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Allyl isothiocyanate actions on populations of *Sitophilus zeamais* resistant to phosphine: Toxicity, emergence inhibition and repellency

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ABSTRACT

The risks associated with the use of synthetic insecticides have caused increased interest in the research of essential oils and their main constituents for use in the pest management of stored products. Allyl isothiocyanate (AITC) is the main component of mustard essential oil and has been reported as a potential replacement pesticide for conventional insecticides that control stored product insect pests. Here, we assessed the toxicity (including emergence inhibition) and repellent actions of AITC on Brazilian populations of the maize weevil *Sitophilus zeamais* (Coleoptera: Curculionidae) resistant to conventional insecticides (e.g., phosphine). We also evaluated physiological (e.g., respiration) and behavioral (e.g., walking and flight) traits of AITC-exposed insects. The AITC showed consistent insecticidal activity against the populations resistant to phosphine and other synthetic insecticides, with LC_{50} values ranging from 1.5 to 2.9 μL L^{-1}. Significant inhibition of the offspring emergence was achieved after the exposure of parental adults to sublethal levels (i.e., LC_1 and LC_5) of AITC. Reductions in respiration rates were also registered in all the populations sublethally exposed to AITC. In all five populations, a high number of insects avoided AITC-treated (1.5 μL L^{-1}) grain masses, and although individuals of a phosphine-susceptible (i.e., Abre Campo) population increased walking and reduced flight activities, individuals of another phosphine-susceptible (i.e., Tunápolis) population exhibited higher flight activity under AITC exposure. Thus, our findings suggest that AITC is a potential tool that may be integrated into the control strategies of maize weevils where resistance to phosphine and other conventional insecticides is a problem.

1. Introduction

Plant-extracted essential oils and their major chemical constituents have become the subject of various investigations aiming to evaluate the potential of these compounds for fungicidal, bactericidal and insecticidal properties because they supposedly pose little threat to the environment or to human health (Isman and Grieneisen, 2014; Regnault-Roger et al., 2012). These natural products have been suggested as serious and important alternative tools to be considered in control strategies for sustainable insect pest management (Isman, 2006; Isman and Grieneisen, 2014; Regnault-Roger et al., 2012).

Allyl isothiocyanate (AITC) is the main component of essential oil extracted from several plant species of the Brassicaceae family. Brassicaceae species such as *Brassica nigra* (black mustard), *B. juncea* (gray or Indian mustard), *B. rapa* (turnip), *B. oleracea* (cabbage, cauliflower, cabbage, and broccoli), *Armoracia rusticana* (horseradish) and *Eutrema japonicum* (Japanese horseradish) are some examples of Brassicaceae plants that produce AITC-rich essential oils with fumigant potential (Dhingra et al., 2004; Isman, 2000; Park et al., 2000; Santos et al., 2011; Zasada and Ferris, 2003). AITC is, therefore, a volatile compound that has shown a wide array of biological effects such as fungicidal (Dhingra et al., 2004), bactericidal (Park et al., 2000), nematicidal (Zasada and Ferris, 2003) and insecticidal (Isman, 2000).

In insects, AITC has shown lethal and sublethal effects including mortality, adult and immature malformation, repellency, and

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altered development (Ellenby, 1945; Isman, 2006; Santos et al., 2011). The mechanisms of action of AITC in insects are still unresolved. While the AITC actions in detoxification enzyme complexes have been reported elsewhere (Wu et al., 2014); recent investigations have proposed the mitochondrial complexes IV and I as the AITC main target sites (Mansour et al., 2012; Santos et al., 2012; Wu et al., 2014; Zhang et al., 2016). As such mitochondrial complexes I and IV are also the target of conventional fumigants such as phosphine (Corrêa et al., 2014; Mansour et al., 2012; Nath et al., 2011; Quistad et al., 2000), AITC and phosphine have been proposed to act by similar mechanisms (Mansour et al., 2012; Zhang et al., 2016), which can compromise the potential of using AITC for controlling insect pest populations that are resistant to phosphine. However, despite the increased attention given to AITC and phosphine mitochondrial actions, no reports exist that relate the AITC action on reproductive, physiological and behavioral responses of insect populations with different susceptibilities to phosphine.

Sitophilus zeamais (Coleoptera: Curculionidae) is one of the most destructive pests of stored food products, and one for which control measures still rely mostly on applications of conventional insecticides (e.g., pyrethroids and organophosphates). Control of this pest also relies on fumigation with phosphine, particularly in tropical regions, and such reliance on these insecticides has led to the selection of resistant populations (Corrêa et al., 2014; Haddi et al., 2015a; Pimentel et al., 2007; Sousa et al., 2008, 2016). Thus, the present study aimed to assess AITC toxicity (including the capacity of the pest to colonize and produce adults when sublethally exposed to AITC) and repellency, as well as the locomotory (i.e., walking and flight activities) and respiratory responses of *S. zeamais* populations with different susceptibilities to phosphine.

2. Material and methods

2.1. Sitophilus zeamais populations and fumigant

Five populations of *S. zeamais* obtained from storage bin collections between 2004 and 2006 were used in this study. They originated from counties of three Brazilian states: Abre Campo (Minas Gerais, MG), Machado (MG), Paracatu (MG), Piracicaba (São Paulo) and Tunápolis (Santa Catarina). The insects had been maintained in the Laboratory of Integrated Grain Pest Management of the Federal University of Viçosa, (MG, Brazil), using 1.5-L glass containers under controlled temperature (30 ± 2 °C), relative humidity (70 ± 5%) and 24-h scotophase. Pesticide-free, whole, maize kernels with 13% (wet basis) moisture content were used as food substrate. The populations from Machado and Paracatu were previously diagnosed as phosphine-resistant (24- and 53-fold, respectively), and the other populations were susceptible to the fumigant relative to the susceptible standard population (Pimentel et al., 2009). The Machado population was also reported to have a high level of fenitrothion resistance and a moderate level of resistance to permethrin and esfenvalerate (Braga et al., 2011; Corrêa et al., 2011), whereas the Piracicaba population was previously shown to be susceptible to traditional insecticides such as phosphine, pyrethroids, and organophosphates (Corrêa et al., 2011; Pimentel et al., 2009).

Commercial AITC formulation was obtained from Petite Marie Química Fina (95% pure; São Paulo, SP, Brazil), where it is produced by the reaction of allyl chloride and potassium thiocyanate, as commonly used in the food industry. An AITC-soybean oil stock solution (0.5% v/v) was prepared for the fumigation bioassays. Soybean oil was used as the solvent to minimize AITC sorption, which takes place when AITC is applied with a more volatile solvent to grains (Paes et al., 2012).

2.2. Toxicity bioassays

Lethal concentrations (LC_{50} and LC_{95}) of AITC were estimated for adults of each *S. zeamais* population using a concentration range that varied from 1.2 μL L^{-1} to 2.8 μL L^{-1}. Bioassays were performed in 0.8-L glass containers (8.0 × 15.0 cm) with four replications for each combination of insect population and oil concentration. A microsyringe (25 μL) (Hamilton Company, Model 702, Reno, Nevada, USA) was used to apply the AITC-containing solution onto 2.25 cm^2 filter paper pieces, which were placed in Petri dishes (5.0 × 1.0 cm) inside the glass containers. To avoid the direct contact of insects with the filter paper pieces impregnated with the essential oil solution, Petri dishes were covered with an organza-type cloth. Fifty unsexed adults of *S. zeamais* were added to each jar. The containers were closed with a metal lid and sealed with silicone rubber (Silicone Multipurpose; Orbived Construction, Sao Paulo, SP, Brazil). Subsequently, the pests were maintained in a growth chamber under controlled conditions (i.e., 27 ± 2 °C; 75 ± 5% of relative humidity; 24 h scotophase) for 48 h. The control treatment was performed under same conditions; however, insects were exposed to only the soy oil. After this period, mortality was assessed, with those insects unable to move when prodded with a fine camel-hair brush being considered as dead.

2.3. Emergence bioassays

This bioassay followed a procedure described elsewhere with slight changes (Guedes et al., 2010; Haddi et al., 2015b). The insects from each population were exposed to three sublethal AITC concentrations (0.5, 1.0, and 1.5 μL L^{-1}, which correspond to the LC_1, LC_5, and LC_{50} of the most susceptible population) following the same procedure as described for the concentration-mortality bioassays. After 48 h of exposure, 20 live insects of each replicate were allowed to colonize 50 g of maize grains. The insects were removed from the grains after 15 days, and the emergence of new adults was recorded daily for 50 days after the first emergence. Five replicates were used for each treatment.

2.4. Respiration bioassay

Respiratory bioassays were performed using a CO_2 Analyzer TR2 (Sable Systems International, Las Vegas, NV, USA) following previously described methods (Guedes et al., 2006; Oliveira et al., 2007). The CO_2 production, indicating the average respiratory rate, was measured for the unsexed adults from each population that were previously exposed to sublethal doses of AITC (0.5, 1.0, and 1.5 μL L-1, which corresponds to the LC_1, LC_5, and LC_{50} of the most susceptible population) or from the population exposed to control (only soy oil) treatments. The insects were placed in 25-mL chambers that were connected to a completely closed system. The chambers were connected to the system for 90 min before injecting CO_2 free air into the chambers for 2 min at a rate of 600 mL/min. The air current directed the CO_2 that was produced by insect respiration to an infrared reader that was connected to the system, which allowed for the immediate quantification of the CO_2 produced. Five replicates of 20 unsexed adults were used for each population*concentration combination. After the CO_2 production had been determined, the insects were removed from the chambers and weighed on an analytical balance (Sartorius BP 210 D, Göttingen, Germany).

2.5. Walking bioassays

The walking bioassays were conducted in a room with controlled temperature (27 ± 2 °C), using methods adapted from