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Repellence and fumigant toxicity of propionic acid against adults of *Sitophilus granarius* (L.) and *S. oryzae* (L.)

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Abstract

The biological activity of propionic acid towards adults of the primary stored-grain pests, *Sitophilus granarius* and *S. oryzae* was investigated by electroantennographic, behavioural and fumigant toxicity assays. Electroantennograms revealed the sensitivity of both sexes of the two species to propionic acid. In two-choice pitfall bioassays, the compound showed dose-dependent repellent effects even in the presence of wheat odours, which attracted the beetles. In the fumigation assay, propionic acid was effective in killing weevil adults. The LC_{50} values, calculated for both species at 23 and 30 °C, ranged from 5 to 10 μg/L air and are comparable to those of other known fumigants. Propionic acid could have potential for applications in IPM programs for stored-grain beetles because of its safety, high volatility, repellency and fumigant activity.

Keywords: Granary weevil; Rice weevil; EAG; Behavioural bioassay; Repellent; Fumigation

1. Introduction

The granary weevil, *Sitophilus granarius* (L.) and the rice weevil, *S. oryzae* (L.) are two primary pests of stored grains which cause severe quantitative and qualitative losses throughout the world. Although several methods have been developed as a part of an Integrated Pest Management (IPM) strategy, at present the fumigation of stored-grain insect pests relies on methyl bromide and phosphine. In the EU, methyl bromide has only been available for specific critical uses since January 2005 because of its depleting properties on the stratospheric ozone layer. Moreover, human health concerns (Garry et al., 1989) and insect pest strains resistant to phosphine have been discovered (Zettler et al., 1989; Bell and Wilson, 1995; Chaudhry, 1997). For this reason, alternative control means are necessary.

Propionic acid occurs in the blend of volatile compounds emitted by barley grains (Maga, 1978), a common food and oviposition substrate for *Sitophilus* spp. Therefore, it may play a semiochemical role in the host finding process of these species. However it is also commonly used by the food industry as a preservative agent in several food products. Propionic acid is known to be a mould inhibitor for stored grains (Milward, 1976; Raecher et al., 1992), hay (Nash and Easson, 1977), and silage (Kung et al., 1998) and it has been used world-wide, under various trade names, for over 30 years to preserve high-moisture products intended for animal feed. In addition, since the compound is Generally Recognized As Safe (GRAS) (by the US Food and Drug Administration), it has been utilized to protect products intended for human consumption. It has also been found to be very effective in inhibiting Gram-negative bacteria strains (Davidson and Juneja, 1990) and in destroying surface-born, fungal conidia that cause postharvest decay of fruit (Sholberg, 1998). Finally, propionic acid has been shown to possess a selective toxicity against some plant-parasitic nematodes which cause serious damage in agriculture (Sitaramaiah and Singh, 1977; Djian et al., 1994). In spite of its toxic activity towards such a variety of parasitic organisms, the effects of propionic acid against insects has been little investigated. Sodium propionate, a salt that readily breaks down into sodium and propionic acid, was tested as an antimicrobial in a synthetic diet for *Agria affinis* (Fallen) (Diptera:

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Sarcophagidae) and its safe consumption level to larvae was determined to be 400 ppm (Singh and House, 1970). Propionic acid showed larvicidal and ovicidal properties against *Trogoderma variabile* Ballion and *Attagenus megatoma* (F.) (Coleoptera Dermestidae) when added (2%) to the food medium (Burkholder et al., 1973).

In the present study, the potential activity of propionic acid as an insect behaviour regulator towards *S. granarius* and *S. oryzae* adults was investigated by electroantennographic and behavioural tests. In addition, as this compound has high volatility, its fumigant toxicity was also evaluated.

2. Materials and methods

2.1. Insects

Sitophilus granarius and *S. oryzae* were reared in the laboratory on whole wheat grains for several generations. Colonies were maintained in the dark in a climatic chamber set at 25 ± 2 °C and 60 ± 5% r.h. Adult beetles, 2–4 weeks old, were used for the experiments.

2.2. Chemicals

Propionic acid, hexanal, mineral oil, and hexane (purity > 99.9%) were purchased from Sigma-Aldrich (Milan, Italy).

2.3. Electroantennography (EAG)

The EAG technique was used to assess the antennal sensitivity of both sexes of the two *Sitophilus* species to propionic acid. The head of the insect was excised from the prothorax and mounted between two glass electrodes filled with Kaissling saline (Kaissling and Thorson, 1980). The recording electrode (diameter ca. 100 μm) was put in contact with the dorsal surface of the terminal antennal segment while the indifferent electrode was inserted into the base of the head. Electrical contact of the preparation to the amplifier (AC/DC UN-6, Syntech Laboratories, Hilversum, The Netherlands) was made with AgCl-coated silver wires. Stimuli were provided by 10-fold dilutions of propionic acid in mineral oil. Test solutions (10 μL) were applied to filter paper (Whatman No. 1) strips (1 cm^2) inserted in a Pasteur pipette (15 cm long). Vapour stimuli (3 cm^3) were puffed for 1 s into a constant stream of charcoal-filtered humidified air (600 mL/min) flowing in a stainless steel delivery tube (internal diameter 1 cm) with the outlet positioned at approximately 1 cm from the antenna. Intervals between stimuli were 1 min. Five antennae of each sex of the two insect species were presented with six propionic acid doses ranging from 10^{-2} to 10^{3} μg. In order to correct for the possible reduction of antennal sensitivity during the experiment, a standard stimulus (10 μL of a 10 μg/μL dilution of hexanal in mineral oil) was applied before and after each propionic acid dose (Van der Pers, 1981; Rotundo and Tremblay, 1993). The highest test dose sometimes elicited a marked positive EAG (hyperpolarization) in both sexes of the two species; in this case the insect EAGs to all doses of the same series were discarded. Based on corrected EAG values, dose–response curves were calculated. The Student's *t*-test was used to compare the mean EAGs of conspecific sexes and the mean EAGs of males and females between the two species to each test dose of the compound.

2.4. Pitfall bioassays

The behavioural responses of *S. granarius* and *S. oryzae* adults to propionic acid were measured by using a two-choice pitfall bioassay similar to that described in Phillips et al. (1993). The test arena was a steel container (32 cm diameter × 7 cm high) with two diametrically opposed holes (3 cm diameter) located 3 cm from the side wall. Test or control (10 μL) stimuli were adsorbed onto a filter paper disc (0.7 cm diameter) suspended at the centre 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 provide a uniform surface and to facilitate insect movements (Pike et al., 1994). 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 high) at the centre of the arena and allowed 30 min to acclimatize prior to release. The arena was covered with a steel lid to prevent insects from escaping. Tests lasted 3 h and were carried out in the dark at 25 ± 2 °C and 60 ± 5% r.h. In a first set of experiments, insects were presented with a propionic acid dose (1, 10, 100, 500, 1000 μg) and mineral oil (control). In a second set of experiments, they were given choices between a propionic acid dose (1, 10, 100, 500, 1000 μg) plus the odours emitted by 200 g of uninfested wheat kernels (m.c. 14.5%), left in the underlying collection flask, as well as mineral oil. Insects were used once. Five replicates of each test were performed.

For each replicate, a response index (RI) was calculated according to Phillips et al. (1993). Positive values of RIs indicate attraction to the treatment and negative RIs indicate repellence. The significance of the mean RI to each treatment of the two-choice pitfall bioassay was evaluated by the Student's *t*-test for paired comparisons (Phillips et al., 1993). Mean RIs to wheat odours alone and in the presence of increasing doses of propionic acid were subjected to analysis of variance (ANOVA) and ranked according to the Duncan's multiple range test. Data were submitted to linear regression analysis in order to evaluate the effect of the dose on the response of the insects.

2.5. Fumigation bioassay

A glass container (600 mL) was used as a fumigation chamber. A filter paper (Whatman No. 1) disc (diameter