z)-3-hexen-1-yl acetate. With maize (VOC induced by *Fusarium* infection) behavioral tests found attraction to synthetic components for adult cereal leaf beetles *Oulema melanopus* L. (*Coleoptera*: *Chrysomelidae*) at 7500 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 (Piesik et al., 2011b). In contrast, Carroll et al. (2006) showed with Y-tube tests that crawling larval *Spodoptera frugiperda* Walker(*Lepidoptera*: *Noctuidae*) were attracted to injured maize over uninjured plants, to a wide range of linalool concentrations, and undamaged plants with linallol added (and even linalool alone) were much more attractive than uninjured plant bouquets. Zoubiri and Baaliouamer (2010) reported that fruit essential oil shows promise as a material for use as a fumigant. This is potentially a useful grain protectant that has fumigant toxicity. The main compounds from coriander fruit essential oil, particularly linalool, can be developed as a potential fumigant for stored-products protection. Also Kordali et al. (2006) found that the essential oils of aerial parts of three *Artemisia* species (*A. absinthium*, *A. santonicum* and *A. spicigera*) can be concluded