INSECTICIDE RESISTANCE OF *Alphitobius diaperinus* (COLEOPTERA: TENEBRIONIDAE) TO β-CYFLUTHRIN AND ASSOCIATED HEAT TOLERANCE

A Thesis

by

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ABSTRACT

The lesser mealworm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) is an important economic pest to poultry producers globally that cause structural damage and spread pathogens to poultry. Adult lesser mealworms were collected from three farms in Mt. Pleasant, TX, USA (A-C) and three farms in Franklin, TX, USA (D-F) in order to assess insecticide resistance across populations, as well its relationship to heat tolerance. Filter papers were treated with a range of doses of the active ingredient (AI) β-Cyfluthrin. Farms B and E displayed much higher LD_{50} of 0.320 mg/mL and 0.627 mg/mL respectively compared to the remaining four farms, which ranged from 0.048-0.161mg/mL. In addition, a field bioassay was conducted to determine adult beetle susceptibility to label rates of formulated permethrin, Vector Ban Plus™ and a pyrethroid, Tempo SC Ultra™. These insecticides were applied to commonly found surfaces in poultry operations (concrete, wood chip particle board, and pressure treated wood). Adult beetles were exposed to the treated surfaces for 2 h and then placed in untreated sterile petri dishes. "Mortality" refers to mortality and morbidity recorded together at 2, 24, and 48 h post-exposure for both bioassays. Insecticide resistance varied greatly based on observation period and compound. The range of mean mortalities measured at 2 h regardless of surface type for Tempo SC Ultra™ was 58-100% and for Vector Ban Plus™ 17-100%. The mean mortality range at 24 h regardless of surface for Tempo SC Ultra™ (91-100%) had less than 10% variation, while Vector Ban Plus™ (0.00-49.73%) displayed almost 50% variation. The mean mortality range at 48 h regardless of surface

for Tempo SC Ultra™ (72-100%) showed high knockdown and increased in variation by 30%, and Vector Ban Plus™ (0-29%) had a similar variation, but with low knockdown. Mortality was similar for Tempo SC Ultra™ for each substrate. β-Cyfluthrin (AI) had varying effectiveness depending on the population's resistance levels, however all the farms tested had LD_{50} well above the equivalent formulation dosage of 0.02mg/mL. The β-Cyfluthrin formulation had high mortality on all the surfaces tested highlighting the importance of the other ingredients in a formulation. Heat tolerance experiments were conducted on the F_1 progeny of populations B, D, and E (n=14). The heat shock results were inconclusive. Modification to the experimental design may be needed to yield comparable results.

DEDICATION

I dedicate this to my parents for always believing that I can do it.

ACKNOWLEDGMENTS

I would like to thank my committee members, Dr. Jeff Tomberlin, Dr. Pete Teel, Dr. Tawni Crippen, Dr. Sonja Swiger, and Dr. Craig Coufal, for their patience and guidance. I want to thank Dr. Jeff Tomberlin especially for seeing my potential and pushing me to become a better student and researcher. I also want to thank the members of FLIES lab for their help and friendship throughout the years.

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CHAPTER I

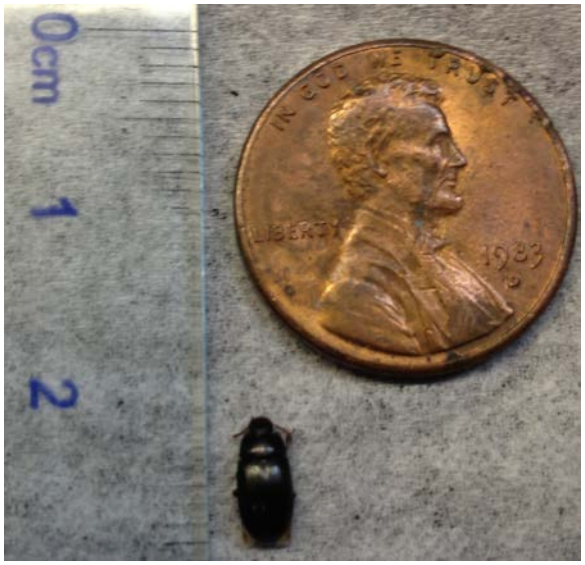
INTRODUCTION AND LITERATURE REVIEW

Introduction

Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae) (Fig. 1), commonly known as the darkling beetle or lesser mealworm, originated from Sub-Saharan Africa (Hogsette et al. 1994, Lambkin 2001). A common grain product pest (Green 1980), this beetle species likely dispersed aboard ships to Europe and then to North America. The beetle is now a cosmopolitan species and is a known poultry house pest in locations around the world, such as Denmark, United Kingdom, United States, and Australia (Green 1980, Lambkin 2005).

Sub-Saharan Africa is a tropical climate. Consequently, successful establishment of *A. diaperinus* in other temperate regions is partially due to artificial environments conducive to their development and survivorship. *A. diaperinus* develops optimally at temperature of ~25°C and 70% RH (Green 1980), which falls within the range typically found inside a broiler house (Lambkin 2001).

Figure 1. Adult *A. diaperinus*



The life cycle of *A. diaperinus* is heavily temperature dependent ranging from five-eight weeks (Rueda and Axtell 1996). All life stages can be found in the litter of the poultry houses (Axtell and Arends 1990). Over the course of 40-60 d larvae will pass through six-ten instar stages, although more stages are possible. Pupation occurs over 7-12 days usually in the soil, but late stage larvae also will go into the walls of the broiler house and pupate in the insulation. The physical damage to facilities by *A. diaperinus* is an important economic factor resulting in its label as a pest. Larvae damage the poultry facility as they burrow into the insulation to pupate (Axtell and Arends 1990). Larvae and adults have been observed moving from the floor up unpainted wood posts of poultry houses (Kaufman et al. 2008). The number of late instar larvae that will climb to pupate in the insulation is impacted primarily by the availability of soil as a pupation

site, and density of beetles in the litter (Geden and Axtell 1987). Given enough time, the damage to the insulation from pupation of multiple generations can increase energy costs by as much as 67% (Loftin 2011).

Alphitobius diaperinus can develop large populations within broiler facilities. A population of *A. diaperinus* has been recorded to be as dense as 1000 beetles per m^2 inside broiler houses (Arends 1997). Using the typical house size in Maryland, USA of 15-18 m wide by ~ 183m long (Rhodes), the total house population can be as large as ~ 2.7-3.6 million. Another estimate puts the population in a 120,000 cage laying or broiler house at ~19 million (Kaufman et al. 2005). Females are highly reproductive depositing 3.6-7.3 eggs/day at 25°C (Rueda and Axtell 1996) resulting in potentially 2000 eggs deposited during her lifespan, which is a year at most (Axtell and Arends 1990); however an adult usually deposits only 200-400 eggs (Dunford and Kaufman 2006).

Managing litter inside poultry operations can serve as a cultural method for managing *A. diaperinus* populations. Standard litter utilization practices by poultry producers usually include used litter being applied to fields. Unfortunately, in such instances, adult beetles begin dispersing from the litter approximately seven days after field application. However, flights have only been found to occur during the summer months when beetle densities are at their highest (Calibeo-Hayes et al. 2005). The spread of beetle populations between broiler houses on the same farm and nearby facilities can lead to several issues related to pathogen transmission and insecticide resistance.

Alphitobius diaperinus serve as a reservoir for various pathogens (Axtell and Arends 1990, Despins and Axtell 1994, Bates et al. 2004, Crippen et al. 2009). Birds

become contaminated due to consumption of beetles containing *Salmonella* (Despins and Axtell 1994, Roche et al. 2009). Other pathogens that can be transmitted to poultry due to consuming *A. diaperinus* include Leukosis, which causes Marek's Disease, *Escherichia*, *Streptococcus*, and fungi (Axtell and Arends 1990). *Alphitobius diaperinus* has been suggested as a reservoir for *Camplyobacter*, which is an important causal agent of human gastroenteritis (Bates et al. 2004).

Salmonella transmission from *A. diaperinus* to broilers is a widely known health concern for the industry. Roche et al. (2009) demonstrated the transmission of *Salmonella* from *A. diaperinus* to birds by lacing feed with marker *Salmonella* and exposing adult and larval beetles to the contaminated feed for 72 h. The larvae and adults were then fed to chicks. Resulting cecal samples were taken from the challenged birds and the pen mates for three and six weeks into the experiment. Roche et al. (2009) concluded that the larvae and adults were contaminating the birds with *Salmonella*, and it spread to the pen mates over the six-week period. The beetle proved to be an adequate reservoir for the marker *Salmonella*, and the spread between the high density of beetles and birds inside a poultry house was a concern (Roche et al. 2009). *Salmonella* at a density of 10^4 cfu/mL was sufficient to allow internalization in the gastrointestinal tract (GIT) of 4-wk-old beetles within 30 min (Crippen et al. 2009). At a rate of 10^3 cfu/mL, on average, 80% of beetles were found to be positive for *Salmonella* within their GIT. All beetles were positive for *Salmonella* within the GIT at a rate of 10^5 cfu/mL.

The presence of *A. diaperinus* in a broiler house can economically impact the production of a facility in other ways in addition to contamination with pathogens.

Chicks will consume beetles rather than feed resulting in distress from over consumption and reduced weight gain (Axtell and Arends 1990, Despins and Axtell 1995). If given feed in addition to the beetles in the litter, a much smaller number of beetles was consumed and weight gain appeared unaffected (Despins and Axtell 1995). Turkey poults fed larvae showed less weight gain than those not fed larvae at all (Despins and Axtell 1994).

Integrated Pest Management (IPM): The core concept of IPM strategy is to know and understand the biology of the specific pest that is to be controlled and then use this information to tailor a comprehensive program that is the most environmentally friendly to achieve control of the insect (EPA 2012). The program incorporates various components for each pest species: a method to monitor the population, a threshold population size at which action must be taken against the pest is established, a method to prevent the population from reaching the threshold size (cultural control), and a method to control the pest once the population threshold has been reached or exceeded. A poultry facility operator can use these concepts to control *A. diaperinus* populations.

Biological control for use against *A. diaperinus* has been suggested in the past. *Beauveria bassiana* (Moniliales: Moniliaceae), a fungus, showed effectiveness against *A. diaperinus* when treated on various surfaces inside the poultry house (Crawford et al. 1998, Geden et al. 1998). A possibility of using a protozoa was suggested as a control method, but variability inside the poultry houses could pose a problem for this method (Apuya et al. 1994).

Cultural control methods to prevent *A. diaperinus* from achieving a high population are mostly accomplished by periodically removing all the litter from the facilities periodically. During colder months when the birds are not present, the house is can be left unheated, which kills many of the beetles (Axtell and Arends 1990). The super-cooling temperature, or low temperature where all movement ceases, of *A. diaperinus* for males and females is -9.5°C and -9.2°C respectively (Salin et al. 1998). When beetles were placed inside a refrigerator at various temperatures for a month, reduced survivability was shown at 8°C and were all individuals were completely dead at 5°C. At a constant 0°C, half the population was dead at approximately six days (Lalouette et al. 2007). At 6°C half the population was dead in 9 d, and 99% in 21 d. At 10°C half the population succumbs in 49 d and 99% in 106 d (Renault et al. 1999). Those individuals exposed to the ambient temperature during cold winter days during cleanout periods would be dead if it reached -10°C quickly inside the house, but on the warmer winter days it may take a few days to kill those individuals. However, in a poultry facility the litter and soil underneath the litter would trap heat allowing some reprieve for the beetles until the birds return and heat is turned back on. Salin et al. (1998) took note of soil temperature being higher than the air temperature and that adults and larvae were in the 0-10 cm layer of soil.

A relatively limited number of pesticides are directed for use against *A. diaperinus*. Many compounds, such as permethrin, have been used for an extensive period of time and insecticide resistance has translated to low knockdown rates. Talstar WP™, Bifenthrin active ingredient (AI), showed wide variation in knockdown rates of

A. diaperinus across six farms in Titus Co. In addition most of these compounds are also only to be used during cleanout when the birds are not inside the houses (Tomberlin et al. 2008). Insecticide resistance and application restrictions hinder the effectiveness of solely using insecticides to control this pest.

To increase the efficacy of the treatments, novel approaches must be undertaken. A promising area of research is inducing heat shock as a method of controlling beetles in grain houses (Fields 1992, Fleurat-Lessard and Dupuis 2010). Enzyme imbalance at high temperatures is one of many concepts as to what exactly causes death in insects at their upper temperature threshold (Fields 1992). An effective heat control program at grain storage and collection points and/or inducing heat shock coupled with insecticide treatment at a poultry farm during cleanout would be key aspects of an *A. diaperinus* IPM strategy. One strategy by itself would have limited results, but using chemical treatment to kill and weaken individuals, followed shortly thereafter with heat treatments to kill the resistant individuals and those knocked down from the insecticide treatment.

Understanding the behavior of the insect is very important to developing an IPM strategy. Prasifka et al. (2008) demonstrated *Scarites quadriceps* Chaudior (Coleoptera: Carabidae) exhibit a period of hyperactivity after coming in contact with sub lethal levels of a pyrethroid. This period of hyperactivity can affect the efficacy of the pesticide application if the beetles are able to quickly avoid the application sites. Similar behavior may be a concern with *A. diaperinus* with regard to heat stress. If similar hyperactivity of resistant individuals is exhibited in regards to heat stress, then localized applications of heat may be less successful. The goal of this research is to: 1) conduct a limited

evaluation of insecticide resistance to β-Cyfluthrin in poultry houses in Texas, USA, and 2) investigate a possible relationship between insecticide resistance and heat tolerance. This experiment will hopefully lead to an understanding of behavior of the insect as they undergo the biological stress as they approach their upper temperature tolerances. Using this information, perhaps an IPM strategy can be adapted to better control the lesser mealworm beetle and lessen the burden on poultry producers and increase animal safety.

Objectives and Hypothesis

H0: There will not be a difference in insecticide resistance of *A. diaperinus* collected from different poultry facilities within a county.

H1: There will be a difference in insecticide resistance of *A. diaperinus* collected from different poultry facilities located within a county.

Rationale: Resistant ratios between adults to β-Cyfluthrin from differing locations along the eastern USA vary from 1.7-9.5 times more than a susceptible colony (Hamm et al. 2006). Another study showed β-Cyfluthrin LC_{99} varied from 80-100% survival across seven counties in New York, USA (Scott et al. 2000). Tomberlin et al. (2008) conducted a survey of insecticide resistance across 6 poultry farms in Titus County, TX, USA. Tempo SC Ultra SC Ultra™, with β-Cyfluthrin AI, showed knockdown rates of individuals for each of the six farms 4 h after exposure to range from 46-100%. Farms A, B, and D were statistically similar in the knockdown rates, and the other three farms were statistically related to each other. Farms A, B, D and C, E, and F were statistically

different. Based on this research, statistical variation is expected between farms located within a county.

H0: There will be no difference of insecticide effectiveness when applied onto different surfaces commonly found inside poultry houses.
H1: There will be a difference in insecticide effectiveness when applied onto various surfaces found inside a poultry house.

Rationale: The time an insect is on a surface can be a short duration, therefore insecticides used as a surface treatment should be able to quickly knockdown that insect (Arthur 1998). This keeps the insect in contact with the insecticide for a longer time increasing mortality. Therefore, producers must carefully research the chemicals they use and how to apply them. This is important because prolonged exposure to a treated surface due to the slow efficacy of the chemical or the individual's resistance to the chemical leads to increased resistance of the population if the individual is allowed to survive (Barson 1991). Most studies use technical grade chemicals on filter paper to measure efficacy of an insecticide (Fletcher and Axtell 1993). While Fletcher and Axtell (1993) acknowledge this is an important step, they showed that how the pesticides were formulated and on what surface they were applied greatly affected the mortality outcome.

H0: There will be no difference in heat tolerance between susceptible and resistant *A. diaperinus* populations.

H1: There will be a difference in heat tolerance between susceptible and resistant *A. diaperinus* populations.

Rationale: The biological effects of resistance have been postulated to be a result of changing the sodium channel function properties, therefore reducing the functionality of the pyrethroid insecticide (Ffrench-Constant et al. 1998, Soderlund and Knipple 2003). Also, dichlorodiphenyltrichloroethane (DDT) and pyrethroid resistance has been correlated to temperature sensitivity (Ffrench-Constant et al. 1998). This heat sensitivity can have a noticeable impact on the survivability of the individual in the wild. A delay in recovery from heat stress by Cyclodiene resistant *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) was found when compared to non-resistant strains (Ffrench-Constant et al. 1993). Based on these studies a significant difference between susceptible and resistant individuals with regards to heat tolerance.

CHAPTER II

INSECTICIDE RESISTANCE

Introduction

An IPM plan has three main components with the goal to keep the population density below a threshold of damage costs while maximizing production (Axtell 1981). These components are biological, chemical, and cultural control. Monitoring of the populations inside broiler houses in an important tool to design your program. Monitoring should include damage assessments, knowing where the colonies are living, and resistance levels. With chemical control such an important component especially to the control of *A. diaperinus* resistance level monitoring is key. Resistance monitoring can be done through bioassays with AI or formulations.

Pesticide application is limited in the field to every 6-8 wk when the birds are removed from the houses (Tomberlin et al. 2008). Pesticide use is also hindered by the limited amount of chemicals available for use against *A. diaperinus.* Some chemicals that have been on the market for longer periods of time have experienced overuse resulting in low knockdown and mortality.

This study will be comparing the efficacy of β-Cyfluthrin AI and a formulation as its active ingredient. Permethrin shares the same mode of action as β-Cyfluthrin as it is in the same insecticide class. β-Cyfluthrin is a α- cyano pyrethroid insecticide. It is a Type II pyrethroid with regard to Na^{+}, Cl^{-}, Ca^{2+} channels and Functional Observatory

Battery (FOB) results (Breckenridge et al. 2009). The FOB results were taken from a previous study in which several parameters were studied in rats to determine the type of toxicity by different chemicals (Breckenridge et al. 2009, Weiner et al. 2009). Specifically this pesticide class targets the sodium channel of the insect. The knockdown resistance (*kdr)* causes a decreased sensitivity of the insect's nervous system to the pyrethroid (Soderlund and Knipple 2003). *Kdr* is a mutation that occurs in the subunits of the sodium channel of the insect.

Materials and Methods

Adult beetles were collected from three farms in Mt. Pleasant, TX, USA (A, B, and C) and three farms near Franklin, TX, USA (D, E, and F). Three houses on each farm were randomly chosen for collection of about 3000 beetles each. Beetles were randomly collected along the edges and nearest feeder lines. Beetles were scooped up with a container and sifted with a kitchen colander. At cracks and crevices, a pen cap or stiff paper was used to remove beetles into a container. All individuals on a farm were homogenized into one container to represent the whole farm (Fig 2.)

Figure 2. Buckets used to transport adult *A. diaperinus* beetles from farm to laboratory. Each bucket contains beetles from three houses from one farm.

Active Ingredient Bioassay: (Fig. 3). Methods were adapted from Tomberlin et al. (2002) and Sheppard and Hinkle (1987) for the preparation of filter papers with insecticide. The label rate of an insecticide was used as a basis for the middle of the concentration curve. Each 9 cm diameter filter paper (Fisherbrand, Loughborough, UK) was inoculated with 1 mL of the proper concentration of insecticidal compound diluted in acetone. The control for each replication was 1 mL acetone. For the compounds three replicates of 10 or 19 concentrations were tested on adult beetles collected from each farm.

Figure 3. Active Ingredient (AI) Bioassay- petri dishes for each Concentration spread on top of aluminum foil for each farm

Preliminary Bioassay: Methods were adapted from Tomberlin et al. (2002) for the bioassay. Under laboratory conditions for the broad range assay, nine concentrations and a control were used to determine the LD_{50}, or lethal dose at which half the population is dead, rates for each beetle population. Thirty adult beetles were placed on

filter paper for ~2 h, transferred to a clean 9 cm diameter petri dish and mortality recorded at 24 h. Mortality assessment was reused from Lambkin (2005) in which those deemed alive can "walk straight in a forward motion using all 6 legs with no jerky movements" and those that do not meet this criteria will be deemed dead. Results from these trials were used to develop a narrower range of doses to test on the beetle populations.

Bioassay: Under laboratory conditions, and based on the preliminary data, 18 concentrations and a control were used to achieve a more sensitive assay. Thirty adult beetles were placed on filter paper for ~ 2 h then transferred to a clean petri dish. Mortality, as previously defined, was recorded at 2, 24, and 48 h post exposure.

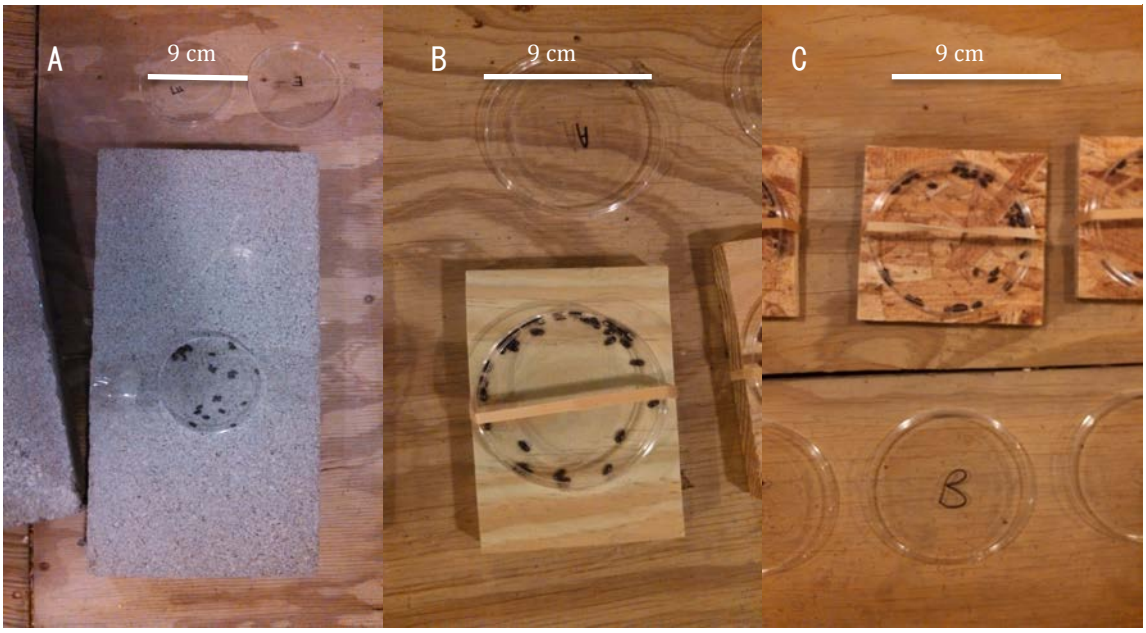
Surface Bioassay: Tempo SC Ultra™ (β-Cyfluthrin) and Vector Ban Plus™ (Permethrin) were purchased from a local feed store in College Station, TX, USA. These insecticides were selected, as they are commercially available for use by poultry producers. The surfaces chosen are commonly found inside poultry houses (Fig. 4); 1) concrete, 2) pressure treated wood, and 3) wood chip particle board.

The surfaces were treated inside an empty poultry barn at Texas A&M University Poultry Center to protect from ultraviolet light degradation of the chemicals. The temperature was kept at 21°C. Following protocols by Kaufman et al. (2008), the surfaces were treated with the low dose label rate for *A. diaperinus* control. The low dose label rate used for Tempo SC Ultra™ is 8.0 mL/3.7 L of water. The low dose label rate used for Vector Ban Plus™ is 0.095 L/ 3.7 L water. After treatment the surfaces were allowed to dry for 1 h at room temperature prior to conducting the assay. The control was tap water. A chemical sprayer (B&G Equipment Company, Jackson, GA) was used with a fan spray pattern that distributes 100 mL of chemical in 10 s.

Thirty adult beetles were placed in clean 9.0 cm diameter petri dishes which were attached upside down to the surfaces tested by rubber bands for the particle board and pressure treated wood, and by packing tape for the concrete blocks. After 2 h, the beetles were transferred to clean petri dishes and mortality recorded at 2, 24, and 48 h.

Analysis: The data from the filter paper assay and the surface treatments was analyzed using JMP Pro 10.0 software (SAS Institute Inc., Cary, North Carolina, USA) for ANOVA. Least significant difference (LSD) was used to separate means ($p \leq 0.05$).

Figure 4. Concrete block (A), pressure treated wood (B), and wood chip particleboard (C)



Results

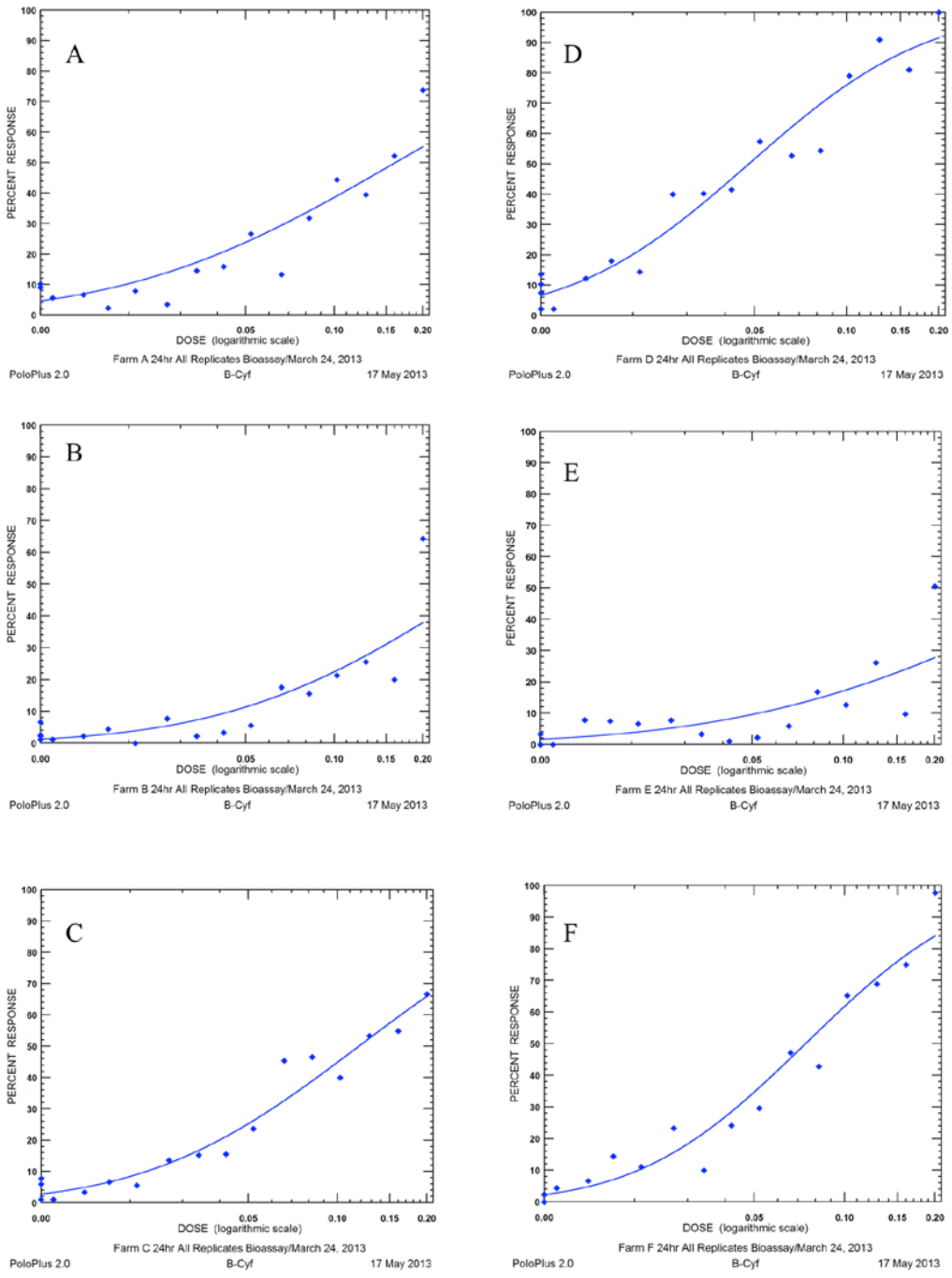
β-Cyfluthrin Bioassay: This study used the Lambkin (2005) definition of mortality. Lambkin's study was only concerned with mortality at 72 h, but our study recorded mortality with his definition at 2, 24, and 48 h after contact with AI or formulation. Since there is a time component and the definition includes mortality and morbidity, our measurement is better described as knockdown. However, the use of mortality and lethal dose will be used throughout our results and discussion rather than knockdown. This study will highlight the sometimes-misleading reference to mortality in other studies by comparing "mortality" between various times.

The LD_{50} for each population is presented in Table 1. Individual probit farm responses are in Fig. 5. A 50% mortality response was only achieved for adult beetles sampled from farms D, F, and C. Farm C had ~60% response and farms D and F ~90%. Farm B and E had a significantly higher LD_{50} when compared to the other farms tested in the filter paper bioassay. The LD_{50} of farm B was 2 fold higher than the third highest LD_{50} of 0.161mg/mL recorded from farm A. Another 2-fold increase in LD_{50} from farm B was recorded for farm E, which was the highest at 0.627mg/mL. The lowest LD_{50} was 0.048mg/mL from Farm D and subsequently was the most susceptible population.

Table 1. 24 h LD_{50} of *A. diaperinus* from six farms in Mt. Pleasant, TX (A-C) and Franklin, TX (D-F) with 95% Confidence Interval.

Farm	LD_{50} (mg/mL)	95% Confidence Interval (mg/mL)
A	0.161	0.114-0.271
B	0.320	0.193-0.838
C	0.118	0.099-0.146
D	0.048	0.041-0.056
E	0.627	0.276-4.777
F	0.074	0.064-0.870

Figure 5. Observed (closed circles) and expected mortality (line) probit of beetles from farm (A-F) exposed to β-Cyfluthrin



A high heterogeneity score was obtained when a probit analysis was conducted on dosage data from all 6 farms. Whereas the score should be 1 or below, the data in this study returned scores 3 to 4 fold higher. This prevents the use of an F test or a Fischer test on the data; however, LD_{50} was still available to compare resistance across most farms (Table 2).

Table 2. 24 h LD_{50} of *A. diaperinus* from six farms in Mt. Pleasant, TX (A-C) and Franklin, TX (D-F) with 95% Confidence Interval with doses removed to keep Probit heterogeneity score < 1.00.

Farm	LD_{50} (mg/mL)	95% Confidence Interval (mg/mL)
A	0.170b	0.134-0.238
B	0.434c	0.278-0.870
C	0.120b	0.107-0.103
D	0.050a	0.044-0.056
E	0.475c	0.260-1.373
F	0.075b	0.067-0.840

*Different under case letters indicate statistical significance (LSD, $p \leq 0.05$).

The probits for each farm with the responses removed to keep heterogeneity ≥ 1 is in Fig. 6. On farms B and E a 0.20 mg/mL dose achieved a 50% response but the output curve best-fit line does not go above 40% and 30% respectively. LD_{50} was still estimated on the output from Polo Plus 2.0 (LeOra Software Co, Petaluma, CA). On a toxicology study on earthworms researchers also achieved Chi squared numbers that were too high, but were able to use the LD_{50} to compare their insecticide's efficacy

(Mostert et al. 2002). The Polo Plus output of the averaged response data was chosen to compare the expected response with the actual. Those that appeared to have a significant difference were removed from the subsequent Polo input and then the probit analysis was rerun. Points were removed one at a time until the heterogeneity score was 1 or less. The probit curve of this corrected data is shown in Fig. 6. An F test or Fischer test could then be run with this data.

Individual farm results by treatment are given in Tables 3-5. Beetle populations from farms A, B, D, and E showed an increase in mortality rate at either 24-48 h except for beetles from farm B which had ~100% mortality indicating the population is highly susceptible. Beetles from farm F showed a near 400% increase in mortality at 0.027mg/mL from the 2-24 h observation before decreasing ~100% at 48 h. All other dosages across the farms showed an increasing rate of beetle recovery between the 24 and 48 h time points. Fig. 7 displays the percent recovery across all 19 treatments between 2-24 h and 24-48 h.

Figure 6. Observed (closed circles) and expected mortality (line) probit of beetles from farm (A-F) exposed to β-Cyfluthrin, with outliers removed to reduce heterogeneity

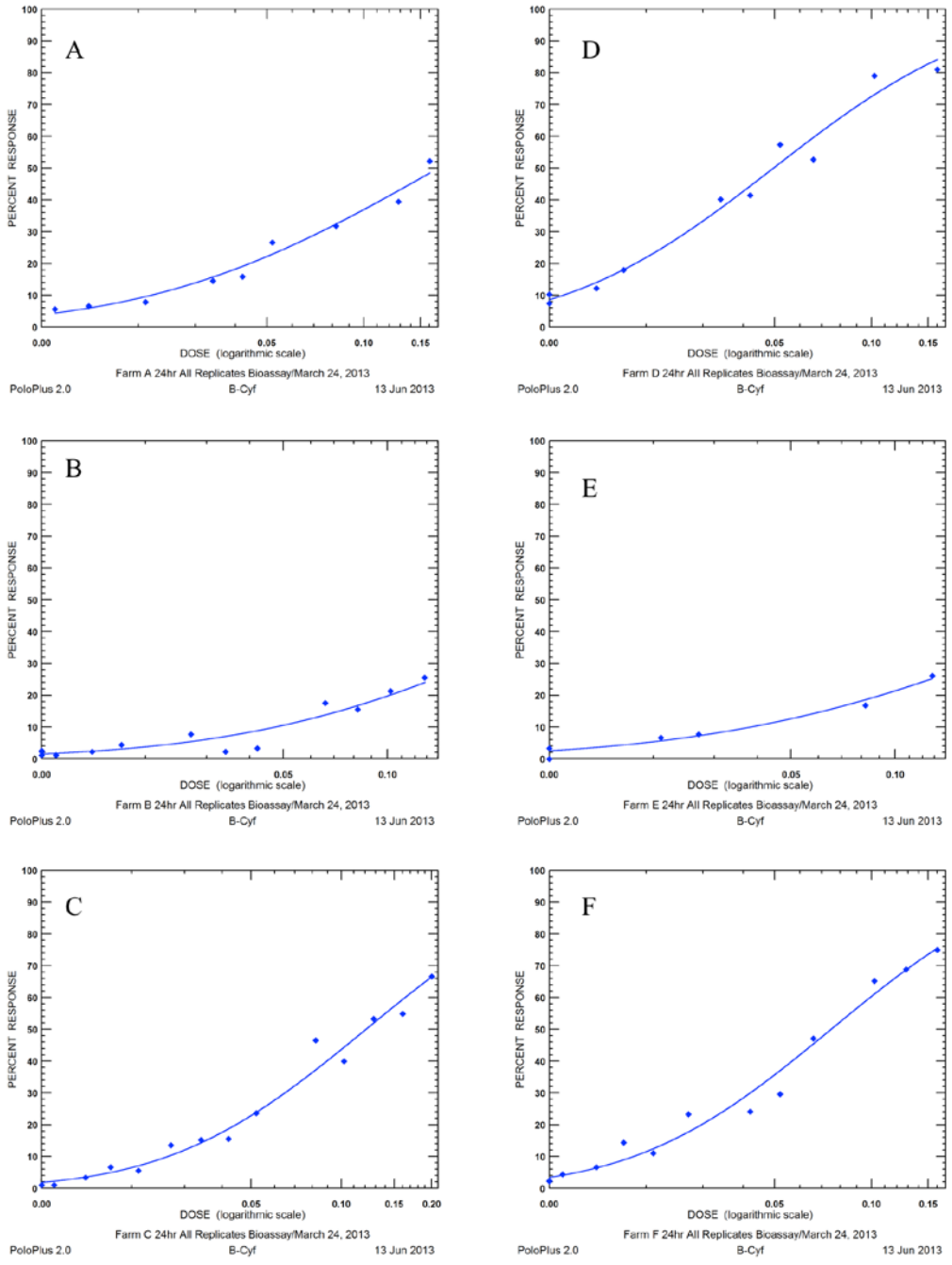


Table 3. Mean percent mortality ± SD for adult *Alphitobius diaperinus* beetles collected from six farms in Texas, USA 2 h after exposure to filter papers treated with β-Cyfluthrin at different doses under laboratory conditions (27°C).

	Dose mg/mL								
Farm	Control	0.005	0.006	0.007	0.009	0.011	0.014	0.017	0.021
A	0.00 ± 0.00	21.92 ± 2.23	11.26 ± 3.99	6.67 ± 11.55	21.98 ± 18.01	16.90 ± 6.31	8.89 ± 6.94	15.33 ± 10.76	25.08 ± 4.50
B	0.00 ± 0.00	6.67 ± 3.33	6.67 ± 3.33	5.68 ± 6.88	11.11 ± 5.09	16.11 ± 7.88	12.22 ± 3.85	11.11 ± 3.85	14.04 ± 5.84
C	0.00 ± 0.00	16.67 ± 8.82	15.92 ± 5.14	7.78 ± 8.39	12.22 ± 10.72	13.45 ± 3.16	19.52 ± 8.01	19.10 ± 5.14	12.22 ± 3.85
D	0.00 ± 0.00	26.51 ± 14.26	12.92 ± 8.36	11.11 ± 10.18	15.06 ± 4.48	14.37 ± 7.06	27.78 ± 11.71	31.49 ± 4.22	32.14 ± 11.15
E	2.22 ± 3.85	7.71 ± 1.99	6.70 ± 3.28	5.68 ± 6.88	1.11 ± 1.92	5.52 ± 1.99	12.45 ± 7.33	2.39 ± 2.09	8.89 ± 3.85
F	0.00 ± 0.00	21.11 ± 50.9	17.43 ± 9.40	4.44 ± 1.92	10.00 ± 3.33	8.89 ± 5.09	13.33 ± 0.00	26.67 ± 23.33	27.78 ± 21.69

Farm	0.027	0.034	0.042	0.052	0.066	0.082	0.102	0.128	0.16	0.20
A	21.05 ± 4.97	34.75 ± 6.42	27.16 ± 8.41	38.89 ± 5.09	25.56 ± 9.62	48.93 ± 4.84	64.44 ± 10.18	84.70 ± 4.76	88.89 ± 6.94	96.67 ± 5.77
B	15.56 ± 3.85	13.45 ± 3.16	11.11 ± 11.71	18.01 ± 10.27	36.24 ± 5.44	34.44 ± 7.70	34.64 ± 15.43	58.81 ± 24.38	65.56 ± 11.71	60.12 ± 29.48
C	20.78 ± 14.76	32.71 ± 6.38	34.44 ± 8.39	33.25 ± 1.07	39.68 ± 9.01	63.49 ± 14.42	60.00 ± 11.55	73.33 ± 24.04	88.96 ± 7.76	91.11 ± 8.39
D	31.11 ± 24.57	53.58 ± 18.06	67.32 ± 15.65	63.40 ± 29.18	73.41 ± 34.70	87.78 ± 10.72	97.78 ± 3.85	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
E	8.89 ± 5.09	13.43 ± 3.01	18.89 ± 10.18	26.67 ± 6.67	20.24 ± 6.57	33.37 ± 7.32	46.91 ± 20.70	62.46 ± 26.71	59.72 ± 35.08	94.44 ± 9.62
F	4.44 ± 5.09	8.89 ± 3.85	32.47 ± 7.35	37.62 ± 9.29	61.83 ± 18.27	78.21 ± 17.74	91.90 ± 7.33	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

Table 4. Mean percent mortality ± SD for adult *Alphitobius diaperinus* beetles collected from six farms in Texas, USA 24 h after exposure to filter papers treated with β-Cyfluthrin at different doses under laboratory conditions (27°C).

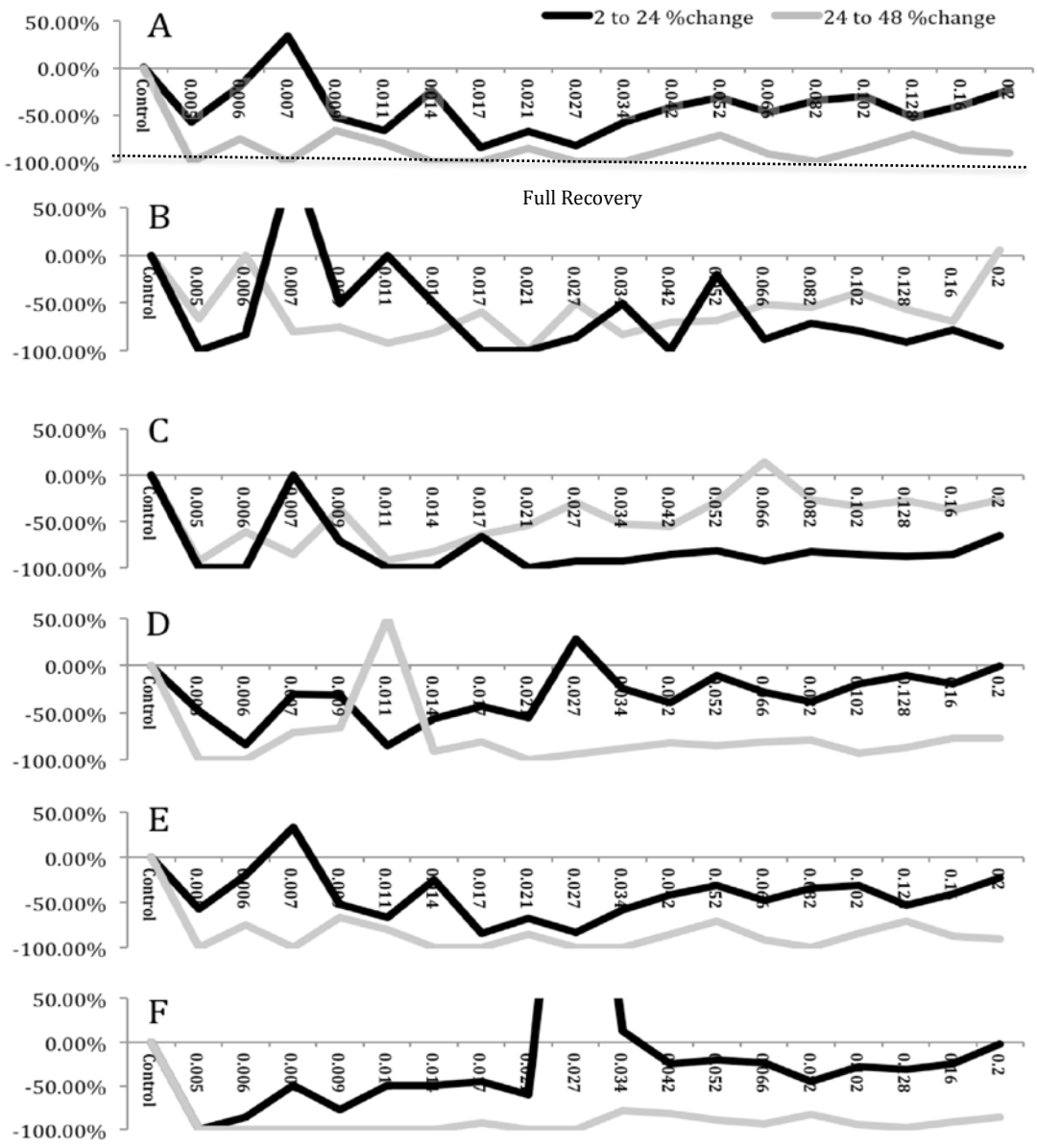
	Dose mg/mL									
Farm		Control	0.005	0.006	0.007	0.009	0.011	0.014	0.017	0.021
A		0.00 ± 0.00	9.09 ± 3.34	8.89 ± 8.39	9.00 ± 2.03	10.24 ± 0.41	5.71 ± 2.08	6.67 ± 0.00	2.26 ± 1.96	8.02 ± 2.34
B		0.00 ± 0.00	2.22 ± 1.92	6.67 ± 6.67	1.11 ± 1.92	2.22 ± 1.92	1.39 ± 2.41	2.22 ± 3.85	4.44 ± 1.92	0.00 ± 0.00
C		0.00 ± 0.00	1.11 ± 1.92	5.97 ± 1.88	1.11 ± 1.92	7.78 ± 8.39	1.15 ± 1.99	3.33 ± 3.33	6.54 ± 2.90	5.56 ± 5.09
D		0.00 ± 0.00	13.89 ± 10.84	2.15 ± 1.87	7.47 ± 3.62	10.25 ± 2.97	2.19 ± 1.89	12.22 ± 5.09	17.97 ± 5.04	14.57 ± 5.61
E		0.00 ± 0.00	0.00 ± 0.00	3.41 ± 3.45	0.00 ± 0.00	3.33 ± 3.33	0.00 ± 0.00	7.93 ± 5.34	7.63 ± 6.73	6.67 ± 5.77
F		0.00 ± 0.00	0.00 ± 0.00	2.44 ± 2.14	2.22 ± 1.92	2.22 ± 1.92	4.44 ± 1.92	6.67 ± 6.67	14.44 ± 5.09	11.11 ± 5.09
Farm	0.027	0.034	0.042	0.052	0.066	0.082	0.102	0.128	0.16	0.20
A	3.68 ± 3.86	14.60 ± 1.81	15.82 ± 8.31	26.67 ± 5.77	13.33 ± 5.77	31.76 ± 4.58	44.44 ± 25.02	39.66 ± 5.50	52.22 ± 16.78	73.73 ± 23.88
B	7.78 ± 1.92	2.30 ± 3.98	3.30 ± 3.33	5.59 ± 3.82	17.56 ± 1.55	15.56 ± 3.85	21.34 ± 8.35	25.72 ± 7.84	20.00 ± 6.67	63.58 ± 29.34
C	13.94 ± 3.49	15.28 ± 4.11	15.56 ± 12.62	23.57 ± 3.11	45.56 ± 3.85	46.51 ± 3.57	40.00 ± 17.64	53.33 ± 17.32	54.66 ± 23.05	66.67 ± 20.28
D	40.00 ± 23.33	40.25 ± 3.36	41.50 ± 5.01	57.71 ± 17.64	52.76 ± 10.05	54.44 ± 5.09	79.10 ± 2.14	90.18 ± 8.62	81.11 ± 21.17	100.00 ± 0.00
E	7.78 ± 3.85	3.46 ± 3.58	1.11 ± 1.92	2.22 ± 1.92	6.24 ± 8.09	16.23 ± 14.04	12.59 ± 1.28	26.19 ± 2.65	9.86 ± 5.90	50.62 ± 3.50
F	23.33 ± 12.02	10.00 ± 6.67	23.95 ± 4.70	29.33 ± 13.63	47.08 ± 4.45	42.94 ± 11.90	65.71 ± 8.14	68.89 ± 10.72	75.02 ± 20.74	97.78 ± 3.85

Table 5. Mean percent mortality ± SD for adult *Alphitobius diaperinus* beetles collected from six farms in Texas, USA 48 h after exposure to filter papers treated with β-Cyfluthrin at different doses under laboratory conditions (27°C).

	Dose mg/mL								
Farm	Control	0.005	0.006	0.007	0.009	0.011	0.014	0.017	0.021
A	0.00 ± 0.00	0.00 ± 0.00	2.22 ± 3.85	0.00 ± 0.00	3.41 ± 3.34	1.19 ± 2.06	0.00 ± 0.00	0.00 ± 0.00	1.11 ± 1.92
B	0.00 ± 0.00	0.00 ± 0.00	1.11 ± 1.92	2.22 ± 3.85	1.85 ± 3.21	1.11 ± 1.92	1.11 ± 1.92	0.00 ± 0.00	1.11 ± 1.92
C	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.11 ± 1.92	2.22 ± 1.92	0.00 ± 0.00	0.00 ± 0.00	2.15 ± 1.87	0.00 ± 0.00
D	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.22 ± 1.92	3.33 ± 3.33	3.33 ± 5.77	1.11 ± 1.92	3.41 ± 3.45	0.00 ± 0.00
E	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.39 ± 2.09	2.22 ± 3.85
F	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.11 ± 0.02	0.00 ± 0.00

Farm	0.027	0.034	0.042	0.052	0.066	0.082	0.102	0.128	0.16	0.20
A	0.00 ± 0.00	0.00 ± 0.00	2.26 ± 1.96	7.78 ± 1.92	1.11 ± 1.92	0.00 ± 0.00	6.67 ± 5.77	11.62 ± 5.00	6.67 ± 3.33	6.75 ± 5.71
B	1.11 ± 1.92	1.11 ± 1.92	0.00 ± 0.00	4.44 ± 5.09	2.19 ± 1.89	4.44 ± 5.09	4.48 ± 1.89	2.19 ± 1.89	4.44 ± 5.09	3.33 ± 3.33
C	1.11 ± 1.92	1.11 ± 1.92	2.22 ± 3.85	4.44 ± 7.70	3.33 ± 3.33	7.78 ± 8.39	5.56 ± 6.94	6.67 ± 3.33	7.63 ± 4.86	23.33 ± 8.82
D	2.22 ± 3.85	4.69 ± 2.35	7.39 ± 3.56	8.40 ± 6.42	9.93 ± 3.44	11.11 ± 3.85	5.45 ± 3.66	11.21 ± 1.51	18.89 ± 13.47	22.87 ± 3.66
E	0.00 ± 0.00	0.00 ± 0.00	2.22 ± 3.85	1.11 ± 1.92	0.00 ± 0.00	0.00 ± 0.00	3.33 ± 3.33	5.63 ± 3.78	2.15 ± 1.87	0.00 ± 0.00
F	0.00 ± 0.00	2.22 ± 0.02	4.57 ± 0.05	3.45 ± 0.06	3.41 ± 0.03	7.74 ± 0.05	4.29 ± 0.02	2.22 ± 0.04	6.86 ± 0.06	14.44 ± 0.07

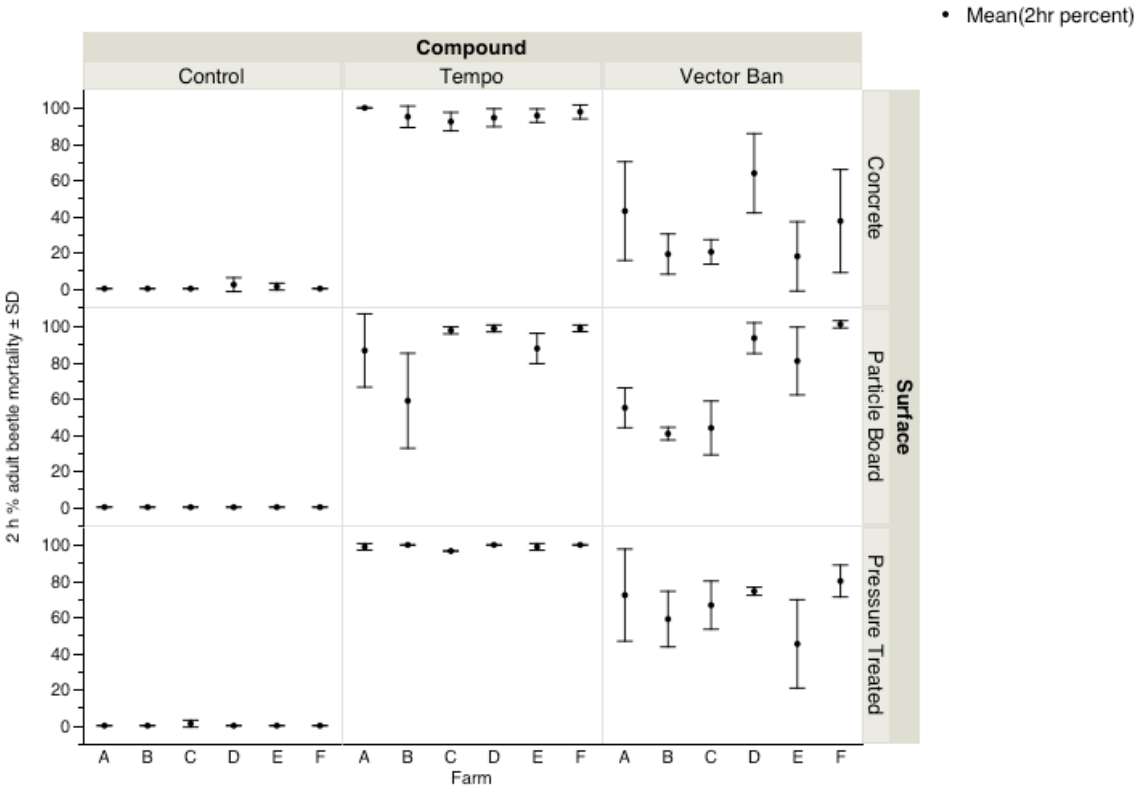
Figure 7. Percent change in mean mortality from 2 to 24 h (black line) and 24 to 48 h (gray line) for adult beetles exposed to filter papers treated at varying doses (x-axis)



In the Franklin, TX, USA area farms D and E were significantly different from one another with Farm D (0.048mg/mL) displaying the lowest resistance of the six farms tested and farm E (0.627mg/mL) having the highest resistance of all farms tested. This highlights that within a region resistance levels can vary and the spread of beetles between houses and farms should be controlled to prevent resistance from spreading. This scenario is also repeated in Mt. Pleasant, TX, USA where farm B (0.320mg/mL) had a much higher level of resistance compared to the remaining two populations, A (0.161mg/mL) and C (0.118mg/mL). A study by Tomberlin et al. (2008) found similar results with Talstar WP™ and its wide variation in knockdown rates in Titus County, the same county where farms A, B, and C represent.

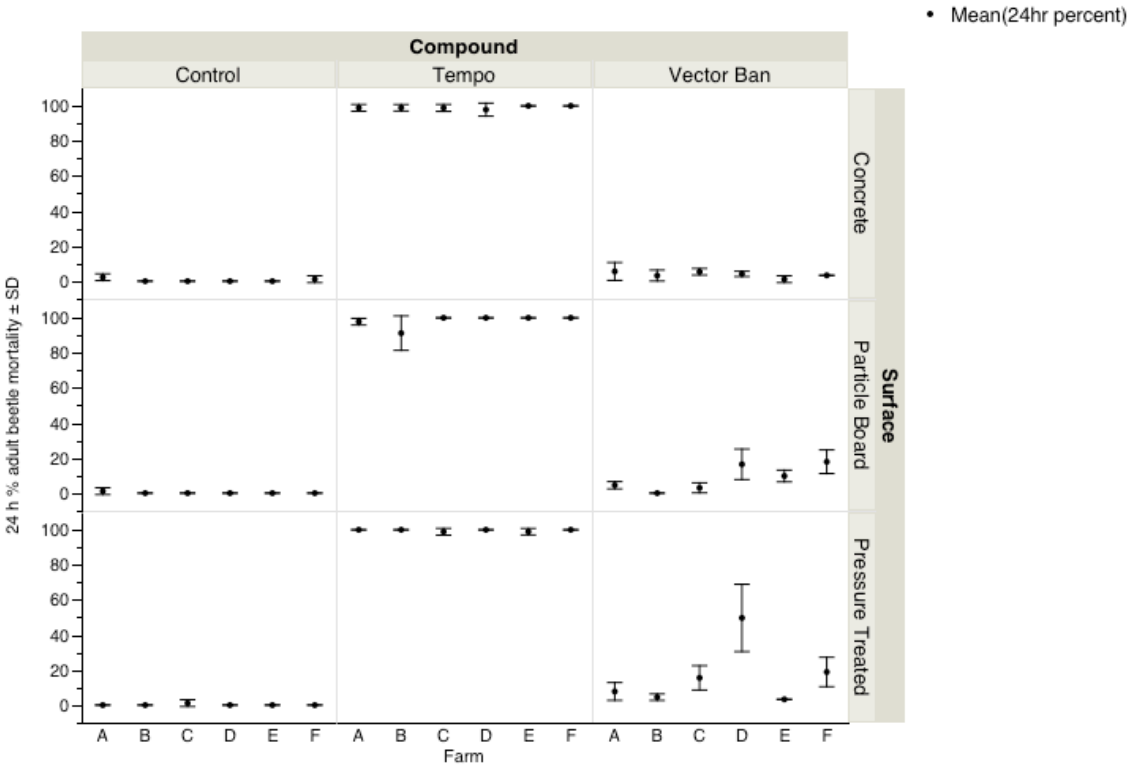
A normal ANOVA was performed with the modified data and the variation with farms D and F was found to be similar to each other, but different from the remaining farms. The ANOVA test assumes similar variation between the tests. The p-value from the Brown-Forsythe test combined with the boxplots of data necessitates the use of a Welch's ANOVA that assumes the standard deviation is not the same between all populations.

Figure 8. Mean mortality ± SD of adult *Alphitobius diaperinus* adults 2 h after exposure to surfaces not treated or treated with Tempo SC Ultra™ or Vector Ban Plus™ at their low dose label rates (Tempo SC Ultra™**:** 8.0 mL/3.7 L of water; Vector Ban Plus™: 0.095 L per 3.785 L water) under field conditions (21.11°C).



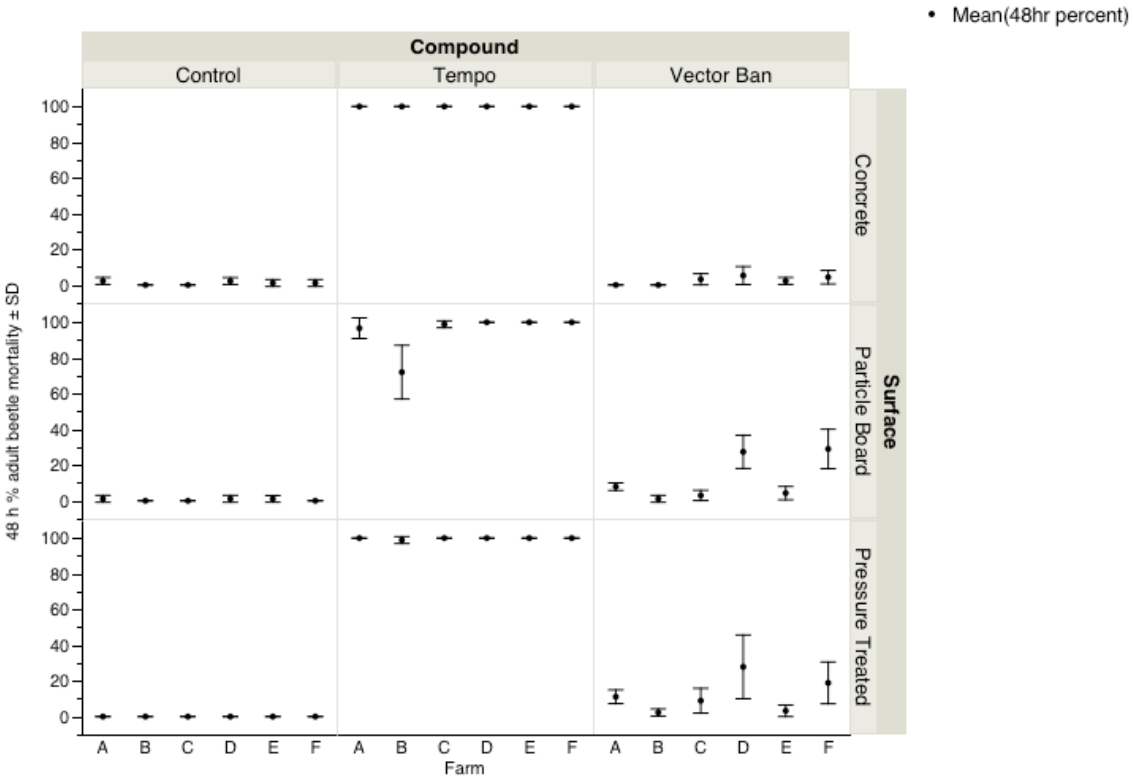
Each error bar is constructed using 1 standard deviation from the mean.

Figure 9. Mean mortality ± SD of adult *Alphitobius diaperinus* adults 24 h after exposure to surfaces not treated or treated with Tempo SC Ultra™ or Vector Ban Plus™ at their low dose label rates (Tempo SC Ultra™**:** 8.0 mL/3.7 L of water; Vector Ban Plus™: 0.095 L per 3.785 L water) under field conditions (21.11°C).



Each error bar is constructed using 1 standard deviation from the mean.

Figure 10. Mean mortality ± SD of adult *Alphitobius diaperinus* adults 48 h after exposure to surfaces not treated or treated with Tempo SC Ultra™ or Vector Ban Plus™ at their low dose label rates (Tempo SC Ultra™**:** 8.0 mL/3.7 L of water; Vector Ban Plus™: 0.095 L per 3.785 L water) under field conditions (21.11°C).

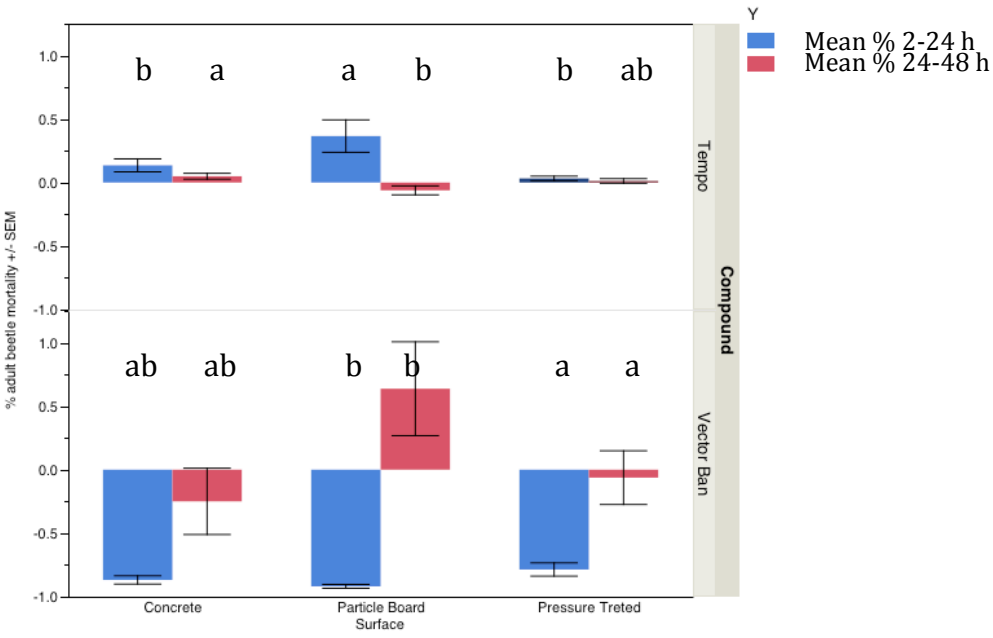


Each error bar is constructed using 1 standard deviation from the mean.

Surface Bioassay: Figs. 8-10 visually displays mean percent mortality ± SD for the Control, Vector Ban Plus™, and Tempo SC Ultra™ on the surfaces tested. Fig. 11 shows percent change in mortality by surface type for both formulations. The range of mean mortality at 2 h regardless of surface type for Tempo SC Ultra™ (58.86-100.00%) and Vector Ban Plus™ (17.85-100.00%) had wide variation. The range of mean mortality at 24 h regardless of surface for Tempo SC Ultra™ (91.25-100.00%) had less than 10% variation, while Vector Ban Plus™ (0.00-49.73%) displayed a near 50% variation. The range of mean mortality at 48 h regardless of surface for Tempo SC Ultra™ (72.06-100%) varied by 30%, and Vector Ban Plus™ (0.00-29.03%) had similar variation in mortality. Mortality was similar for Tempo SC Ultra™ within each substrate. The most recovery, or decrease in mortality, seen from 2-24 h was 1.11% (farm A on concrete) for Tempo SC Ultra™**,** but in many instances mortality increased. The most recovery seen from 2-24 h for Vector Ban Plus™ was 82.11% (farm F on particle board) and the least recovery was 24.63% (farm D on pressure treated wood). The most mean recovery from 24-48 h mean for Tempo SC Ultra™ was 19.19% (farm B on particle board), but in all other instances recovery did not exceed 2%. The most mean recovery from 24-48 h for Vector Ban Plus™ was 21.87% (farm D on pressure treated wood), but in all other instances the maximum recovery did not exceed 7.00%. In some instances with Vector Ban Plus™ mean mortality increased from 24-48 h, such as farm F (17.89-29.03) and farm D (16.45-27.42), both on particle board. Other occurrences with Vector Ban Plus™ mean mortality increases did not exceed 3% with all three surfaces.

On concrete, farms A, B, C, and D increased mean percent mortality from 24-48 h by 1.15% or less, and farm C on pressure treated wood increased over the same period by 1.11%. Tables 6-8 displays mean percent mortality ± SD by farm for 2, 24, and 48 h by compound and surface type.

Figure 11. Mean percent change ± SEM of mortality from 2 h to 24 h and 24 h to 48 h of adult *Alphitobius diaperinus* adults (n=30) by surface type and compound (Tempo SC Ultra™ or Vector Ban Plus™ at their low dose label rates (Tempo SC Ultra™**:** 8.0 mL/3.7 L of water; Vector Ban Plus™: 0.095 L per 3.785 L water)) in field conditions (21.11°C).



*Different letters in each column indicate statistical significance (LSD, $p \leq 0.05$). Means comparisons of percent change in either 2 h to 24 h or 24 h to 48 h within a compound between the three surface types. Each error bar is constructed using 1 standard error from the mean.

Table 6. Mean percent mortality ± SD for adult *Alphitobius diaperinus* beetles collected from six farms in Texas 2 h after exposure to three surfaces treated with Tempo SC Ultra™ or Vector Ban Plus™ at their low dose label rates (Tempo SC Ultra™**:** 8.0 mL/3.7 L of water; Vector Ban Plus™: 0.095 L per 3.785 L water) under field conditions (21.11°C).

Farm	Pressure Treated Wood			Particle Board			Concrete		
	Control	Tempo SC Ultra	Vector Ban	Control	Tempo SC Ultra	Vector Ban	Control	Tempo SC Ultra	Vector Ban
A	0.00 ± 0.00a	98.96 ± 1.80a	72.22 ± 25.46ab	0.00 ± 0.00a	86.67 ± 20.28a	54.94 ± 11.01c	0.00 ± 0.00a	100.00 ± 0.00a	42.91 ± 27.31ab
B	0.00 ± 0.00a	100.00 ± 0.00ab	58.97 ± 15.39ab	0.00 ± 0.00a	58.86 ± 26.27b	40.65 ± 3.52c	0.00 ± 0.00a	95.08 ± 5.95a	19.10 ± 11.14b
C	1.11 ± 1.92a	96.63 ± 0.07b	66.67 ± 13.33ab	0.00 ± 0.00a	97.78 ± 1.92a	43.81 ± 14.88c	0.00 ± 0.00a	92.39 ± 5.12a	20.31 ± 6.78b
D	0.00 ± 0.00a	100.00 ± 0.00ab	74.36 ± 2.23a	0.00 ± 0.00a	98.89 ± 1.92a	93.51 ± 8.51ab	1.67 ± 3.85a	94.55 ± 4.95a	63.82 ± 21.96a
E	0.00 ± 0.00a	98.92 ± 1.86ab	45.23 ± 24.43b	0.00 ± 0.00a	87.78 ± 8.39a	80.83 ± 18.76b	1.11 ± 1.92a	95.69 ± 3.73a	17.85 ± 19.18b
F	0.00 ± 0.00a	100.00 ± 0.00ab	80.00 ± 8.82a	0.00 ± 0.00a	98.92 ± 1.86a	100.00 ± 0.00a	0.00 ± 0.00a	97.78 ± 3.85a	37.39 ± 28.49ab

*Different under case letters in each column indicate statistical significance (LSD, $p \leq 0.05$).

Table 7. Mean percent mortality ± SD for adult *Alphitobius diaperinus* beetles collected from six farms in Texas 24 h after exposure to three surfaces treated with Tempo SC Ultra™ or Vector Ban Plus™ at their low dose label rates (Tempo SC Ultra™**:** 8.0 mL/3.7 L of water; Vector Ban Plus™: 0.095 L per 3.785 L water) under field conditions (21.11°C).

Farm	Pressure Treated Wood			Particle Board			Concrete		
	Control	Tempo SC Ultra	Vector Ban	Control	Tempo SC Ultra	Vector Ban	Control	Tempo SC Ultra	Vector Ban
A	0.00 ± 0.00a	100.00 ± 0.00a	7.78 ± 5.09bc	1.11 ± 1.92a	97.78 ± 1.92b	4.52 ± 2.06bc	2.26 ± 1.96a	98.89 ± 1.92a	5.56 ± 5.09a
B	0.00 ± 0.00a	100.00 ± 0.00ab	4.52 ± 1.86bc	0.00 ± 0.00a	91.25 ± 8.81c	0.00 ± 0.00c	0.00 ± 0.00b	98.92 ± 1.86a	3.19 ± 3.13a
C	1.11 ± 1.92a	98.89 ± 1.92b	15.56 ± 6.94bc	0.00 ± 0.00a	100.00± 0.00a	3.02 ± 2.87bc	0.00 ± 0.00b	98.85 ± 1.99a	5.38 ± 1.88a
D	0.00 ± 0.00a	100.00 ± 0.00ab	49.73 ± 19.19a	0.00 ± 0.00a	100.00± 0.00ab	16.45 ± 8.70a	0.00 ± 0.00b	97.85 ± 3.72a	4.15 ± 1.50a
E	0.00 ± 0.00a	98.92 ± 1.86b	3.30 ± 0.06c	0.00 ± 0.00a	100.00± 0.00ab	9.79 ± 3.35ab	0.00 ± 0.00b	100.00± 0.00a	1.11 ± 1.92a
F	0.00 ± 0.00a	100.00 ± 0.00ab	18.89 ± 8.39b	0.00 ± 0.00a	100.00± 0.00a	17.89 ± 6.75a	1.11 ± 1.92ab	100.00± 0.00a	3.34 ± 0.11a

*Different under case letters in each column indicate statistical significance (LSD, $p \leq 0.05$).

Table 8. Mean percent mortality ± SD for adult *Alphitobius diaperinus* beetles collected from six farms in Texas 48 h after exposure to three surfaces treated with Tempo SC Ultra™ or Vector Ban Plus™ at their low dose label rates (Tempo SC Ultra™**:** 8.0 mL/3.7 L of water; Vector Ban Plus™: 0.095 L per 3.785 L water) under field conditions (21.11°C).

	Pressure Treated Wood			Particle Board			Concrete		
Farm	Control	Tempo SC Ultra	Vector Ban	Control	Tempo SC Ultra	Vector Ban	Control	Tempo SC Ultra	Vector Ban
A	0.00 ± 0.00a	100.00 ± 0.00a	11.11 ± 3.85bc	1.11 ± 1.92a	96.67 ± 5.77a	7.89 ± 2.12b	2.26 ± 1.96a	100.00± 0.00a	0.00 ± 0.00b
B	0.00 ± 0.00a	98.89 ± 1.92b	2.30 ± 1.99c	0.00 ± 0.00a	72.06 ± 15.06b	1.11 ± 1.92b	0.00 ± 0.00a	100.00± 0.00a	0.00 ± 0.00b
C	0.00 ± 0.00a	100.00 ± 0.00b	8.89 ± 6.94bc	0.00 ± 0.00a	98.89 ± 1.92a	3.02 ± 2.87b	0.00 ± 0.00a	100.00± 0.00a	3.19 ± 3.13ab
D	0.00 ± 0.00a	100.00 ± 0.00ab	27.86 ± 17.87a	0.83 ± 1.92a	100.00 ± 0.00a	27.42 ± 9.33a	1.67 ± 1.92a	100.00± 0.00a	5.29 ± 5.03a
E	0.00 ± 0.00a	100.00 ± 0.00ab	3.26 ± 3.23bc	1.08 ± 1.86a	100.00 ± 0.00a	4.31 ± 3.73b	1.11 ± 1.92a	100.00± 0.00a	2.26 ± 1.96ab
F	0.00 ± 0.00a	100.00 ± 0.00ab	18.89 ± 11.7ab	0.00 ± 0.00a	100.00 ± 0.00a	29.03 ± 11.07a	1.11 ± 1.92a	100.00± 0.00a	4.37 ± 3.79ab

*Different under case letters in each column indicate statistical significance (LSD, $p \leq 0.05$).

Discussion

Methods for monitoring pest populations represent important tools for IPM (Axtell 1981). Monitoring techniques for *A. diaperinus* have included estimating the size of the population and their location within a poultry house with geographical information systems (GIS) and Arends tube traps (Strother and Steelman 2001). Monitoring could also include measuring insecticide resistance levels exhibited by *A. diaperinus* populations inside a poultry house. Bioassays with AI and formulations, like those presented in this research, allow producers to determine what insecticides might be most effective for their specific case for suppressing *A. diaperinus* populations.

The number of insecticides available for suppressing *A. diaperinus* in poultry operations is limited and is further hindered by the fact that the compounds can only be applied during cleanout periods of the facilities between flocks of birds every 6-8 wk. Identification of insecticide resistance could allow producers to rotate chemicals based on the AI in order to reduce the likelihood of resistance development in a given population (Prabhaker et al. 1998, He et al. 2012). For example, *Bemisia argentifolii* (Homoptera: Aleyrodidae) treated with only bifenthrin developed a resistance ratio (RR) of 8 fold greater than the unselected population within one generation from the baseline of 5 RR with the parents, and increased to 752 RR after 27 generations (Prabhaker et al. 1998). However, when bifenthrin was used as part of a rotation with endosulfan and chlorpyriofos, resistance ratios did not exceed 10 until after the 24^{th} generation.

Estimated level of resistance of *A. diaperinus* adults to an insecticide, or an associated AI, is highly dependent on the amount of time allowed to pass after their

treatment (Figs. 7-10, Tables 3-8). Previous studies assessed mortality of *A. diaperinus* 48 h after exposure to an AI or its formulation (Hamm et al. 2006, Kaufman et al. 2008). Hamm et al. (2006) determined the LD_{50} for *A. diaperinus* exposed to cyfluthrin 48 h after treatment ranged from 0.04-0.17 μg/cm^2. This study observed mortality for *A. diaperinus* adults at 2, 24, and 48 h after exposure to filter papers treated with varying doses of β-Cyfluthrin. Mortality at the highest treatment dose (20 μg/cm^2) varied from 60.12 to 100.00% across all farms 2 h after treatment; however, after 48 h the range dropped substantially from 0-23.00% mortality (Tables 3-5). For example, farm A (Fig. 7), mortality decreased 22.94% from the 2-24 h observation and an additional 66.98% by the 48 h observation. Furthermore, when comparing mortality for beetles from five of the farms (excluding farm B), the 2 h observation indicated no difference in susceptibility to these compounds at 0.20 mg/mL ($p \geq 0.05$); however at 24 h the beetle mortality at the same dose differed between populations (D was different from B and E). Furthermore at 48 h mortality, farms C and D were statistically the same ($p \leq 0.05$). These two farms (C and D) were, however, statistically different ($p \leq 0.05$) from Farms B and E. These results also demonstrate variation between farms.

Resistance levels were determined for *A. diaperinus* adults exposed to various surfaces treated with the formulated insecticides (Tables 6-8). Fig. 11 shows percent change in mortality between 2-24 h and 24-48 h. Tempo SC Ultra™ and Vector Ban Plus™ had the greatest variability in mortality during the 2 h observation. Particle board treated with Tempo SC Ultra™ resulted in *A. diaperinus* mortality ranging from 58.86% (farm B) to 98.92% (farm F). On concrete, Vector Ban Plus™ mortality ranged from

17.85% (farm E) to 63.62% (farm D). In general, surfaces treated with Tempo SC Ultra™ exhibited *A. diaperinus* mortality similar to what was recorded during the 2 h and 24 h observation. *Alphitobius diaperinus* beetles that were treated with Vector Ban Plus™ had significant levels of recovery (farm A on concrete decreased 37.35%) after the same period ($p \geq 0.05$). The maximum 2-24 h recovery for Vector Ban Plus™ was 82.11% for beetles from farm F on Particle Board, and the minimum recovery was 24.63% for beetles from farm D on pressure treated wood. Maximum 24 to 48 h mean recovery for Tempo SC Ultra™ was 19.19% for *A. diaperinus* beetles from farm B on particle board, but in all other instances the maximum recovery did not exceed 2.00%. Maximum 24 to 48 h mean recovery for Vector Ban Plus™ was 21.87% for beetles from farm D on pressure treated wood, but in all other instances the maximum recovery did not exceed 7.00%. Tempo SC Ultra™ produced high mortality through the 48 h observation period on all surfaces, but with Vector Ban Plus™ ™ the knockdown decreased with time of exposure in a similar pattern as in the paper assay. For example, the concrete surface treated with Vector Ban Plus™ at 2 h showed a knockdown range of 17.85-63.82% at 2 h, 1.11-5.56% at 24 h and 0.00-5.29% at 48 h.

A previous study recorded an 81.30% decrease in mortality from 4 to 24 h in one farm with Talstar SFR™ (Tomberlin et al. 2008). Their farm D had a 4 h knockdown of 87.00%, but by 24 h observation mortality dropped to 5.70%. Their farm E saw a 48.70% (maximum for all farms) drop in mortality with Tempo SC Ultra™ but of all their compounds tested it had the least amount of recovery. Permethrin did not have as much success in killing three *Liposcelis* species (Psocoptera: Liposcelididae) when used

on concrete compared to the steel surface it which it was applied (Nayak et al. 2002), and both Malathion (EC) and pirimi- phos-methyl (EC) had lower knockdown rates on concrete and plywood when compared to steel (Jankov et al. 2013). When examining eight stored products pests, (Toews et al. 2003) used 24 and 48 h as the time points to assess their susceptibility to select insecticides. When examining eight stored product pests, Toews et al. (2003) used 24 h to measure knockdown and 48 h to measure mortality. Of the eight species tested, the knockdown ranged from 89-100%. For all the species, except *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), mortality was 100.00%. *Sitophilus oryzae* (Linnaeus) (Coleoptera: Tenebrionidae) experienced 100% knockdown and mortality at the time intervals examined. With *T. confusum* recovery on concrete at 48 h was 0.2% and on the other surfaces tested recovery ranged from 13.00-20.00%. *T. castaneum* also had higher mortality on concrete (99.50%) compared to steel (84.00%), unwaxed tile (87.00%) and waxed tile (80.00%). While surface type may affect insecticide activity, the environment in which the surfaces are subjected to can also be a factor (Gudrups et al. 1994). High temperature and humidity can affect volatilization of the solution applied. Gudrups et al. (1994) also noted that higher respiration of insects in high temperatures could increase movement on surfaces and therefore increase contact with compound. The conditions described match those of the lesser mealworm inside poultry houses and the surfaces inside them. Future research should focus more on matching these conditions and with various poultry house materials to get a better assessment of insecticide efficacy specific to the poultry industry.

Variation in insecticide resistance was observed within region (Table 1). The 24 h LD_{50} indicates that two farms from Mt. Pleasant, TX, USA were statistically different from one another ($p \leq 0.05$), and all three in Franklin, TX, USA were statistically different. Tomberlin et al. (2008) determined populations of darkling beetles from facilities in the Mt. Pleasant, TX, USA region had highly variable levels of resistance to Tempo SC Ultra™ and Talstar WP™. Hamm et al. (2006) observed wide variation in resistance ratios RR_{95} (1.7-9.5) for *A. diaperinus* beetles from across the east coast, USA.

Mortality results from laboratory assays of AI do not convey mortality for compounds sprayed in the field. The data presented here show a high recovery rate by 48 h (Fig. 7) when *A. diaperinus* beetles were subjected to β-Cyfluthrin AI, but when a formulation was used (Tempo SC Ultra™) the mortality was near 100% until the last observation at 48 h (Figs. 8-10). A previous study with β-Cyfluthrin AI showed some adults had 7.7-9.5 fold resistance ratios compared to the susceptible population (Hamm et al. 2006), but a follow-up study shortly after used Tempo WP 20™ (β-Cyfluthrin AI) and measured a 91.8% mortality in the susceptible population and 100.00% mortality with a resistant population from the AI assay (Kaufman et al. 2008). Formulations can include synergists to improve the product's effectiveness (NPIC 1999). Inactive ingredients, or inert ingredients, include carriers and solvents. Carriers can help the AI have better contact with insects. Three synergists, Diethyl maleate (DEM), S,S,S-tributyl phosphorotrithioate (DEF), and piperonyl butoxide (PBO) were shown to decrease resistance (He et al. 2012). In their RA11 population treated with fipronil DEF was

reduced resistance by 43.30% and 40.40% when PBO was used. Synergists and inactive ingredients coupled with β-Cyfluthrin AI can be responsible for the complete turnaround in mortality from the filter paper assay to the surface treatments.

Data collected from this study indicate that filter paper assays are an important tool for toxicology studies, but the formulation has a notable impact that can differ from simple AI assays. This echoes Kaufman et al. (2008) as they highlighted the importance for both assay types, but be cautious of what you take from the data recorded. For whichever assay is conducted, determining appropriate observation times is critical. Recovery was seen in both assays conducted during this research. While morbid individuals were able to regain movement, this study did not take into account lasting physiological effects of the beetles after they "recovered." These sub-lethal effects include behavior that make them more prone to desiccation and predation (Van Herk et al. 2008), and reproduction losses (Sohrabi et al. 2011, Ugine et al. 2011). In their LC_{50} study, Van Herk et al. (2008) noted individuals from the five species of wireworms (Coleoptera: Elateridae) in clothianidin treated cups were on the surface in an attempt to escape the compound and were all in a moribund state. *Bemisia tabaci* (Hemiptera: Aleyrodidae) individuals had ~ 4 fold decrease in number of eggs laid in individuals exposed to buprofezin (Sohrabi et al. 2011) and daily doses of imidacloprid up 30 ppm over ten weeks on *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) reduced the number of viable eggs by 23-38% (Ugine et al. 2011). If the goal of IPM is to keep the mean population density below a threshold level (Axtell 1981), then a bioassay study should focus on sub lethal effects on individuals. This study

saw a high level of recovery with some compounds, and if these individuals have reduced reproductive capacity or will be unable to survive post treatment for a prolonged period then the treatment may still be judged a success when the IPM strategy is considered. Data from this study and other studies discussed above demonstrate that this failure to implement a conservative observation (waiting 24 to 48 h post treatment) period could lead to false confidence in the ability of a compound to suppress an arthropod pest population while continuing added pressure for greater selection of resistance.

CHAPTER III

HEAT TOLERANCE

Introduction

The core concept of an integrated pest management (IPM) strategy is to know and understand the biology of the specific arthropod that is to be controlled and then use this information to tailor a comprehensive program that is the most environmentally friendly to achieve control of the targeted arthropod (EPA 2012). The program is made up of various components for each pest species. A method to monitor the population, a threshold population size in which action must be taken against the pest, a method to prevent the population from reaching the threshold size (cultural control), and an approach to control the pest once a threshold has been reached or exceeded.

To increase the efficacy of the treatments, novel approaches must be undertaken. Such approaches have been researched for cultural control of *A. diaperinus.* A promising area of research is inducing heat shock as a method of controlling beetles in grain houses (Fields 1992, Fleurat-Lessard and Dupuis 2010). Enzyme imbalance at high temperatures is one of many concepts as to what exactly causes death in insects at their upper temperature threshold (Fields 1992).

Understanding the behavior of the insect is very important to developing an IPM strategy. Prasifka et al. (2008) demonstrated ground beetles exhibit a period of hyperactivity after coming in contact with sub lethal levels of a pyrethroid. This period

of hyperactivity can affect the efficacy of the pesticide application if the beetles are able to quickly avoid the application sites. Similar behavior may be a concern with *A. diaperinus* with regard to heat stress. This experiment will hopefully lead to an understanding of behavior of the insect as they undergo the biological stress as they approach their upper temperature tolerances.

Heat temperature paralysis has been documented in *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) (Ffrench-Constant et al. 1993). Homozygous resistant *Rdl* (*Resistance to dieldrin)* gene flies were shown to take longer than heterozygous *Rdl,* which took longer than wild type susceptible *Rdl* genes when placed in a 38°C water bath and then returned to room temperature. The biological effects of resistance have been postulated to be a result of changing the function of voltage sensitive sodium channel properties therefore reducing the functionality of the pyrethroid insecticide (Ffrench-Constant et al. 1998, Soderlund and Knipple 2003). The substitution of alanine for serine occurs in *Rdl* mutations(Ffrench-Constant et al. 1993). An additional hydroxyl group is added in the process and is thought that the conformation of this protein is affected at high temperatures and may affect the function of the channel. The *PARA* voltage gated channel is the target site of pyrethroids and DDT (Ffrench-Constant et al. 1998). Mutations to *PARA* have been identified using their temperature sensitive phenotype (Suzuki et al. 1971, Ffrench-Constant et al. 1998). The well known link of at least two insecticide resistant mutants, *Rdl* and *PARA*, to temperature sensitivity is the basis for the theory that heat will affect a highly resistant population differently than a susceptible population.

Heat shock proteins have been found in every plant and animal for which it was searched within (Lindquist and Craig 1988). HSP 70 and HSP 90 genes are expressed in response to elevated temperatures. These proteins are among the most conserved known to exist. These genes, or close relatives, are present at normal temperatures for their species. When HSP's have to be released in elevated levels in response to stresses, it is a quick and intense expression. The expression could be triggered by a moderate temperature stress to help protect from further increasing temperatures. The temperature that will trigger HSP's differs by species due to the varying natural habitats of its host.

Materials and Methods

Beetle Colonies: Populations B (LD_{50} 0.320) and E (LD_{50} 0.627) from chapter II were chosen to represent the resistant populations to β-Cyfluthrin, and population D (LD_{50} 0.0480) was chosen to be the field susceptible colony. Individuals were taken from the F_1 generation of the B, D, and E colonies. Fourteen individuals from each population were subjected to increasing ambient temperatures ranging from 31.5-47°C in an attempt to meet the thermostupor point of 46.75°C as recorded by Salin et al. (1998).

Experiment Design: *Alphitobius diaperinus* adults 1-2 months of age were taken from the colony room and placed inside a glass graduated cylinder for each population and then moved to an incubator set to 27°C until used for experiment. For the experiments, 9 cm diameter filter papers (Fisherbrand, Loughborough, UK) were cut to 5.0 cm to fit inside a watch glass. The resized filter papers were placed inside each of 14

watch glasses. One beetle was placed inside a single watch glass. The water bath temperature followed the schedule set on Table 9.

Table 9. Water bath and ambient temperature in relation to experiment time

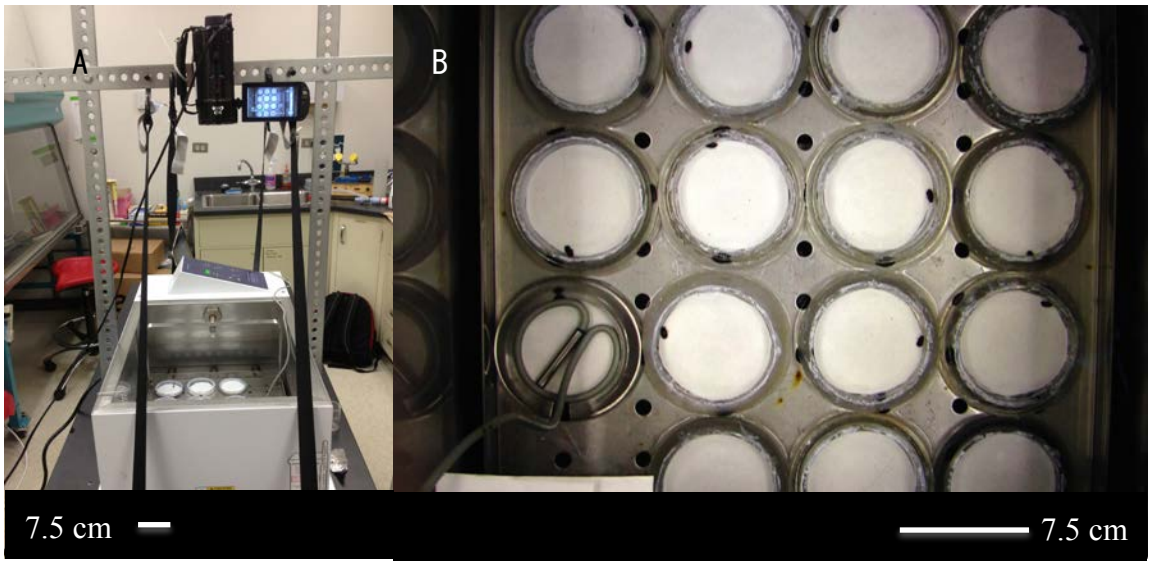
Water bath temperature setting (°C)	Ambient temp setting (Hobo), (°C)	Time spent at temperature (min)
31.00	31.50	5
36.00	34.00	5
41.00	37.00	10
43.00	38.00	10
44.50	39.00	10
45.50	40.00	10
46.50	41.00	10
47.50	42.00	10
48.50	43.00	10
49.50	44.00	10
51.00	45.00	10
52.50	46.00	10
55.00	47.00	10

A high definition video recorder (Canon Vixia HD HFM21 64gb, Canon Inc., Tokyo, Japan) was placed above the watch glasses about 0.60 m such that all 14 were observable, but close enough to maintain enough resolution for the computer to calculate data (Fig. 12). Four ratchet straps were attached to the table below the water bath to prevent accidental movement of the camera during filming. The experiment lasted approximately two hours. A Hobo unit (Onset Computer Corporation, Bourne, MA, USA) was used to measure the ambient temperature during the experiment and was placed inside its own watch glass with filter paper. Preliminary experiments were conducted to verify the water temperature correlation to ambient temperature for these

experiments. Water level was set approximately 5 mm below the top of the watch glasses. Before the experiment began, fluon (Insect-a-Slip, Bioquip Products, Rancho Dominguez, CA, USA) was coated on the inside of the watch glasses.

Statistical Analysis: Video from the experiments was analyzed by Ethovision XT (Noldus, Wageningen, Netherlands) software. Ethovision has been used to measure beetle behavior in previous studies (Vacha 2004, Prasifka et al. 2008). The output is in Excel (Microsoft Corporation, Redmond, WA, USA) spreadsheet format and includes distance, direction, and velocity data. Using the output, arenas were disqualified for analysis based on the total time the beetle was tracked within the arena. An arena is defined as the area inside a watch glass in which one beetle was placed. If the beetle was tracked 89% or less of the total time of the experiment the beetle's data was not used for analysis. Distance traveled was chosen for analysis of the insect's behavior. Three analyses were performed; Models to examine percent change from initial temperature (37°C) to final temperature (47°C), percent change between subsequent temperatures, and percent change from 37-41°C were analyzed using ANOVA with JMP Pro 10 (SAS Institute Inc., Cary, NC, USA). Means were separated with Least Significant Difference (LSD) ($p < 0.05$) if ANOVA was significant.

Figure 12. Water bath setup (A) and arrangement of 14 watch glass arenas (B)



High Definition Camcorder was placed about 0.60 m above water bath (A) to record *A. diaperinus* movement in the watch glasses (B). Fourteen watch glasses were arranged in four columns and 4 rows. Note the Hobo sensor in lower left corner used to record ambient temperature.

Results

Fig. 13 and Fig. 14 shows that both farm B and E displayed a similar trend in the line from 37.00-40.00°C. The model for percent change between two consecutive temperatures was not statistically significant ($F = 1.0126$, $df = 32, 231$, $p = 0.4544$). The model for the percent change from initial temperature was also not significant ($F = 1.2470$, $df = 32, 231$, $p = 0.1800$). The percent change from 37°C and 41°C was not significant ($F = 1.4124$, $df = 2, 21$, $p = 0.2658$).

Fig. 15 shows the mean difference between the 37 °C and 47°C distance observation was 22.13% and 24.99% for farms E and D respectively. Farm B underwent a 29.82% increase between the same two temperatures. Fig. 16 shows the percent change by population from 37-40°C. Farm D had an overall downward trending line between the starting and ending temperature.

Figure 13. Mean distance (mm) ± SD traveled of adult *Alphitobius diaperinus* beetles between 37°C and 47°C (ambient temperature) within individual watch glasses in water bath experiment setup under laboratory conditions (27°C).

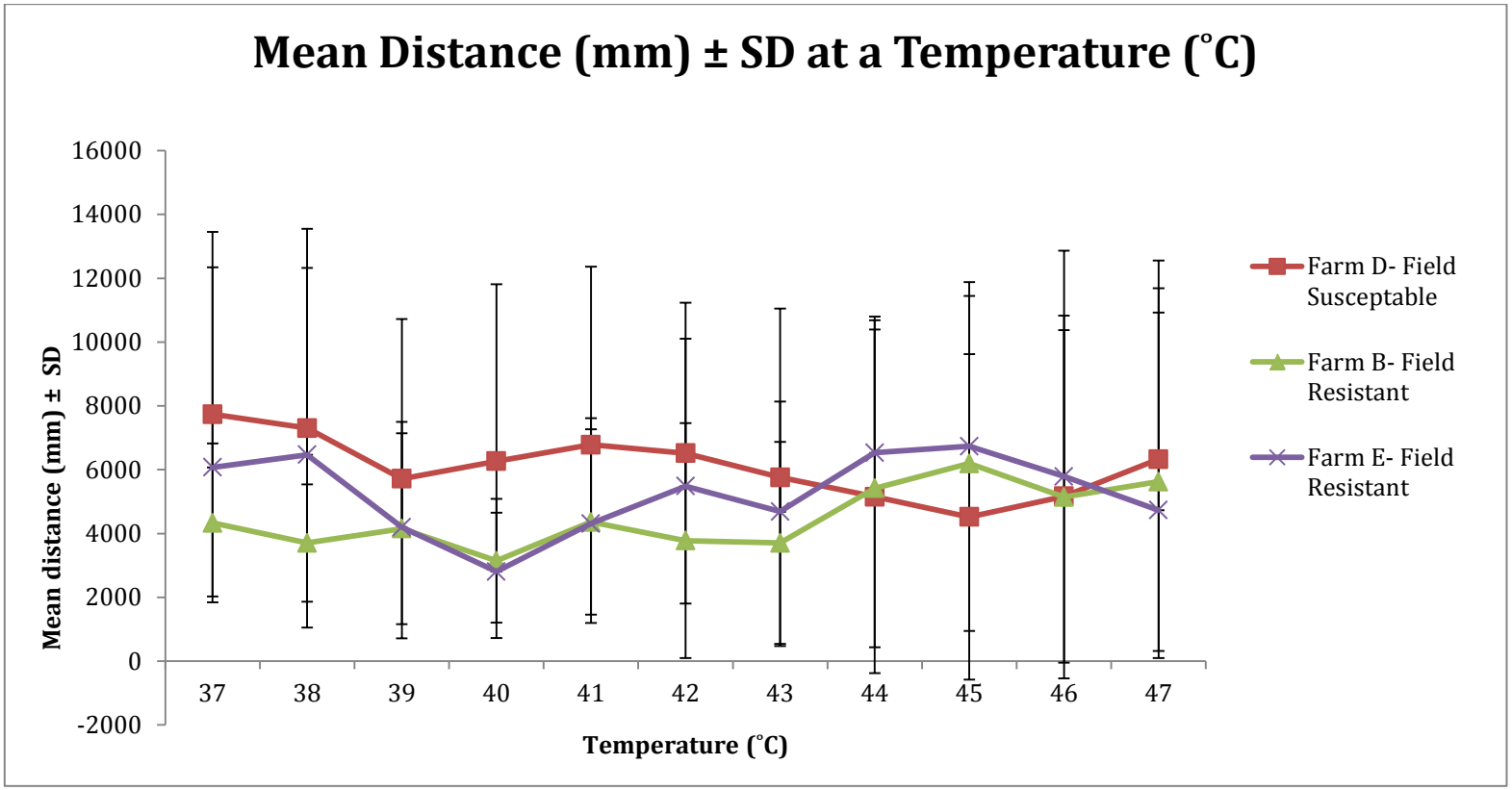
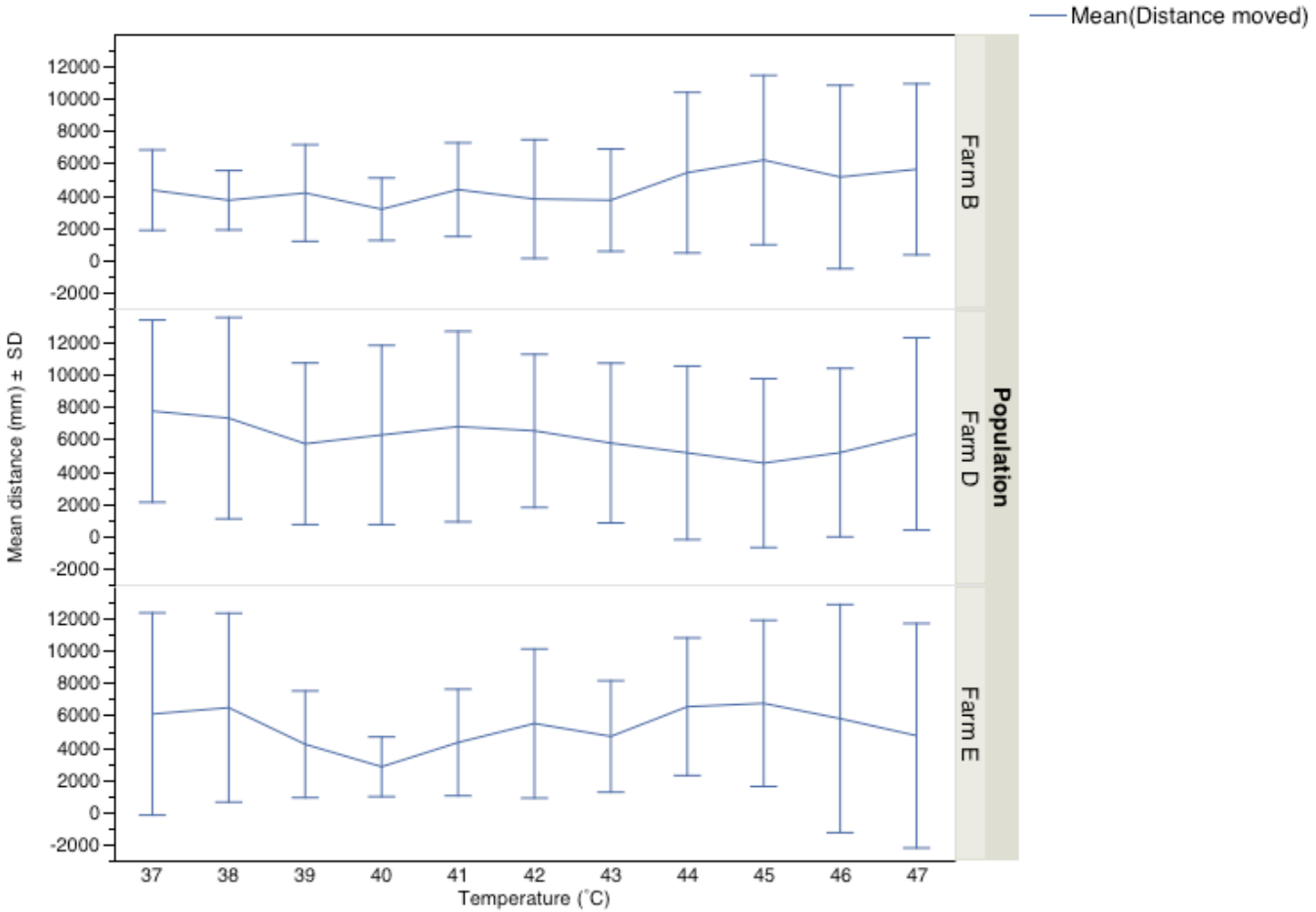


Figure 14. Mean distance (mm) ± SD traveled of adult *Alphitobius diaperinus* beetles between 37°C and 47°C (ambient temperature) within individual watch glasses in water bath experiment setup under laboratory conditions (27°C).



Each error bar is constructed using 1 standard deviation from the mean.

Figure 15. Mean percent change of distance (mm) ± SD traveled of adult *Alphitobius diaperinus* between 37°C and 47°C (ambient temperature) within individual watch glasses in water bath experiment setup under laboratory conditions (27°C).

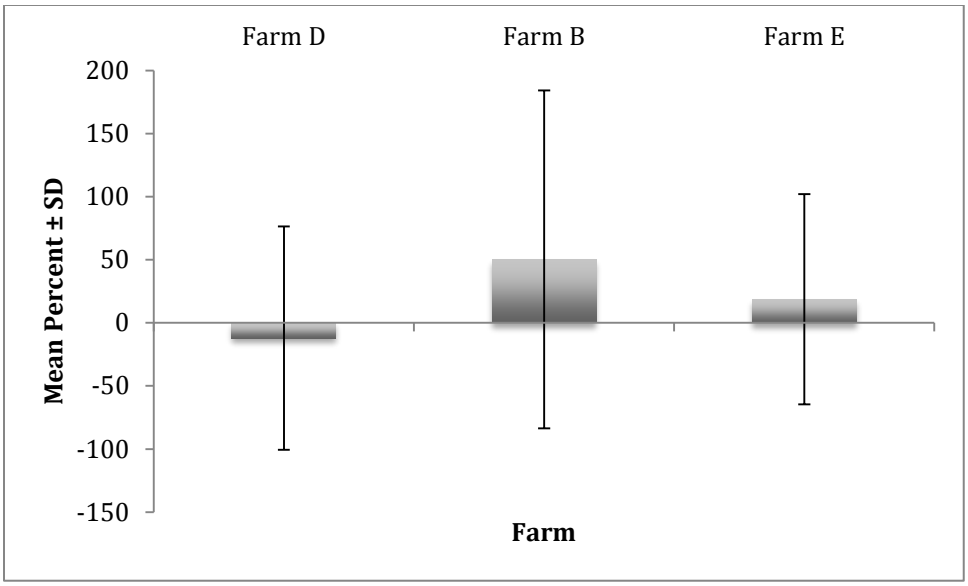
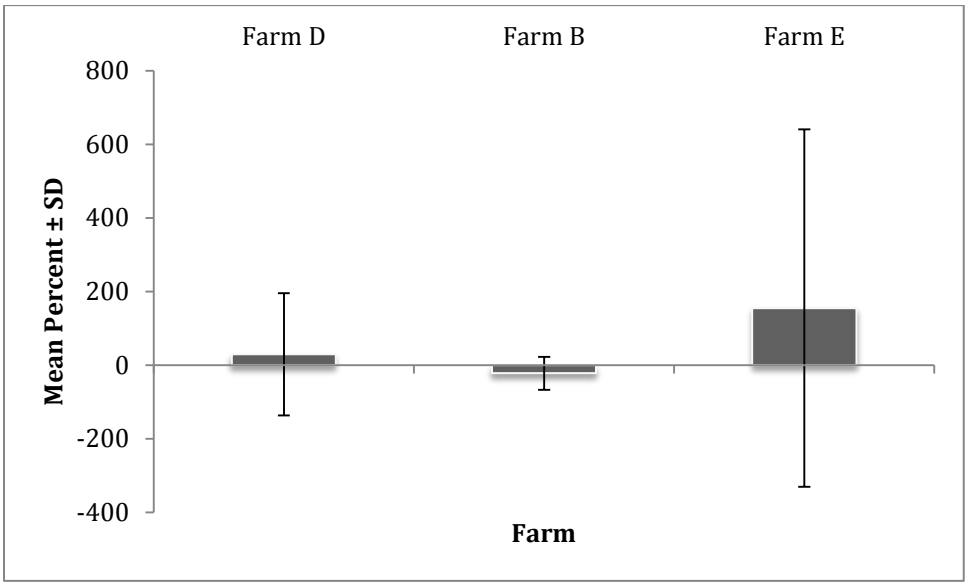


Figure 16. Mean percent change of distance (mm) ± SD traveled of adult *Alphitobius Diaperinus* between 37°C and 40°C (ambient temperature) within individual watch glasses in water bath experiment setup under laboratory conditions (27°C).



Discussion

The average distance (mm) traveled at 37°C between the farms were statistically different ($p \leq 0.05$), and at 47°C the distance (mm) average were all statistically the same. For further analysis the percent change from the initial temperature and percent change between each temperature jump was analyzed with ANOVA. The percent change from 38-39°C was statistically different ($p \leq 0.05$) between B and the other two farms. No other statistical differences were seen between temperature differences and no statistically differences were seen between the farms when the percent change was compared from the initial temperature (37°C). The high variability in the populations is the reason why no separation was statistically determined. The percent change for farm D from 37.00-41.00°C was -17.09-405.87%. It can be hypothesized that heat shock proteins (HSP) are the factor influencing why the results of this study were not indicative of population differences. The slow heating and prolonged periods of time between temperature increases allowed HSP to repair temperature damage to proteins of individuals in the experiments. A prior heat stress can trigger the expression of HSP that can protect the insect from future heat stress (Zhao and Jones 2012). HSP bind to other proteins that have been denatured due to stress, such as temperature, and make sure they do not behave inappropriately inside the body (Feder and Hofmann 1999). A study of the HSP gene expression levels correlation with temperature with two populations of *Bemisia tabac*i (Gennadius) (Hemiptera: Aleyrodidae) saw HSP 90 begin elevated expression levels at 35°C for both populations and peaked at 43°C for the Q population (Yu et al. 2012). The Q population had the highest peak temperature for peak expression

for HSP 70 (43°C) and HSP 20 (41°C). The expression levels for both of the latter HSP's began at 37°C for both populations. The expression levels suddenly drop to near pre elevated expression levels by 45°C. The expression of one or more HSP proteins was likely offering thermal protection, by denatured protein repair, for the individuals in the current study. This may explain partially the dip in distance moved by farm B and E from 37-40°C. In experiments conducted by Yu et al. (2012) one population had peak expression levels at 39.00°C or 41.00°C.

Previous experiments such as Salin et al. in (1998) conducted rapid increases in temperature to find the TSP, but for this experiment the goal was to detect behavior at and around the TSP. The time between temperature increases (ramp rate) was increased from previous studies to allow ambient temperature to increase following water bath temperature and to record behavior changes. Ten minutes was allotted, which may have been too long allowing the beetles to adapt to the temperature increase, therefore increasing the TSP points and altering the behavior previously recorded for TSP points of this beetle. Cold TSP work with *A. diaperinus* has noted that a slower ramping rate of temperature will affect the TSP point making it a lower temperature than with a fast ramping rate of 0.75 $^{\circ}$C min^{-1} (Renault et al. 2012) For *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) acclimation to cold temperatures for flies kept at 25.00°C was shown to increase cold tolerance (Rako and Hoffmann 2006). The extended amount of time in the temperature ramping used in this study may have allowed the beetles to acclimate and subsequently increase the heat shock threshold. The impact for IPM would be that it is imperative to have a sufficiently rapid heating of the facility to

reduce the number of adults that may survive treatment. Previous research by Salin et al. (1998) is 1.00°C per two minutes and had successful heat shock results, whereas this experiment's rate was 1.00°C per 10 min and was not successful. Future research should focus on shortening the time frame between temperature changes, while still monitoring the beetles with a camera, to see if behavior differences can be observed between resistant and susceptible colonies. Comparisons of behavior between temperature changes, such as distance traveled and speed, may help IPM practices when heating a facility.

While a whole house can be heated potentially to temperatures necessary for heat shock treatment, it may prove to be cost prohibitive. A method for localized application of heat to aid in this IPM strategy may be a better solution, however an additional cost for manpower, fuel and machinery would still be required. Therefore monitoring population resistance, densities and damage levels would help producers find a threshold where a more costly IPM strategy would be implemented on an as needed basis.

CHAPTER IV

SUMMARY AND FUTURE RESEARCH

IPM practices include cultural, chemical, and biological control (Axtell 1981). The focus of this research is on the chemical and cultural control of adult *Alphitobius diaperinus.* Monitoring the resistance levels of populations being treated is important for proper chemical treatment, since application practices and formulations impact the chemical efficacy.

Conservative measurement of mortality is critical to resistance monitoring. This research demonstrates that a measurement at 2 h or 4 h post application is an important indicator of knockdown by the chemical. Tomberlin et al. (2008) and this study saw wide variation in our first observation periods. While both studies referred to these measurements, as mortality, the wide variation and later recovery suggests that the terminology needs clarification. This study attempts to prove that mortality can be misleading and appropriate use of toxicology terms is important. Knockdown is important, as individuals will typically have limited contact with a surface that the compound was sprayed on. This research is supported by Tomberlin et al. (2008) and suggests that a 48 h measurement post application is useful for measuring mortality. For future studies however, recording a 36 h measurement for comparison to the 24 h and 48 h should improve the accuracy of mortality measurements. Recovery from exposure to a compound is seen when looking at AI assays or with formulations sprayed on surfaces. The conclusion presented in this research, that inferring a formulation's efficacy based

on AI assay may not be appropriate, is supported by previous studies by Hamm et al. (2006) and Kaufman et al. (2008). Recovery from the knockdown by AI at 48 h was significant, whereas the commercial formulation containing the same AI in combination with inert ingredients displayed only slight recovery.

This research suggests, as others have also indicated, that surface type impacts chemical insecticide efficacy (Nayak et al. 2002, Jankov et al. 2013). In addition, the current research and previous researchers have tested surfaces have been in laboratory conditions, but surfaces inside a poultry house are subject to additional factors that may affect the efficacy of a sprayed-on compound when applied. For instance, dust and feathers can be heavy inside a poultry production facility and these particles coating a surface would block the chemical spray from sufficiently contacting the surface. Future research should mimic the various poultry house surfaces and the dirt found on these surfaces during a common insecticide treatment period for conducting formulation efficacy tests.

This research focused on heat treatment as a cultural control of adult *Alphitobius diaperinus*. The wide variability in response within the beetle populations did not allow the statistical separation of the populations. Future research should begin by repeating the heat shock experiment multiple times and selecting individuals that had the most movement. Progeny from these individuals would then be compared with this experiment. The genetic selection should reduce variability, therefore allowing differences to be seen when analyzed. The artificial selection that this would require is not applicable to the field, but it could help us better understand the behavior for future

research. The experimental design could also be improved upon by employing a faster ramp rate between temperatures to allow for less adaptation by the individuals and provide different results. Expansion of the temperature range tested from poultry house conditions (27°C) to beyond the TSP measured by Salin et al. (1998) (47°C) should also be tested. While the results of this study were inconclusive on the value of understanding the relationship of heat shock and insecticide resistance, future research will hopefully be able to understand this behavior and adapt it to an applicable IPM strategy.

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