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Susceptibility of the life stages of cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) to ozone

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ABSTRACT

The ban of methyl bromide and problems associated with the use of other stored-product pest control methods have demanded search for potential alternatives. Ozone, an oxidizing gas, is one of such potential alternatives. In this study, the susceptibility of life stages of the cigarette beetle, *Lasioderma sericorne* (F.) (Coleoptera: Anobiidae) to ozone, was investigated. The concentration-mortality relationships for all life stages exposed to 100–400 ppm at 50 ppm increments for 1 h were determined. The time-mortality relationships for adult *L. sericorne* exposed to 100 ppm ozone concentration for 1–6 h were also determined. Mortality was recorded as percentages of eggs that failed to hatch 10 days after treatment (DAT), larvae or pupae that failed to develop into adults 28 or 15 DAT, respectively, and adults that died 2 DAT. The concentration-mortality estimates suggested that, generally, higher concentrations were required to kill 99% of insects when treated without food compared to when treated with food made of whole wheat flour and brewer's yeast. In the absence of food, larvae were the most tolerant and adults were the least tolerant to ozone treatment and required 15, 974 ppm and 3769 ppm to kill 99% of the individuals respectively. In the presence of food, eggs and pupae were the most and least tolerant respectively. An exposure time of 7.1×10^{30} h was required to kill 99% of adults treated in the presence of food and 99 h in the absence of food. When adult insects were exposed to 100 ppm ozone for 1–6 h in the absence of food, a weak relationship between survival rate and exposure duration observed initially became stronger in subsequent days. The present study suggests that ozone treatment could be a potential alternative for the management of all life stages of *L. sericorne*.

1. Introduction

Pest management operators have relied heavily on chemical pesticides to manage stored product insects. Currently, insect pests are managed by different methods including cleaning and sanitation, modified atmosphere treatment, heat treatment, contact insecticides, and fumigation. The ban of methyl bromide, the most effective fumigant for the control of many stored product insect pests (USDA, 2000; Fields and White, 2002; EPA, 2006), has necessitated the search for other potential alternative management methods (Bond et al., 1984; Price and Mills, 1988; Brigham, 1998, 1999; Mills, 2001; Fields and White, 2002; Phillips and Schilling, 2013). Fumigation with phosphine is effective to some extent, but insects are developing resistance (Savvidou et al., 2003; Sağlam et al., 2015; Fukazawa and Takahashi, 2017). Aside from this, phosphine is corrosive (Phillips and Schilling, 2013) and its use is therefore limited. Although effective, repeated use of fumigants has disrupted biological control by natural enemies and led to outbreaks of insect species, development of resistance to the chemical, undesirable effects on non-target organisms, and environmental and human health concerns (Champ and Dyte, 1976; Phillips and Throne, 2010). Therefore, there is a need for alternatives to be identified.

One of the potential alternatives is ozone (O_3), a highly oxidative, environmentally safe and unstable gas. Ozone is formed by the excitation of molecular oxygen (O_2), into atomic oxygen (O), and then combination of three atomic oxygen to form ozone. The gas is formed naturally in the upper atmosphere from oxygen by ultraviolet radiation and by atmospheric electrical discharges, i.e. corona discharge, such as lightning. For commercial applications, ozone is usually generated using the corona discharge method, which consists of forcing oxygen from the air between high voltage plates. The

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oxygen is then broken apart and recombines to form ozone (Weavers and Wickramanayake, 2001). Humans are usually exposed to very low levels of ozone produced by electrical devices such as photocopiers (Xiu, 1999; Guzel-Seydim et al., 2004).

Ozone is a powerful antimicrobial agent that has been generally recognized as a safe (GRAS) compound and has been employed in the food industry in several applications including disinfection, sterilization and preservation of food and water (Legeron, 1984; Suffet et al., 1986; Majchrowicz, 1998; EPA, 1999; Kim et al., 1999; Weavers and Wickramanayake, 2001; Guzel-Seydim et al., 2004). The use of ozone against stored product insect pests has gained tremendous attention over the past decade (e.g., Leesch, 2003; Mendez et al., 2003; Frazer, 2004; Leesch et al., 2004; Rajendran and Parveen, 2004; Mahroof et al., 2018). Ozone can be generated on-site with inexhaustible, no-cost and abundant air in the surrounding. It decomposes rapidly into oxygen thereby leaving no residue on treated products (Kim et al., 1999; Kells et al., 2001; Mendez et al., 2003), hence there is no need for post-fumigation disposal or aeration of treated commodity.

Lasioderma serricorne (F.) (Coleoptera: Anobiidae), commonly known as the cigarette beetle, is a stored product insect pest of economic importance. This species causes significant damage to grain-based products, tobacco products, spices, and other commodities and dried substrates of animal or vegetable origin. It is therefore a common pest of feed mills and retail stores. The damage caused by this pest can account for millions of dollars in the food and feed industries (Arbogast, 1991). The cigarette beetle ranked second after the Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) in terms of its abundance in five selected retail stores that have grocery and pet departments (Arbogast et al., 2000).

Like many other stored product insect pests, the control of the cigarette beetle had primarily dependent upon continued applications of fumigants including phosphine and methyl bromide (White and Leesch, 1995; Abdelghany et al., 2010). However, with the ban of methyl bromide and its known resistance to phosphine, (e.g. Savvidou et al., 2003; Sağlam et al., 2015; Fukazawa and Takahashi, 2017), many studies have focused on non-chemical alternatives in the management of *L. serricorne* (Adler, 2003; Roesli et al., 2003; Conyers and Collins, 2006; Yu, 2008; Mahroof and Phillips, 2007, 2014; Masukwedza et al., 2016; Kumar et al., 2017). Heat treatment studies on *L. serricorne* have shown that different life stages respond differently to elevated temperatures. Temperatures higher than 45 °C elicit different response based on its level, duration of exposure, and the life stage of the insect (Adler, 2003; Conyers and Collins, 2006; Yu, 2008). Trap catches of stored product insects after subjecting a feed mill to heat treatment of a target of 50 °C recorded no *L. serricorne*, an indication that heat treatment may be effective in controlling this insect among other insects (Roesli et al., 2003). Mahroof and Phillips (2014) studied the effect of synthetic serricornin, the predominant sex pheromone, on the mating disruption of *L. serricorne* and reported a significant reduction in the population size of subsequent generations. This was as a result of the failure of males to properly orient to females, thereby disrupting mating, delaying mating and reducing mating success rate. Controlled or modified atmosphere technology has also been investigated. Vacuum packaging with carbon dioxide flush treatment resulted in 95% adult mortality of *L. serricorne* compared to 7% in normal packaging of stored tobacco (Masukwedza et al., 2016). Kumar et al. (2017) exposed cured turmeric rhizomes artificially infested with *L. serricorne* to various CO_2 concentrations and reported that 50–80% CO_2 could control *L. serricorne* infestation and prevent progeny development up to nine months after treatment.

There have been few studies (including Hasan et al., 2012; Anandakumar et al., 2016) on the effect of ozone on *L. serricorne*. Moreover, published studies did not investigate the effect of ozone in the presence or absence of food. Hasan et al. (2012) also focused on adult *L. serricorne,* which is short-lived, rarely feed, and mates immediately after eclosion. Damage and contamination to commodities is therefore caused exclusively by larval feeding (Minor, 1979). Anandakumar et al. (2016) investigated the effect of ozone on *L. serricorne* on a different commodity, turmeric rhizome. In addition, the authors did not report their research findings on the effect of ozone on *L. serricorne* eggs, although they had apparently exposed all life stages of the insect to ozone. Therefore, the study described here was conducted using all life stages of *L. serricorne* exposed to different concentrations of ozone for different durations in the presence and absence of food. The objectives of this study were to evaluate the relative susceptibility of all life stages to different ozone concentrations, determining concentration-mortality and exposure time-mortality relationships.

2. Materials and methods

2.1. Ozone generation and application

A bench top model ozone generating equipment that produces up to 8000 ppm with a continuous flow rate of 1–2 L min^{-1} was obtained from Ozone Solutions Inc. (Hull, IA, USA). The equipment was fully described and used by Mahroof et al. (2018). The equipment consists of the oxygen concentrator, the control box, the ozone analyzer, the ozone chamber and ozone destructing unit. Briefly, ambient oxygen is taken up by the oxygen concentrator. Ozone is then produced by the ozone generator enclosed in the control box using the corona discharge method. The amount of ozone is regulated by the ozone analyzer (ozone monitor Model 465M) by adjusting the regulator buttons on the control box. Test specimens are placed inside the ozone chamber (50 cm long, 20 cm wide and 60 cm high), in which the set ozone concentration is maintained. Ozone gas build-up time, gas exhaustion-time along with temperatures within the ozone fumigation chamber at various ozone concentrations were previously determined. On turning on the equipment, the set ozone concentration reaches equilibrium within 6 min. It takes up to 55 min for ozone concentration to return to 0 ppm after the equipment is turned off by the user (Mahroof et al., 2018). A temperature and relative humidity (RH) data logger (HOBO UX100-003, Onset Computer Corporation, Bourne, MA, USA) was placed inside the ozone chamber to record the temperature and RH every 30 min.

2.2. Test insects

Test insects were maintained at the Stored Products Entomology Research Laboratory at South Carolina State University since 2010. Prior to bioassays, the *L. serricorne* were reared on food made of whole wheat flour and powdered yeast in the ratio of 95:5. This food was used in all experiments except in egg experiments where cornmeal was used instead. New colonies were established by transferring newly emerged adults to 473 ml rearing jars (Ball Corporation, Broomfield, CO, USA) with the diet mix. The adults were removed after 48 h and the rearing jars were kept in an incubator (I-36VL; Percival Scientific Inc., Perry, IA, USA) at 27.4 ± 0.1 °C and 50.9 ± 0.7% RH until testing of larvae, pupae, and adults was done. Third instar larvae and 1–5 d old pupae were collected from rearing jars 21–24 and 31–35 days after establishing the colonies, respectively. Pupae had their cocoon, which is formed from food and other debris intact. The adults used in the experiments were 0–4 d old. For eggs, newly emerged adults were allowed to oviposit on a thin layer of white flour sifted through sieve # 60 (VWR International, Center Valley, PA, USA) and the eggs were collected after 24 h for use in the experiments.