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Ozone toxicity to *Sitophilus zeamais* (Coleoptera: Curculionidae) populations under selection pressure from ozone

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ABSTRACT

Two populations of *Sitophilus zeamais* (Coleoptera: Curculionidae) were subjected to selection pressure with ozone (O_3), and the possibility of acquiring resistance to O_3 was investigated. The pattern of locomotion and the rate of respiration were evaluated following each selection cycle. Two source populations were used in the study: one was a mixture composed of 30 populations (MP), and the other was composed of the population that was the least susceptible to O_3 among these 30 populations (LSP). The beetles from each source population experienced selection cycles with O_3 using the lethal time for 80% (LT_{80}) of the insect population from each generation. The O_3 toxicity (50 ppm at a continuous flow rate of 2 L min^{-1}) to each generation was calculated using time-response bioassays. The locomotor pattern (distance traveled, resting period, and walking speed) and the respiratory rate (CO_2 production) were also evaluated. The *S. zeamais* populations that were subjected to successive cycles of selection with O_3 did not acquire resistance to O_3, and the pattern of locomotion and the rate of respiration did not change following the selection cycles with O_3.

1. Introduction

The evolution of pest resistance to insecticides is a major obstacle in pest control programs that use chemical products (Groeters and Tabashnik, 2000). In the grain storage industry, the increased levels of resistance are primarily caused by the limited availability of different insecticides, the indiscriminate use of these insecticides for long periods, and the lack of appropriate structures for the application of fumigants (Collins et al., 2005; Sousa et al., 2009). Recent studies demonstrated that the Brazilian populations of *Tribolium castaneum* (Coleoptera: Tenebrionidae), *Sitophilus zeamais* (Coleoptera: Curculionidae), *Rhyzopertha dominica* (Coleoptera: Bostrichidae), and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) were highly resistant to phosphine, with rates of resistance that were 32.2–186.2-fold that of susceptible populations (Pimentel et al., 2007, 2009).

The enrichment of the atmosphere with ozone (O_3) is recognized as an important alternative for the control of stored products pests (Kells et al., 2001; Sousa et al., 2008; Isikber and Öztekin, 2009; Lu et al., 2009) because pests of stored products do not show cross-resistance between phosphine and O_3. Additionally, O_3 does not leave a residue in the grain because oxygen (O_2) is the degradation product (Zhanggui et al., 2003; Sousa et al., 2008). Ozone is a gas that is derived from the rearrangement of oxygen atoms that occurs during electrical discharges or from exposure to high-energy electromagnetic radiation (ultraviolet light) in the atmosphere (Khadre et al., 2001; Liu et al., 2007). Ozone is an unstable molecule with a half-life of 20–50 min (Isikber and Öztekin, 2009) that can be generated locally, which eliminates the requirements for its handling, storage, and transport.

Although the toxic effects of O_3 to stored products insect pests are well documented, further studies are required to assess the risk for the development of resistance in the populations that are exposed to selection pressure with O_3. Based on evolutionary theory, resistance to insecticides is predicted to evolve in the populations that are maintained under selection pressure from chemicals because different traits are selected to increase the probability of survival in harsh environments (Foster et al., 2000;

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Coustau et al., 2000; Arnaud and Haubruge, 2002; Guedes et al., 2010). Moreover, the selection of a particular trait may be associated with pleiotropy, i.e., the ability of a single gene to affect more than one trait in an individual (Boivin et al., 2001; Raymond et al., 2005; Guedes et al., 2009). Therefore, in this study, the susceptibility of populations of *S. zeamais* to develop resistance when subjected to selection pressure with O_3 was evaluated. Additionally, we evaluated the locomotor pattern and the metabolic rate of the beetles during each selection cycle, considering that the toxicity of these insecticides has a known association with physiological and behavioral mechanisms.

2. Materials and methods

2.1. Insects

Two source populations were used in this study: (1) a mixture of 30 populations that were collected in 10 Brazilian states and two Paraguayan regions (MP), and (2) the population that was the least susceptible to O_3 among these 30 populations (LSP), which was collected in Guaxupé in the state of Minas Gerais. The toxicity to these populations was previously established by Sousa et al. (2012). Both populations were maintained in 1.5-L glass vials under controlled conditions (27 ± 2 °C and 70 ± 5% r.h.). Previously fumigated corn grain, with a moisture content of 13% (wet basis), was used as the food substrate, and the corn was maintained at −18 °C to prevent re-infestation.

2.2. Selection and O_3 toxicity bioassays

The adults from the MP and the LSP populations were submitted to selection cycles with O_3. Initially, the lethal time for 80% (LT_{80}) of the parental generation of each population was used. Subsequently, the corresponding LT_{80} for each generation was used. The insects that survived the final selection cycle were collected to obtain the progeny. Approximately 2000 beetles, aged 1–2 weeks post-emergence, were used in each selection cycle.

The O_3 toxicity was determined with the estimations of the lethal exposure times for 50% and 95% of the populations (LT_{50} and LT_{95}, respectively), using the adapted methodology from Sousa et al. (2008). The O_3 concentration was set at 50 ppm (≈0.11 g m^{-3}) at a continuous flow rate of 2 L min^{-1}. The O_3 was administered inside plastic chambers (width, 13 cm; height, 20 cm) at 27 °C ± 2 °C and a relative humidity of 70% ± 5%. The insects from each population were placed in plastic cages (width, 4 cm; height, 3.5 cm) that were suspended 10 cm from the base of the fumigation chamber. The cover and bottom of the cages were composed of organza-type fabric. For the controls, O_2 with a minimum purity of 99.99% was used. The experiments were performed in three replicates, each with 50 unsexed adults that were 1–2 weeks old. The mortality of the beetles was assessed after eight days of exposure to O_3.

The O_3 was obtained with an O&L3.ORM (Ozone & Life, São José dos Campos, São Paulo, Brazil), which used compressed O_2 (minimum purity of 99.99%) as the source for the O_3. The O_3 concentration that was indicated by the O_3 generator was confirmed using a continuous O_3 monitor (BMT Messetechnik GMBH–BMT 930), with accuracy of 0.001 ppm_v, and the iodometric method with indirect titration (Eaton et al., 2000).

2.3. Locomotor behavior

The methods used were adapted from Watson et al. (1997) and Pereira et al. (2009). The walking of individual male and female insects was observed over 10 min in acrylic arenas (3.5 cm high × 15 cm wide), with walls coated with Teflon® polytetrafluoroethylene (PTFE; DuPont, São Paulo, Brazil) to avoid the insects' escape. The O_3 concentration was fixed at 50 ppm (≈0.11 g m^{-3}) at a continuous flow rate of 1.3 L min^{-1}. This concentration is in the sublethal range for the two populations. The gas injection and exhaustion were through two connections installed on the opposite sides of each arena. For the controls, O_2 with a minimum purity of 99.99% was used.

The movement of the insects within the arena was recorded by a tracking system that consists of a CCD camera that registers and digitally transfers the images to a coupled computer (ViewPoint Life Sciences Inc., Montreal, Canada). Each insect was individually placed in the center of the arena, 2 min before the beginning of the test to allow the chamber to reach ozone saturation and the insect to acclimate to the arena. The evaluated characteristics were distance (cm), resting period (s), and walking speed (mm/s). Twenty repetitions were conducted for each population. The tests were performed in a climate-controlled room (27 ± 2 °C and 70 ± 5% r.h.), between 7:00 am and 7:00 pm.

2.4. Respiratory rate

The production of carbon dioxide (CO_2) was measured using a TR3C CO_2 analyzer (Sable Systems International, Las Vegas, USA). For this purpose, 25-mL respiratory chambers that contained 20 adults of each population were used, with each connected to a completely closed system. Four replicates were used for each population, and the CO_2 production was measured in each chamber at a controlled temperature of 27 °C ± 2 °C after a 15-h acclimation period. CO_2-free air was injected into the chambers for 2 min at a flow rate of 100 mL min^{-1}. An infrared sensor reader was connected to the system output for the quantification of the CO_2 (μL CO_2 h^{-1} per insect). The controls were empty respiratory chambers, and the values were used to normalize the respiratory rate data for each population.

2.5. Statistical analyses

The data of the time-response bioassays were subjected to probit analysis (PROC PROBIT; SAS Institute, 2011). The confidence intervals for the toxicity ratios (TRs) were calculated according to Robertson and Preisler (1992), and the lethal time (LT) values were considered significantly different ($P < 0.05$) when the confidence intervals of the TRs did not include the value 1. For the locomotor parameters, multivariate covariance analyses were performed (population × generation × treatments, with and without O_3) (PROC GLM with MANOVA procedure; SAS Institute, 2011). The CO_2 data were subjected to an analysis of covariance (population × generation) (PROC GLM; SAS Institute, 2011).

3. Results

3.1. O_3 toxicity

For the two populations, the variation in the toxicity of O_3 between generations was markedly low (<2-fold; Table 1), which indicated the lack of variation in the populations in the susceptibility to O_3. Because no resistance to O_3 was observed, the tests were terminated in the F_2 generation. The TRs of the LT_{50} values varied between 1.00- and 1.09-fold for the mixed population (MP) and between 1.00- and 1.03-fold for the population least susceptible to O_3 (LSP). The slopes of the time–mortality curves were similar between the populations, which indicated homogeneity in the responses to O_3 in the selection cycles.