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Potential of ozone technology for German cockroach (bBlattella germanica (L.)) management

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POTENTIAL OF OZONE TECHNOLOGY FOR GERMAN COCKROACH

(*BLATTELLA GERMANICA* (L.)) MANAGEMENT

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ABSTRACT

Yanlin Tian. M.S., Purdue University, May 2015. Potential of ozone technology for german cockroach (*Blattella germanica* (L.)) management. Major Professors: Linda J Mason and Ameya D Gondhalekar.

German cockroaches (*Blattella germanica* (L.)) are important urban pests that adversely impact human health. Previous studies indicated that ozone technology (ozone gas) can be used as a management tool to control insect pests in grain storage facilities, stored foods and some finished products. The goal of my study was to broaden the applicability of ozone from traditional stored grain pests to *B. germanica*. The first part of my study determined the concentration-time (CT) relationship of ozone required to achieve 100% mortality in various life stages (adults, nymphs and eggs) of *B. germanica*. Results showed that eggs were the most ozone-tolerant life stage and 100% mortality was only achieved after 24 h exposure at 480 ppm ozone (CT of 691200 ppm-min). Second part of my research determined the effects of sub-lethal ozone exposure on reproduction and population growth rate of *B. germanica*. In general, crosses that included ozone-treated females produced significantly fewer F1 nymphs. Exposure of both sexes to sub-lethal ozone environment caused significant population growth reduction in comparison to the other three pairings at 7, 11 and 15 weeks post exposure.

CHAPTER 1 OVERVIEW AND AIMS

Cockroaches are the most successful insects on our planet (Brenner 1995). They are found in houses, apartments, restaurants, hotels and most structures around the world. About 70 species are found in United States (Mallis, 2004). The most common are the German cockroach (*Blattella germanica* (L.)) and American cockroach (*Periplaneta Americana* (L.)). German cockroaches are the most important cockroach pest species in US. They can carry many microorganisms such as bacteria, protozoans and viruses which cause food poisoning, dysentery, diarrhea, and other illnesses (Cochran, 1994; Mallis, 2004). Gel bait formulations of insecticides like abamectin, hydramethylnon, indoxacarb, imidacloprid, and fipronil are widely used for *B. germanica* control (Appel, 2003; Buczkowski et al., 2001; Buczkowski et al., 2008; Gondhalekar et al., 2011; Wang et al., 2008; Wang et al., 2004). However, recent reports have shown that *B. germanica* have developed low levels of resistance to active ingredients in newer insecticides (Cochran, 1994; Gondhalekar et al., 2011). Therefore, new ecofriendly methods are essential for *B. germanica* IPM program.

As a gas, ozone consists of three oxygen atoms. It is very unstable andbreaks down into atmospheric oxygen in a matter of minutes. Since it leaves no residue, it has a significant advantage over hazardous residual chemicals (Kim et al., 1999). Ozone technology has been widely used to sanitize water and kill bacteria in the food industry.

Compared to its widely usage in food industry, ozone is relatively a new method for pest management. Recently, research on the use of ozone to control pests of stored grains has increased (Holmstrup et al., 2011; Kells et al., 2001; McDonough et al., 2011a; McDonough et al., 2011b; Sousa et al., 2008; Tiwari et al., 2010) but it has not been examined as a control method for urban insect pests. For cockroach management, ozone has the ability to rapidly spread in enclosed spaces and may reach hidden *B. germanica* harborages. This ability to reach hidden pests would be advantageous for managing *B. germanica* in areas of structures with limited human access.

1.1 Specific objectives

1) Determine the concentration-time (CT) relationship of ozone needed to achieve 100% mortality for various life stages (adult, nymph and egg) of *B. germanica*.

2) Determine the effects of sub-lethal ozone exposure on reproduction and population growth rate of *B. germanica*.

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CHAPTER 2 TOXICITY OF OZONE ON DIFFERENT
LIFE STAGES OF THE GERMAN
COCKROACH (*BLATTELLA GERMANICA* (L.))

Abstract

German cockroaches (*Blattella germanica* (L.)) are important urban pests that adversely impact human health. Since use of insecticides is associated with environmental contamination and threat of resistance development, new safe and sustainable methods are needed for *B. germanica* management. Ozone technology (ozone gas) has been widely used to sanitize water, remove odors from structures, kill bacteria and other pathogenic microorganisms. Recent studies have also shown that ozone technology can be used as a management tool for insect pests in grain storage facilities, stored foods and some finished products. The objectives of this study were to determine the concentration-time (CT) relationship of ozone required to achieve 100% mortality in various life stages (adults, nymphs and eggs) of *B. germanica* and provide baseline information needed to develop ozone technology for cockroach management. Insects were treated with five ozone concentrations between 80 and 900 ppm at various time intervals until 100% mortality was achieved.

Results showed that higher ozone concentrations significantly reduced treatment times. For example, when ozone concentration was increased from 80 to 180 ppm, the

exposure time needed to attain 100% mortality in adults and nymphs decreased from 72 h to 48 h. Eggs were the most ozone-tolerant life stage and 100% mortality was only achieved after 24 h exposure at 480 ppm ozone (CT of 691200 ppm-min). The CT determined for 100% mortality of adult and nymphs were 518400 ppm-min and 345600 ppm-min, respectively.

Keywords: Ozone, *Blattella germanica,* CT value

2.1 Introduction

German cockroaches, *Blattella germanica* (L.) (Blattodea: Blattellidae) are important urban pests commonly found in human residences, restaurants, food-processing facilities and other structures all over the world (Brenner, 1995). They serve as carriers of several disease causing pathogens, including bacteria, viruses, fungi and protozoa that threaten human health (Cloarec et al., 1992; Fotedar et al., 1991). In addition, allergen proteins produced by cockroaches have been linked to increased incidences of childhood asthma and other allergic conditions (Sohn and Kim, 2012). The presence of *B. germanica* infestations is also associated with social stigma and reduced psychological well-being (Brenner, 1995). Since the late 1990s, *B. germanica* management in the United States has been mainly based on use of bait formulations of insecticides like abamectin, hydramethylnon, fipronil, imidacloprid, and indoxacarb (Appel, 2003; Buczkowski et al., 2001; Buczkowski et al., 2008; Gondhalekar et al., 2011; Wang et al., 2008; Wang et al., 2004). However, recent reports indicate that *B. germanica* have already developed low levels of resistance to newer insecticide active ingredients

(Cochran, 1994; Valles et al., 2000; Wang et al., 2004; Gondhalekar and Scharf 2012; Gondhalekar et al., 2013). Additionally, the use of pesticides for cockroach control is also associated with contamination of indoor environments and unintended exposure of humans to insecticide residues. Thus, new ecofriendly methods are needed for *B. germanica* management.

Benefits of ozone, a strong oxidizing agent, include such uses as water purification and odor mitigation (Khadre et al., 2001; Kim et al., 1999). With limited control strategies available for stored grain, especially with the loss of methyl bromide, examination of post-harvest ozone use is on the rise. In recent years ozone has been increasingly investigated for its potential use in the control of insect pests of stored grains (Holmstrup et al., 2011; Kells et al., 2001; McDonough et al., 2011a; McDonough et al., 2011b; Sousa et al., 2008; Tiwari et al., 2010). The use of ozone for insect control has several advantages. It is rapidly and spontaneously degraded to oxygen and therefore does not lead to accumulation of toxic residues in the environment (Kim et al., 1999). Ozone also has the ability to rapidly spread in enclosed spaces and also reach hidden insect harborages that are difficult for human access. Although ozone does not penetrate substrates, its ability to reach hidden pests would be advantageous for managing *B. germanica* in structures with limited human access. Lastly, ozone treatments have been effective against insecticide resistant populations of certain stored grains pests (Jian et al., 2013). Thus, ozone could be a good alternative to manage cockroach resistant populations in specific situations.

Previous research has shown that the ozone concentrations and exposure times required to achieve 100% mortality can be significantly different between insect species

(Isikber and Athanassiou, 2014; Kells et al., 2001; McDonough et al., 2011b; Tiwari et al., 2010). Moreover, ozone toxicity data for urban pests like *B. germanica* are not available. Thus, the objective of this study was to determine the concentration-time (CT) combination of ozone required to achieve 100% mortality in various life stages (adult, nymph and eggs) of *B. germanica* and provide baseline information needed to develop ozone technology for cockroach management. My hypothesis was that ozone would be able to control *B. germanica* with a sufficient concentration and an appropriate treatment time.

2.2 Materials and Methods

2.2.1 Insects

A laboratory strain of the *B. germanica*, Johnson Wax susceptible (JWax-S) was used in all bioassays. The JWax-S strain has been maintained in the lab without insecticide exposure for >70 years. Rearing was conducted in plastic containers (37.2 cm × 21.7 cm × 18.1 cm) with corrugated cardboard harborages, lab diet (Harlan Teklad Rodent Diet #8604) and a water source. Rearing containers were held at 25 °C ± 0.5 °C, 60% rh and 12:12 h light: dark photoperiod. A thin barrier layer of petroleum jelly and mineral oil (2:3) was placed around the inner top portion of the rearing box to prevent escape.

2.2.2 Ozone exposure system and treatments

Ozone concentrations between 80-900 ppm (80, 180, 480, 700, and 900) were used with exposure times ranging from 2 h to 96 h at 25 °C ± 0.5 °C and 60% rh. For

ozone exposure tests, adults, nymphs or females carrying egg cases (10 per life stage) were placed in Petri dishes (94.4 mm diameter; Scientific Equipment of Houston, Navasota, TX) with screened lids. Adult treatments consisted of 5 one-week-old adult males and 5 one-weekold non-gravid adult females. Bioassays with immature insects consisted of ten 3rd-5th instar mixed sex nymphs. Egg bioassays were performed with 10 gravid females each carrying a 1-week old ootheca. Each concentration and exposure time combination was replicated three times. Controls consisted of insects that were handled similar to the exposed insects, but were held under lab conditions during ozone exposure.

Bioassays requiring ozone concentration of 80 ppm, 180 ppm and 480 ppm were carried out by a corona discharge generator provided by Medizone International (Sausalito, CA) and built by Biozone Corportation. Ozone was generated from 99% pure medical grade oxygen and passed through the bench top ozonation chamber (60.0 cm $\times$ 30.0 cm $\times$ 30.0 cm). The airflow through the chamber was regulated by a computer to obtain the intended ozone concentration. An ozone analyzer (Eco sensors UV-100) provided by 2B Technologies, Inc (Boulder, Colorado, USA) was connected to the outlet of the chamber at the end of the series to monitor ozone concentration. Experiments requiring ozone concentration of 700 ppm and 900 ppm were carried out by an ozone generator provided by O_3Co (Idaho Falls, ID, USA) using the corona discharge method. Ozone was passed through three identical plastic boxes (101.6 mm $\times$ 101.6 mm $\times$ 25.4 mm) connected to the ozone generator by plastic tubes (Tygon®; 12.7 mm $\times$ 2.38 mm diameter). Air used for ozone generation was dried with a column of anhydrous calcium sulfate prior to being passed through the generator. An ozone analyzer (L2-LC Model

040977; IN USA Inc., Needham, MA, USA), was connected to the outlet of the box at the end of the series. Ozone (1 L/min) was flowed into the boxes at a rate of 1 L/min and vented through a filtered fume hood to the outside.

Immediately after ozone exposure, insects were placed in rearing containers with corrugated cardboard harborages, lab diet (Harlan Teklad Rodent Diet #8604) and a water source. The rearing boxes were held in an environmental chamber at 25 °C ±0.5 °C, 60% rh and a12:12 h light:dark photoperiod. Twenty-four hours post-exposure, insects were scored for mortality and this number was used to determine percent mortality. Insects were scored as dead if they were unable to right themselves upon disturbance. Control adults and nymphs were held under the same conditions as treated insects and scored in the same time period and manner. Egg cases (deposited or carried by adult females) were placed in rearing boxes which were held in the environmental chamber after exposure. Mortality was determined by counting the total number of emerged and unemerged nymphs. Mortality was scored as 100% if no nymphs emerged. Once all nymphs emerged from control (untreated) egg cases, the ozone-treated egg-cases were scored for mortality and discarded (generally between 7-14 days post-exposure).

2.2.3 Data analyses

Abbott's formula was used to correct for control mortality (Abbott, 1925). Analysis of variance (ANOVA) and Tukey's honestly significance design (HSD) test were used to analyze the percent mortality data at all ozone concentrations and exposure times ($\alpha < 0.05$).

The concentration X time products (ppm-min) required to achieve 100% mortality were calculated for all life stages and all concentrations. Additionally, mortality data at 480 ppm ozone concentration and different exposure times (CT products) were also analyzed by using the PROC PROBIT function in the SAS software package (SAS Institute, Cary, NC). Significance of probit CT values between life stages was determined on the basis of the criterion of non-overlap of 95% fiducial limits.

2.3 Results

All adult *B. germanica* were killed after a treatment at 480 ppm with an exposure time of 18 h, while all nymphs were dead after 12 h of ozone exposure at 480 ppm. Percent mortality levels for all life stages of the *B. germanica* at 480 ppm ozone concentration and a range of treatment times is shown in Table 2.1. Eggs were the most ozone-tolerant stage and required a treatment of 24 h to reach 100% mortality.

Based on empirical results shown in Table 2.1, theoretical concentration X time products (CT products; ppm-min) for obtaining 50% mortality in different life stages of *B. germanica* at 480 ppm were calculated and are presented in Table 2.2. The empirical CT50 value was obtained by multiplying concentration (480 ppm) by treatment time (12 h for eggs, 5 h for nymphs, 6 h for adults) to achieve ca. 50% mortality. The theoretical CT50 was estimated by using the PROC PROBIT function in the SAS software package (SAS Institute, Cary, NC). From this table, it was found that there were no significant differences between nymphs and adults. However, the CT50 vaules of eggs are much higher than nymphs and adults. It was also found that empirical and theoretical CT estimates were similar for all three life stages.

Table 2.1 Percent mortality of all life stages of *Blattella germanica* exposed to 480ppm ozone for various time periods.

Life stage	Treatment time (h)	Mortality(%) ± *SE*	ANOVA
Eggs	2	1.1 ± 0.6 a	df = 20,
	3	5.7 ± 3.3 a	F = 85.03
	4	3.6 ± 1.5 a	P= < 0.0001
	6	26.9 ± 2.9 b	
	12	46.6 ± 6.6 b	
	18	77.5 ± 2.7 c	
	24	100 ± 0.0 d	
Nymphs	2	0.0 ± 0.0 a	df = 20,
	3	30.0 ± 4.7 b	F = 43.99
	4	40.0 ± 4.7 b,c	P= < 0.0001
	6	63.3 ± 9.8 c	
	12	100 ± 0.0 d	
	18	100 ± 0.0 d	
	24	100 ± 0.0 d	
Adults	2	0.0 ± 0.0 a	df = 20,
	3	16.7 ± 2.7 b	F = 167.10
	4	26.7 ± 2.7 b	P = < 0.0001
	6	56.7 ± 2.7 c	
	12	90.1 ± 4.7 d	
	18	100 ± 0.0 d	
	24	100 ± 0.0 d	

Different letters in each column and within life stage indicate significant differences between treatment times (a = 0.05, Tukey's HSD test).

Table 2.2 Empirical and theoretical CT values (ppm-min) required to achieve 50% mortality in different life stages of *Blattella germanica* at 480 ppm ozone.

Life stage	Empirical CT50	Theoretical CT50
Eggs	345,600 b	255,780 b (141,900-408,600)
Nymphs	144,000 a	144,180 a (118,440-164,940)
Adults	172,800 a	171,420 a (146,640-198,780)

Different letters for life stage indicate significant CT differences between life stages (a = 0.05, Tukey's HSD test).

Based on experimental results in Table 2.1, CT values for 100% mortality at 480 ppm in different life stages of *B. germanica* were calculated (Table 2.3). Differences were observed in the susceptibility of all life stages. Again, results showed that the most ozone-tolerant stage of *B. germanica* were the eggs, which required a treatment of 24 h at 480 ppm ozone to reach 100% mortality for a CT of 691200 ppm-min. The CT determined for adult and nymphs were 518400 ppm-min and 345600 ppm-min, respectively.

Table 2.3 CT values (ppm-min) required to achieve 100% mortality in different life stages of *Blattella germanica* at 480 ppm ozone.

Life stage	Empirical CT (ppm-min)
Eggs	691,200 c
Nymphs	345,600 a
Adults	518,400 b

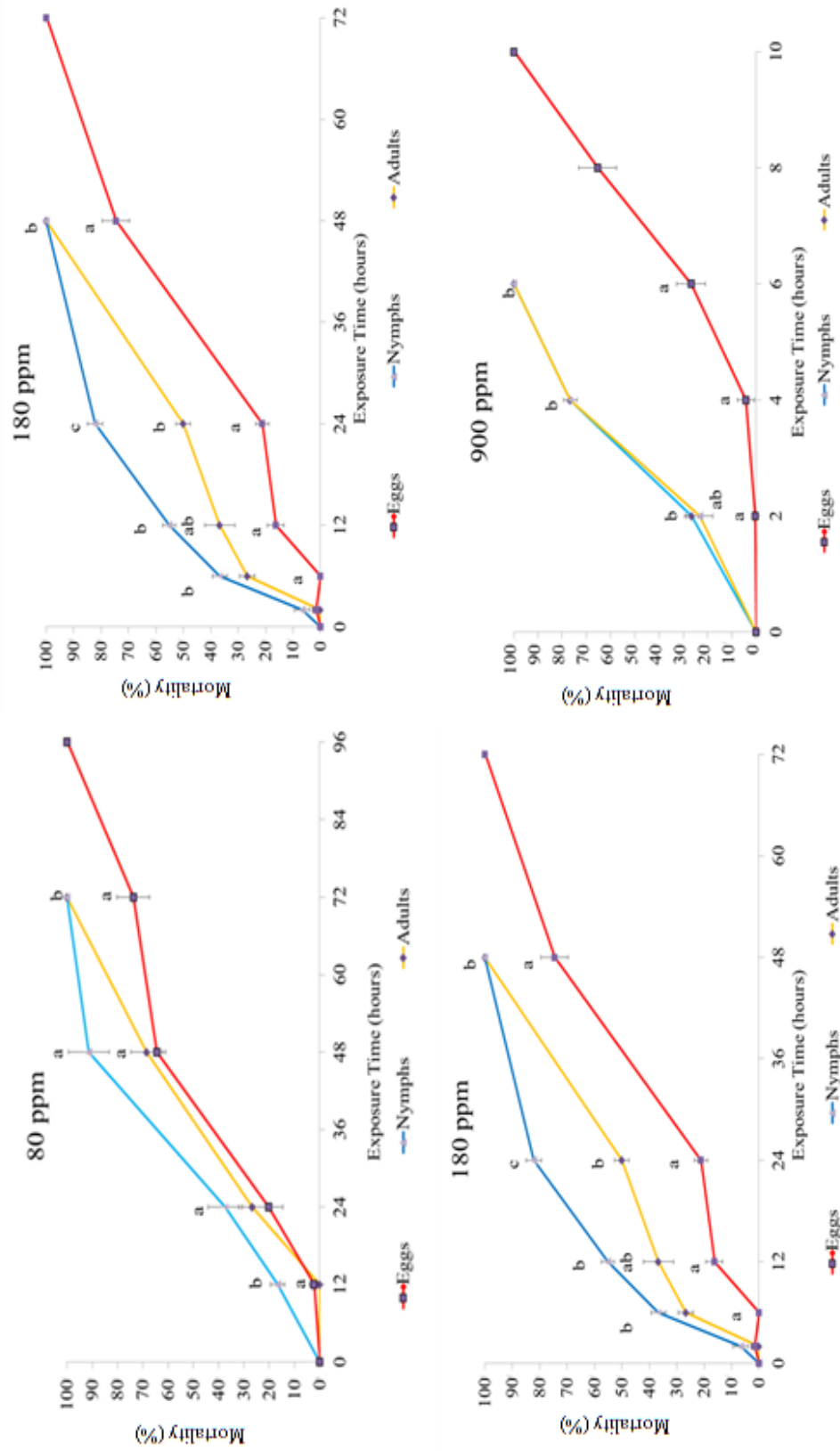Different letters for life stage indicate significant CT differences between life stages (a = 0.05, Tukey's HSD test).

Figure 2.1 Concentration-time curve for all life stages of Blattella germanica treated with ozone concentrations of 80 ppm, 180 ppm, 700 ppm, and 900 ppm. Different letters in each curve and within the same exposure time indicate significant mortality differences between life stages (a = 0.05, Tukey's HSD test)

2.4 Discussion

In this study, five ozone concentrations and a variety of treatment times needed to achieve 100% mortality in three *B.germanica* life stages were examined. In general, within each life stage, longer exposure times were required to attain 100% mortality with lower concentrations (Table 2.1 and Figure 2.1). Results also indicated a significant difference in susceptibility between life stages. *B. germanica* nymphs were more sensitive to the effects of ozone than adults and eggs. Ozone toxicity of *Ephestia kuehniella* (Zeller) and *Tribolium confusum* (Jacquelin du Val) has reported a similar trend wherein eggs were more tolerant to ozone than adults, larvae and pupae (Isikber and Öztekin, 2009). McDonough et al. (2011b) also reported that eggs were the most ozone-tolerant stage of *T. castaneum* (Herbst), which required a treatment of 180 min at 1800 ppm ozone to reach 100% mortality.

Although the actual mode of action of ozone in insects has not been elucidated so far, it is hypothesized that the insect respiratory system is the main target site (Tiwari et al., 2010). The increased tolerance of eggs to ozone might be due to lower respiration rates and underdeveloped respiratory system in this life stage (Hoback and Stanley, 2001; Yu, 2008). It is also possible that the outer layer on eggs provided an additional barrier to ozone (McDonough et al., 2011b). In our experiments, one week old egg cases carried by females were used and hence the females may have further protected the eggs within the egg cases from lethal effects of ozone.

From the CT curves shown in Figure 2.1, an inconsistent trend between the mortality of nymphs and adults was found. At 80, 180 and 700 ppm, the percent mortality

between nymphs and adults were significantly different at shorter exposure times. However, when exposure time was increased, the curves overlapped. Interestingly at the highest ozone concentration (900 ppm), the curves for nymphs and adult were almost similar and overlapped at all treatment times. One of the possible reasons for inconsistent mortality trend was that I tested mixed-age nymphs (3rd-5th instar). Previous research has shown that young cockroach nymphs (3rd instar) have a relatively higher respiratory rates than old nymphs (4th or 5th instar) (Appel and Liu, 2008). Higher respiratory rate could result in increased ozone uptake by small nymphs in comparison to large nymphs and thus higher susceptibility of smaller nymphs to ozone. This factor could have made smaller nymphs more susceptible to ozone, but older 5th instar nymphs likely had ozone tolerance similar to that of adults, thus contributing to observed variation in mortality trends.

As an alternative to traditional fumigants, successful application of ozone technology for insect control requires adequate ozone concentration and an appropriate treatment time (McDonough et al., 2011b). Busvine (1938) first developed a concentration X exposure time (CT) model for insect mortality calculations, which describe the relationship of fumigant concentrations (C) and exposure times (T) needed to obtain a desired mortality level. The model states that the CT value is constant for a specific mortality level. However, from the CT curves shown in Figure 2.1, an inconsistency was found. The CTs needed to reach 100% mortality in these experiments were inconsistent. For adults, a CT at 80 ppm was 345600 ppm-min, CT at 180 ppm was 518400 ppm-min, CTs for 700 ppm and 900 ppm were 336000 ppm-min and 324000 ppm-min, respectively. A similar trend was found for nymphs and eggs. These results

indicate that although the CT model is appropriate for considering cumulative ozone toxicity data for other insect species, it may not be suitable for the *B. germanica* data collected in this study. The mixed aged cohorts, as well as mixed gender cohorts of insects and eggs used in experiments, is probably the primary explanation for variation in CT values obtained at different ozone concentrations and exposure times. To account for this inconsistency, it will be essential to perform probit analysis on CT data obtained for each ozone concentration to determine the theoretical CT99 values. These theoretically CT values for different ozone concentrations can then be statistically compared to test if the CT method can still be used for reporting ozone toxicity in cockroaches.

CTs have been reported in some recently published ozone studies. McDonough et al. (2011), calculated the CT values essential for achieving 100% mortality in all life stages of *Plodia interpunctella* (Hübner), and *T. castaneum* and adult *Sitophilus oryzae* (L.) and *S.* zeamais (Motschulsky). *T. castaneum* adults required higher CTs than *P. interpunctella* adults (216000 ppm-min and 30000 ppm-min, respectively), *S. zeamais* adults required higher CT than *S. oryzae* (216000 ppm-min and 108000 ppm-min, respectively) (McDonough et al. 2011). Sousa et al. (2008) also conducted laboratory experiments with *T. castaneum* adults at 150 ppm ozone and achieved 95% mortality with an exposure time between 23.35 h and 31.98 h (CT95 of 210,150 ppm-min and 287,820 ppm-min, respectively). Kells et al. (2001) treated *T. castaneum* adults hidden between corn kernels with 50 ppm ozone for 3 d and observed 92% mortality with a CT value of 216,000 ppm-min, which also agreed with the results reported by McDonough et al. (2011a,b) and Sousa et al. (2008).

In the current study, the CT value required for obtaining 100% mortality of allthree life stages of *B. germanica* were calculated for 480 ppm ozone concentration. Eggs required a CT equal to 691200 ppm-min, and CT values determined for adult and nymphs were 518400 ppm-min and 345600 ppm-min, respectively. The results of our study indicated that adult *B. germanica* were two to three times more tolerant to ozone than *T. castaneum* and *P. interpunctella* adults. Based on previously published research, the *B. germanica* appears to be more ozone-tolerant than most of the stored product insects except *E. kuehniella* (Isikber and Athanassiou, 2014; Kells et al., 2001; Leesch et al., 2003; Mendez et al., 2003). For *E. kuehniella* adults, a much higher CT (777,840 ppm-min) was required to attain 100% mortality (Isikber and Öztekin 2009). From their results, *E. kuehniella* adults were more ozone-tolerant than *B. germanica* adults, whose CT was 518400 ppm-min.

Several factors are likely contributing to increased tolerance of *B. germanica* to ozone. First, the large body size of the *B. germanica* as compared to stored grain pests could be could be responsible for higher CTs required to attain 100% mortality. Second, differences in respiration rates between cockroaches and stored grain insects may affect the ozone concentration and exposure time required to obtain >95% mortality. And finally, differences in expression and activities of antioxidant enzymes in cockroaches and stored product pests could result in differential ozone tolerance between these species. It is also likely that some combination of all three factors is contributing to increased ozone-tolerance in the *B. germanica*. Future research is needed to determine the cause of ozone-toxicity differences in *B. germanica* and stored product pests.

Future ozone toxicity studies with the *B. germanica* could test the CT combinations reported here, under non-laboratory field conditions. Controlling *B. germanica* with ozone in a large open room would be more challenging than in an ozonation chamber, where insects were easily exposed to ozone. Additionally, *B. germanica* infestations present in a room are often found in difficult to reach areas such as underneath and deep within furniture, behind baseboards, and equipment, and inside wall cracks/voids. Achieving lethal ozone concentrations (>80 ppm) in such protected harborage areas for a prolonged period of time (48 to 72 h) would be a major challenge. It is likely that either very high ozone concentrations or long exposure times might be needed unless other conditions could favor faster mortality.

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CHAPTER 3 SUB-LETHAL EFFECTS OF OZONE EXPOSURE ON
REPRODUCTION AND POPULATION GROWTH OF THE GERMAN
COCKROACH (*BLATTELLA GERMANICA* (L.))

Abstract

German cockroaches, *Blattella germanica* (L.), are the most successful urban
pests infesting human dwellings. Previous laboratory studies have indicated that ozone is
an effective non-chemical alternative for control of insect pests. However, achieving
adequate *B. germanica* control in the field may not be feasible due to design efficiencies.
Thus, in this study, virgin adult males and females of *B. germanica* were exposed to a
sub-lethal ozone concentration-time (CT) combination (480 ppm for 6 h) and paired with
either treated or untreated males or females for mating and oviposition. A total of four
pairings were made, treated females x treated males, treated females x untreated males,
untreated females x treated males and untreated females x untreated males (control).
Further, the number of F1 nymphs hatching from the first egg case of each pairing and
the overall population growth rate were determined in separate experiments. In general,
crosses that included ozone-treated females produced significantly fewer F1 nymphs.
Exposure to a sub-lethal ozone environment both treated females paired with treated
males resulted in significant population growth reduction compared to the other three
pairings at 7, 11 and 15 weeks post exposure. No significant differences were found
between the control pairings and pairings in which only one of the sex was treated.

Key words: *Blattella germanica,* sub-lethal ozone, reproduction

3.1 Introduction

German cockroaches, *Blattella germanica* (L.), are the most successful urban pests infesting human dwellings (Benner, 1995). They are also found in restaurants, food processing facilties, hospitals, and any other indoor structures with food, water and harborage (Wang and Bennett, 2009). Their tendency to hide within small cracks and crevices and the ability to move through small openings makes them the extremely difficult to control. Additionally, their high reproductive rate can lead rapid population growth if left unchecked (Appel and Liu, 2008; Wang and Bennett, 2009). For example, hatching of a single egg case containing ~35 to 40 nymphs is enough to regenerate a nearly exterminated pest population within a few generations.

Blatella germanica management in the United States has primarily relied on the use of bait formulation of insecticides like abamectin, fipronil, and indoxacarb (Appel, 2003; Buczkowski et al., 2001; Buczkowski et al., 2008; Gondhalekar et al., 2011). However, some *B. germanica* may survive insecticide treatments due to issues like insecticide resistance and bait aversion. These survivors might exhibit sub-lethal effects that impact their longevity and fecundity (Cochran, 1985; Haynes, 1988; Moriarty, 1969). Lee et al. (1998) found that egg case production, egg case hatchability, and nymph emergence declined with increasing doses of deltamethrin and propoxur from LD_{10} to LD_{50}. Exposure to insecticides could also cause gravid *B. germanica* females to drop their egg cases prematurely, resulting in decreased nymph emergence rates (Abd-Elghafar et al., 1990; Hamilton et al., 1990; Harmon and Ross, 1987).

An environmentally friendly alternative to insecticides, ozone has been investigated to manage stored product insects within stored grain (Isikber and Öztekin, 2009; Kells et al., 2001; McDonough et al., 2011; Mendez et al., 2003). As a gas, ozone has the ability to fill large spaces and enter cracks and crevices where cockroaches hide. However, like insecticides, previous ozone exposure experiments have showed that satisfactory mortality in the target insect pest (>90%) requires longer buildup times, requiring treated grain to be in phase two condition for efficient treatment times (Kells et al., 2001; McDonough et al., 2011; Sousa et al., 2008). Sub-lethal ozone exposure may however affect reproduction and population growth rate of surviving insects.

To determine if exposure to sub-lethal levels of ozone influences the reproductive ability of the surviving *B. germanica* adults, the objective of this study were to compare offspring production and population growth rates in *B. germanica* adults exposed or unexposed (control) to sub-lethal ozone concentrations and treatment times. I hypothesized that sub-lethal ozone exposure would cause the surviving adult cockroaches to be reproductively less fit which would indirectly affect their population growth rate.

3.2 Materials and Methods

3.2.1 Insects

A laboratory strain of the *B. germanica*, Johnson Wax susceptible (JWax-S) was used in all bioassays. The JWax-S strain has been maintained in laboratory culture without insecticide exposure for >70 years. Rearing was conducted in plastic containers (37.2 cm × 21.7 cm × 18.1 cm) with corrugated cardboard harborages, lab diet (Harlan Teklad Rodent Diet #8604) and a water source. Rearing containers were held at 25°C ±

0.5°C, 60% rh and 12:12 h light: dark photoperiod. A thin barrier layer of petroleum jelly and mineral oil (2:3) was placed around the inner top portion of the rearing box to prevent escape.

3.2.2 Influence of sub-lethal ozone on F1 population numbers

B. germanica nymphs were sexed and held separately for adult emergence. Newly emerged adults (less than 7 d) were exposed to a sub-lethal concentration-time combination (480ppm for 6 h) of ozone. Surviving adults were sexed and assigned to one of three different treatments as follows:

Treatment 1: 10 treated females × 10 treated males; Treatment 2: 10 untreated females × 10 treated males and Treatment 3: 10 treated females × 10 untreated males. The fourth treatment (control) included 10 untreated females × 10 untreated males. The bioassay was replicated five times.

Paired insects (10 males and 10 females) from one of each of the the 4 treatment groups were placed in plastic containers (37.2 cm × 21.7 cm × 18.1 cm) with food and water to allow mating and egg case production. Containers were held in environmental chambers (25 °C ± 0.5 °C, 60% RH and 12:12 h light: dark photoperiod) for 7 d, after which, males were removed from the containers. After nymphs emerged from egg cases, all female adults were removed to prevent any additional oviposition. These containers were checked daily for newly emerged F_1 nymphs and as found, were removed and counted. This was done until nymphs no longer emerged. Total F_1 nymphs in all ten egg cases were counted and compared between the first three treatments and the control.

3.2.3 Influence of sub-lethal ozone exposure on population growth

B. germanica nymphs were sexed and held separately for adult emergence. Newly emerged adults (less than 7 d) were exposed to sub-lethal concentration-time combination (480ppm for 6 h) of ozone. Surviving adults were sexed and assigned to one of three different treatments as described in 3.2.2. Controls consisted of untreated females and males. The test was replicated five times. In contrast to the previous experiment (3.2.2), only 5 females and 5 adult males were used in these tests because the number of F1 progeny produced by 10 females and 10 males was too large. After treatment, containers were held in environmental chambers (25 °C ± 0.5 °C, 60% RH and 12:12 h light: dark photoperiod). The total number of insects in each treartment were counted at 7, 11, and 15 weeks post exposure.

3.2.4 Data analyses

Analysis of variance (ANOVA) was used to analyze the data. The differences between treatment and control group means were compared by using Tukey's honestly significant design (HSD) test ($\alpha < 0.05$).

3.3 Results

Treatments that included treated females paired with either treated males or untreated males produced significantly fewer F1 nymphs in their first egg case compared to the control treatment and pairings that included untreated females. (Table 3.1). At 7 weeks post exposure, the population growth was significantly less when both females and males were exposed to sub-lethal doses of ozone (Figure 3.1). Conversely, the highest

number of nymphs were produced when only one sex of the mating pair (male or female) was treated or neither sex was treated (controls). Although the pairing of treated females and untreated males produced significantly more nymphs than the pairings with both treated females and males, they were still significantly less than controls and the pairing with untreated females at week 7. Week 11 post exposure trends were similar to those at week 7. At all observation intervals, no significant differences were found between the controls and pairings with untreated females x treated males. At 15 weeks post exposure, when both males and females were exposed to sub-lethal ozone, the population size was significantly less than other three pairings..

Table 3.1. Adult progeny (F1) produced in the first egg case following exposure of adult *Blattella germanica* to 480 ppm ozone for 6 h (CT = 172800 ppm-min).

Treatments	Number ± SE
Both treated	199.8 ± 17.06 a
Females treated	194.6 ± 17.48 a
Males treated	321.6 ± 12.85 b
Controls (Neither treated)	357.8 ± 7.67 b

Different letters in each column and within indicate significant differences between treatments (a = 0.05, Tukey's HSD test). (ANOVA value: df = 19, F = 24.06, P = < 0.0001)
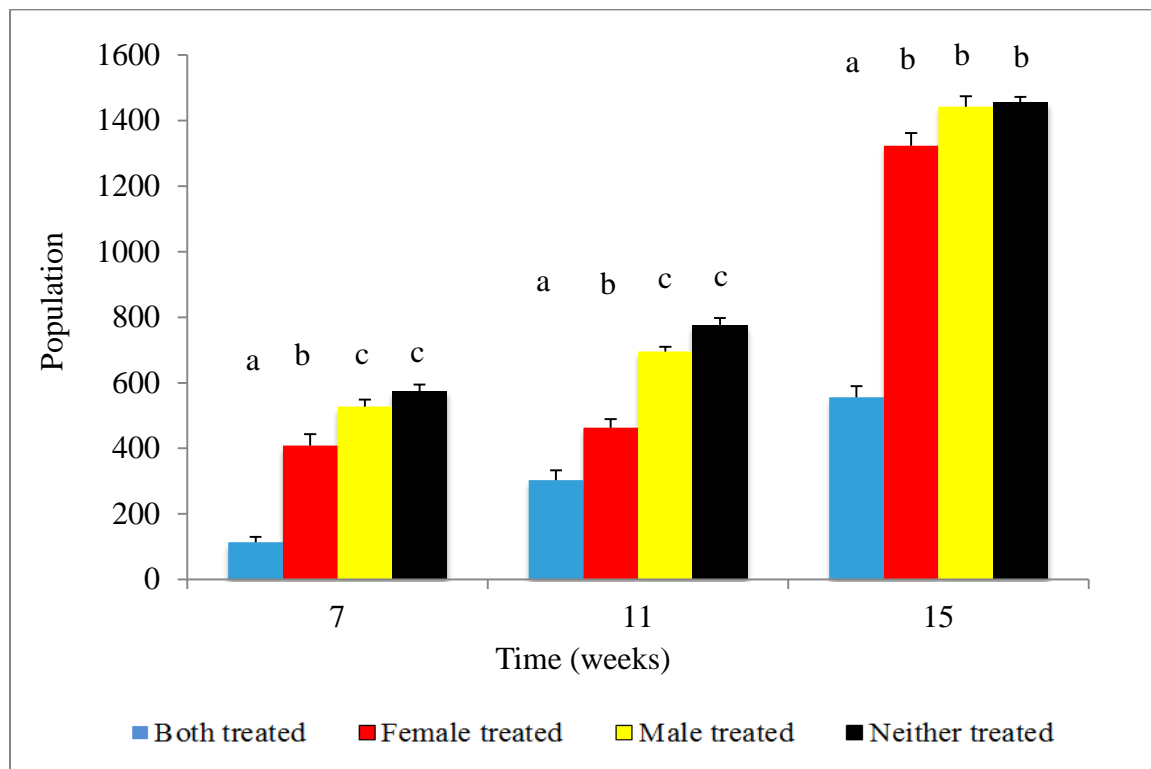
Figure 3.1 Influence of a sub-lethal ozone exposure (480 ppm for 6 h) on population growth of *Blattella germanica* at weeks 7, 11, 15 weeks post exposure. Virgin adult females and males were exposed to sub-lethal ozone concentrations. Different letters in each bar indicate significant differences between treatments (a = 0.05, Tukey's HSD test).

3.4 Discussion

Sub-lethal exposure of adult *B. germanica* to ozone can have a significant influence on progeny production. Females are more sensitive, as the number of F1 nymphs produced from the first egg case was significantly lower in the groups with treated females compared to groups which included untreated females (Table 3.1 and Figure 3.1). Female associated effects on F1 progeny numbers points toward sex-specific effects of sub-lethal ozone exposure on reproduction. This might suggest that physiological impacts of ozone exposure on females made them reproductively less fit.

Similar results have been reported in some sub-lethal insecticide exposure studies with *B. germanica*. One report showed that *B. germanica* females failed to produce egg case when fed with low concentrations of avermectin B_1 (Cochran, 1985). Another study showed that exposure to carbamate or organophosphate insecticides caused gravid females to drop their egg cases prematurely, resulting in decreased hatch (Harmon and Ross, 1987). Exposure to sub-lethal doses of chlorpyrifos-methyl at LD10, LD20 and LD60 significantly reduced the fecundity of *B. germanica* females compared to untreated females (Hamilton et al., 1990). Appel and Abd-Elghafar (1990) found that sulfluramid treatment increased egg case drop, decreased hatch rate (number of progeny) from the egg cases that were dropped or retained, and increased the time to egg hatch in *B. germanica*. They also reported that longevity and fecundity of adult *B. germanica* males and females were reduced with increasing sub-lethal doses of cyfluthrin and hydramethylnon (Abd-Elghafar and Appel, 1992).

Four possible reasons for the reduction of number of F1 nymphs after sub-lethal ozone exposure are: (1) Direct toxic effects of ozone on female *B. germanica* reproductive system. Previous research has reported that anesthesia affected the ability of the females insects to store sperm (Bloch Qazi et al., 1998). Muscular contractions necessary for sperm storage are likely impacted by anesthesia thus leading to loss in the ability to store sperm. (2) It is hypothesized that insect nervous system is likely affected due to ozone exposure and such disturbances in the nervous system might negatively impact reproductive physiology of adults. For example, as reproduction is hormone-regulated, neurohormone imbalances upon insecticide/ozone poisoning might affect normal reproductive function (Maddrell and Reynolds, 1972). (3) Also, disruption of

mating behavior could also have led to formation of unfertilized egg cases and resulted in

decreased fecundity in the treated insects. Deltamethrin-treated *B. germanica* females are

unreceptive to male courtship (Lee et al., 1998). It is possible that the mating behavior of

female cockroaches treated with sub-lethal ozone might also have been affected in a

similar way. (4) Declined movement and possibly metabolism might have affected

mating behavior and/or reproduction causing lower fecundity (Mitcham et al., 2006).

Ozone environments are hypoxic. Hypoxic conditions might suppress movement and

probably respiration in *B. germanica* adults. Limited movement of ozone treated females

may have limited the food consumption and thereby their nutritional status thus leading

to lower fecundity. Future work is needed to determine the exact mode of action of ozone

on *B. germanica* reproduction.

Seven and 11 weeks post exposure, it was observed that the pairing with treated

females and untreated males had significantly less population growth than the control

group and the reverse group (untreated females and treated males). However, at 15 weeks

post exposure, no significant differences were found between these three groups. The

effects of sub-lethal ozone exposure on progeny production were not evident beyond

week 11, which indicated that the treatment with sub-lethal ozone simply possibly

affected viability of the earlier egg cases or that the next generation is not affected. By

week 15 (~3.5 to 4 months) it is likely that the F1 progeny may have molted to adult

stage and were reproductively active. Thus, the lack of population growth differences at

week 15 suggests that sub-lethal ozone exposure did not affect long term reproductive

potential of the F1 progeny. The results presented in Table 3.1 and Figure 3.1, indicate

that although fewer progeny were produced in earlier egg cases, the same number or a

greater number of progeny might have been produced in the later cases. Populations of treated females and untreated males would grow slowly due to reduced number of progeny in the first egg case, but would also rebound with enough time. For the pairing with both sexes were treated, although the population was growing from seven weeks to 15 weeks post exposure, it was at a slower rate than the other three groups. One possible reason for this reduction in population growth compared with the only females treated group, is that ozone reduced both longevity and fecundity of this group. Adult lifespans were significantly shorter after exposure to chlorpyrifos-methyl (Hamilton, 1990). In this study, ozone may had similar effect on adults: female longevity and fecundity may have been reduced after exposure to sublethal doses of ozone. Compared with the female only treated group, in which the population rebounded at 15 weeks post exposure, the population growth in pairings where both sexes were treated group grew slower. Similar results have been found in ozone treated red flour beetle (*Tribolium castaneum* (Herbst))(McDonough, 2010). Although sub-lethal exposure reduce population growth up to 12 weeks post exposure, by 18 weeks post exposure, this effect was lost. Thus, the reproductive effects of sub-lethal ozone on the insects were not sustained past the F1 generation.

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