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Effect of ozone on respiration of adult *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.)

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ABSTRACT

The effect of ozone on the respiration of three species of adult stored-product Coleoptera was tested in an air-tight flask. *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst) adults were exposed to atmosphere containing 0.1, 0.2 or 0.4 μg/ml initial ozone at 23–25 °C and 50% r.h. Carbon dioxide (CO_2) production reflected the respiration rates of insects and was determined with a gas chromatograph (GC). The experiments showed that the effects of ozone on respiration had two distinct phases. Phase 1 involved a lower respiration rate of the adult stored-product Coleoptera under ozone atmosphere and reflected the need for insects to reduce ozone toxicity. After 1 h, CO_2 production of *S. oryzae* was 3.19, 2.63, 2.27 and 1.99 μl/mg for the ozone concentration of 0, 0.1, 0.2 and 0.4 μg/ml, respectively. The results also showed that there were decreases in the rate of respiration in *R. dominica* and *T. castaneum* with an increase in ozone concentration. During phase 2, respiration of *S. oryzae*, *R. dominica*, and *T. castaneum* adults treated with ozone increased as the ozone degraded to oxygen. After 7 h, the effect of ozone on CO_2 production, relative to the control, changed from a decrease to an increase. The findings in relation to control strategies were discussed.

1. Introduction

As gaseous fumigants are mainly absorbed by the exposed insects through their respiratory systems, factors that influence respiration in insects could also affect the uptake of fumigant. Modified atmosphere (MA) technology, such as changes in the concentration of oxygen (O_2) and carbon dioxide (CO_2) in a sealed system has been widely recognized as a means of preserving stored grain. Changes in the concentration of either O_2 or CO_2 have the potential to affect the rate of respiration, hence the rate and biochemistry of metabolism and therefore the rate of incorporation and ultimately the toxicity of a fumigant. For example, the respiration rate of immature stages (eggs, young larvae and old larvae) of *Tribolium castaneum* (Herbst) was suppressed when O_2 concentrations were reduced (Emekci et al., 2002). Similarly, respiration rates of immature stages of *Rhyzopertha dominica* (F.) were proportional to the O_2 level at reduced O_2 levels (Emekci et al., 2004). In some insects, concentrations of CO_2 as low as 1% can elicit a response of increased spiracle opening (Jay and Cuff, 1981; Ren et al., 1994). Caution is needed when interpreting fumigant dosage data obtained from sealed systems where CO_2 concentrations exceed about 1% and changes in respiratory physiology start to occur.

Pure ozone is a toxic, bluish, unstable, potentially explosive gas which is a natural component of the atmosphere. As an oxidizing agent in organic and inorganic reactions, it is used in many fields (Kells et al., 2001), such as water purification, bleaching and disinfection of medical appliances, as well as to eliminate odours, colours, pesticides, inorganic, and organic compounds. The attractive aspect of ozone is that it decomposes rapidly (in about half an hour in atmospheric conditions) to molecular oxygen without leaving a residue. Therefore ozone can safely be used in the food processing industry (Palou et al., 2002; Forney et al., 2007; Wei et al., 2007). Some studies have been published on its efficacy as a fumigant against pests of stored products (Erdman, 1980; Kells et al., 2001; Sousa et al., 2008). The efficacy of ozone against *T. castaneum* and *T. confusum* has been investigated by mortality of larvae (Erdman, 1980). Maize treated with 50 ppm ozone for 3 days resulted in 92–100% mortality of adult *T. castaneum*, *Sitophilus zeamais* and *Plodia interpunctella* larvae (Kells et al., 2001). Sousa

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et al. (2008) assessed ozone toxicity to phosphine-resistant pests of stored products: no tested populations showed resistance to ozone. The ozone flowed freely through the grain with little degradation once the molecular sites responsible for ozone degradation became saturated (Kells et al., 2001; Mendez et al., 2003). All these results demonstrate that ozone is a potential fumigant for stored products.

Information on the respiration rates of stored-product insects in ozone atmospheres has an important role in understanding the effects of sealed storage atmospheres on pests and in developing efficient control strategies based on insect physiology. Rice weevil (*Sitophilus oryzae*), lesser grain borer (*R. dominica*), and rust-red flour beetle (*T. castaneum*) are common stored-product insects. Populations develop very quickly and can cause severe damage both in terms of loss of quantity and quality. There have been several previous studies undertaken on these species where respiration has been measured under a range of conditions and with a range of techniques (Damcevski et al., 1998; Emekci et al., 1998; Emekci et al., 2002, 2004). There is no information in the published literature on respiration rates of *S. oryzae*, *R. dominica*, and *T. castaneum* under varying ozone concentrations. Therefore, this study was undertaken to investigate the effect of ozone on the respiration of adults of these species *S. oryzae*, *R. dominica*, and *T. castaneum* adult in an air-tight system.

2. Materials and methods

2.1. Insects

The insect species tested were *S. oryzae* (L.), *T. castaneum* (Herbst) and *R. dominica* (F.). They were the phosphine susceptible strains (LS2, TC4 and RD2, respectively), and held at CSIRO Entomology, Canberra, Australia. *T. castaneum* was reared on medium comprising 1 part yeast and 12 parts wholemeal flour milled from Australian soft wheat (Rosella). *R. dominica* was reared on a medium containing 40 parts wheat and 1 part wholemeal flour. *S. oryzae* was reared on wheat. The wheat was conditioned to 13.5% m.c. and disinfested by freezing at −20 °C for >2 days. Adult insects used for the bioassays were derived from cultures held at 25 °C and 70% relative humidity (r.h.). Adults 7–14 days post-emergence were used for testing.

2.2. Preparation of gas mixture

To evaluate the respiration of three species of adult stored-product insects, 0.1, and 0.2 or 0.4 ozone in air were chosen as test atmospheres, and normal air served as a control. These reported concentrations were the initial levels because ozone was degrading into oxygen during the experiments. Ozone supply was obtained from an ozone generator (Ozx-B300t, Canada, ozone output 200 mg/h and pump output: 3 L/min). The parameters of Ozx-B300t were tested using iodometric titration and an airflow meter before the experiments. Ozone was collected using a Tedlar® gas sampling bag (3 L) in which all air was evacuated by vacuum before collection. To get the experimental gas concentrations, 30, 60 or 120 ozone was injected into each exposure flask after equivalent air was removed from the flask via the septum using a pressure-lock syringe. The volumes of the flasks were determined calibrated gravimetrically from the amount of water each one contained. The flask lids were sealed with glass stoppers containing a septum. One hundred insects were counted and then weighed before being placed them in the exposure flasks without food. The experiments were conducted in a thermostatically controlled room at 23–25 °C and 50% r.h. Insects, exposed to treated air and the control, were left for 24 h to acclimatize before starting of measurements (Emekci et al., 1998).

2.3. Analysis of gas

During the experiments ozone was decaying to oxygen. This caused the O_2 level to increase slightly. Therefore, CO_2 production was used to measure respiration and expressed in terms of μl/mg. Gas samples of 500 μl were removed from the flasks via the septum after shaking. A Fisher model 1200 Gas Partitioner was used to analyse the gas samples. This was fitted with 80–100 mesh Columpak™ PQ (2 m × 3.18 mm ID) and 60–80 mesh molecular sieve 13*X* (3.35 m × 4.76 mm ID) columns in series and a thermal conductivity detector (TCD). The operating conditions were 50 °C for the oven and detector temperatures and a helium carrier gas flow rate of 30 ml/min. A Hewlett Packard Reporting Integrator model 3390A was used to calculate the concentrations. Concentrations were calculated from the peak areas which were calibrated periodically using a standard gas mixture of known CO_2, O_2 and N_2 concentration. Respiration results were based on averages of three replicates.

2.4. Statistical analysis

Differences in production of CO_2 in different atmosphere were analyzed by analysis of variance (ANOVA), using the statistical package of SAS (version 9.0, SAS Institute 2002) for PC. We conducted separate analyses for each observation time (1, 2, 3, 4, 5, 6, 7 and 8 hrs for *S.oryzae*; 2, 3, 4, 5, 6, 7 and 8 h for *R. dominica* and *T. castaneum*). The relationships between the production of CO_2 in different atmospheres and treatment time were analyzed with regression analysis (PROC REG, SAS Institute 2002). Intercepts and slopes of the regression for different level of ozone were compared by analysis of covariance (PROC GLM, SAS Institute 2000).

3. Results

3.1. The effect of ozone on respiration of S. oryzae

The effect of ozone on the production of CO_2 by *S. oryzae* adults is shown in Fig. 1. After 1 h, CO_2 production (the average for the three replications, the same as below) of *S. oryzae* was 3.19, 2.63, 2.27 and 1.99 μl/mg for the ozone concentrations of 0, 0.1, 0.2 and 0.4 μg/ml, respectively. The respiration of *S. oryzae* decreased with increasing ozone concentration ($P = 0.0002$; $F = 23.68$; df = 3). We observed that *S. oryzae* adults in the ozone atmospheres were able to move limbs but were not mobile presumably due to the toxicity

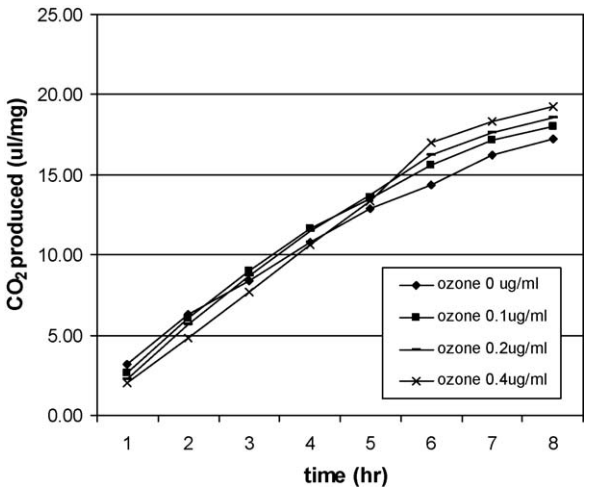

Fig. 1. The production (The CO_2 production is the average for the three replicate experiments for every observed occasion.) of CO_2 by *S. oryzae* adults for different levels of ozone concentrations.