Journal of Stored Products Research 72 (2017) 111–116

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Behavioral and physiological responses induced by ozone in five Brazilian populations of *Rhyzopertha dominica*

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ARTICLE INFO

Article history:
Received 11 April 2017
Accepted 12 April 2017

Keywords:
Fumigation
Toxicity
Locomotor behavior
Respiratory rates
Body mass

ABSTRACT

This study evaluated locomotory and respiratory responses induced by ozone at a concentration of 500 ppm in five Brazilian populations of *Rhyzopertha dominica* (Coleoptera: Bostrichidae). Toxicity and body mass were also assessed to establish their relationship with behavioral patterns. The results indicated that none of the evaluated populations of *Rhyzopertha dominica* showed resistance to ozone. No significant correlations were observed between ozone toxicity and locomotory behavioral patterns. Moreover, no significant correlations were found between ozone toxicity and the respiratory rate of *Rhyzopertha dominica*. Ozone is a potential alternative for phosphine resistance management, and its rapid degradation constitutes an advantage for the environment.

1. Introduction

Due to the growing world population, the food industry has been challenged to develop, among other things, improved management of stored grain and other agricultural products to not only avoid waste but also provide products that protect human health (Nerio et al., 2009). Agricultural products can be used after harvest or stored for use in times of scarcity or when prices are more cost effective. Thus, the storage of these products should be able to maintain their qualitative and quantitative characteristics for long periods of time (Phillips and Throne, 2009).

A considerable part of the food produced in the world is not consumed because it is lost or wasted, which increases concern about the rise of food prices (FAO, 2012). Every year, insects cause significant losses in food quantity and quality. Insect activity results in weight loss and commercial product depreciation due to the presence of live insects, insect fragments, such as chemical excretions or silk, dead insects and chemical residues in food (Phillips and Throne, 2009).

The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), is a primary and widely distributed pest. It is difficult to control because it is an internal feeder, which gives it shelter from insecticides. The fumigant phosphine (PH_3) is widely used to control insect pests in stored grain due to its low cost, effectiveness, rapid spread in the air and ease of use (Chaudhry, 1997; Bell, 2000). However, the long term use of a single fumigant increases the risk of development of resistance in insect pest populations (Benhalima et al., 2004). An association between fumigant phosphine resistance and reduced respiratory rate was observed in *R. dominica*, which suggests that resistance is associated with reduced absorption of the fumigant (Pimentel et al., 2007).

Sustainable strategies should be developed for the management of stored grain pests and the prevention of the development of resistance (Shi et al., 2012). The use of ozone gas (O_3) for atmosphere modification is one of the main alternatives. This gas is a strong oxidant with insecticidal properties. Studies have already demonstrated its efficacy as a fumigant for insect pest control (Kells et al., 2001; Sousa et al., 2008). However, further studies should be conducted to diagnose its degree of toxicity and characterize insect behavioral changes in the presence of ozone gas. Thus, this study aimed to evaluate the toxicity and the physiological and behavioral responses of five populations of *R. dominica* exposed to ozone gas.

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http://dx.doi.org/10.1016/j.jspr.2017.04.006
0022-474X/

2. Material and methods

2.1. Insect populations

We used five Brazilian populations of *R. dominica* (Table 1). All of the insect populations were maintained in glass containers in rearing facilities under controlled conditions (30 ± 2 °C, 70 ± 5% relative humidity, 14 h:10 h lighting regime [L:D]) and reared on insecticide-free wheat.

2.2. Ozone gas

Ozone gas was obtained from a generator developed by the company Ozone & Life (São José dos Campos, Brazil). The gas generation process used oxygen at a purity of 90 ± 3%, was moisture-free, and was obtained by the concentrator Mark 5 Plus Oxygen Concentrator (Oxxisul Industrial Ltd., Curitiba, Brazil) at a continuous flow of 1.5 L min^{-1}.

The concentration of ozone gas was quantified using the iodometric method with indirect titration (APHA et al., 2005), as recommended by the International Ozone Association (IOA).

2.3. Ozone gas exposure chambers

The insects were exposed to ozone gas within a cylindrical plastic chamber (13 cm wide × 20 cm). Gas injection and exhaust were carried out through a connection set up 6 cm from the base of each chamber and another at the top (cap), respectively. The same system was used for the control (atmospheric air). The insects of each population were packed into 4-cm-wide and 3.5-cm-high plastic cylindrical cages and suspended 10 cm from the base of the fumigation chamber on a metal screen, which led to the creation of a plenum. The top and bottom of the cages were made of organza fabric, which allowed the free passage of ozone or atmospheric air.

2.4. Bioassays of ozone toxicity

The toxicity of ozone gas for five populations of *R. dominica* was determined by the estimates of lethal exposure times for 50 and 95% of the insects (LT_{50} and LT_{95}, respectively). Ozone gas was applied at a concentration of 500 ppm (≈1.07 g m^{-3}) at a continuous flow of 1.5 L min^{-1}. Three replicates were used, each with 20 non-sexed adult insects, aged from one to four weeks.

Insect mortality was evaluated after 48 h of each gas exposure time. Preliminary tests were performed to estimate the shortest gas exposure times, where there was no insect death (lower end), and the longest exposure times, where the highest mortality occurred (upper end). Curves were established using time-response bioassays with increasing periods of exposure to ozone gas. After exposure to ozone gas, the insects were removed from the fumigation chamber and placed in Petri plates with ground wheat grain. These plates were kept in growth chambers (BOD type) at a temperature of 30 ± 1 °C and a relative humidity of 60 ± 5%.

2.5. Behavioral (locomotory) responses

Walking bioassays were performed in a climatized room at 28 ± 2 °C and with artificial lighting. The method used in walking bioassays was adapted by Pereira et al. (2009) and Sousa et al. (2012). The chambers used in these bioassays were made of clear acrylic material with a diameter of 15.0 cm and an internal volume of 0.345 L. The injection and exhaust of the gas were performed through two opposite connections installed in each chamber. Atmospheric air was used as the control. The age of the (non-sexed) adult insects ranged from one to four weeks. Each insect was placed in the center of the chamber for 1 min prior to the start of testing, so that the chambers could be saturated with ozone gas.

The walking of each insect inside the chamber was recorded by a tracking system formed by a video camera coupled to a computer (View Point Life Sciences Inc., Montreal, Canada) for a period of 10 min. The behavioral parameters traveling distance (cm), repose time (s) and walking speed (mm s^{-1}) were assessed. The experiment was arranged in a complete randomized design with 20 replications for each population, and the treatments were arranged in a 5 × 2 factorial design structure. Each replication consisted of a single insect.

2.6. Body mass and respirometry bioassays

The respirometry bioassays were carried out in a TR3C respirometer (Sable Systems International, Las Vegas, NV, USA) equipped with a CO_2 analyzer (μL de CO_2 h^{-1} $insect^{-1}$), as described by Guedes et al. (2006) and Sousa et al. (2008). The insects used in the measurement of the respiratory rate were subjected to ozone gas or atmospheric air for a period of 5 h under conditions equal to those of the toxicity bioassays. This choice was based on the time-mortality curves determined for the five populations. Thus, this time was sublethal, considering the exposure time used to estimate the time-mortality curves.

Five replicates of 20 (non-sexed) adult insects of each population were used, ranging from one to four weeks of age, and packed in respirometry chambers with volumetric capacity of 25 mL, which were connected to a completely closed system. The carbon dioxide (CO_2) produced by the insects was measured after 3 h at the temperature of 28 ± 2 °C. To scan all CO_2 produced inside each chamber, the passage of carbon dioxide-free air (600 mL min^{-1}) for 2 min was performed. This air stream caused all produced CO_2 molecules to pass before an infrared reader connected to the system, which continuously measured the CO_2 produced by the insects within each chamber. After the measurement of CO_2, the insects were removed from the chambers and were weighed by an analytical balance (Sartorius BP 210D, Göttingen, Germany). The assays were conducted at the temperature of 27 ± 2 °C and a humidity of 70 ± 5%.

2.7. Statistical analysis

Time-mortality curves were estimated in probit analyses using

Table 1
Origin of the populations of *Rhyzopertha dominica*.

Code	City	State	Collection site	Product
1	Belo Horizonte	Minas Gerais	Flour factory	Wheat
2	Campos de Júlio	Mato Grosso	Metal bottom silo	Maize
3	Cornélio Procópio	Paraná	Laboratory	Maize
4	Rio Pomba	Minas Gerais	Animal feed factory	Wheat
5	Viçosa	Minas Gerais	Laboratory	Maize and Wheat