LESSER MEALWORM (*ALPHITOBIUS DIAPERINUS*) ASSOCIATION WITH *ENTEROCOCCUS CECORUM* AND PEST MANAGEMENT CONSIDERATIONS FOR THIS PEST IN BROILER HOUSES

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Abstract: The lesser mealworm *Alphitobius diaperinus* is a worldwide pest of poultry. Lesser mealworms transmit various viral and bacterial pathogens. The economic impact of the beetle is a problem for broiler growers. Consumption of the beetle by young birds leads to reduced weight gain and gastrointestinal issues. The boring out of insulation by the larvae to prepare a pupation site reduces the insulating abilities and increases energy costs. This study indicates that the lesser mealworm is a mechanical vector for the causative agent of enterococcus spondylitis (ES) disease in poultry. With 30 min of exposure to *Enterococcus cecorum* inoculated agar, the lesser mealworm was able to transfer bacteria phenotypically similar to *E. cecorum* to sterile agar plates within 30 min of exposure. Future research should expand beyond mechanical transmission to determine the vector competency of this pest to *E. cecorum*. A new set of primers was developed for detection of *E. cecorum* by use of PCR. A Knowledge, Attitudes, and Practices (KAP) survey was conducted on broiler growers in one county of Oklahoma. Eighty-three percent of growers observed *A. diaperinus* present in their broiler houses. Ninety-two percent of growers monitor their houses for pests. Fifty-eight percent of the growers targeted beetles for their insecticide treatments, mostly targeting under feeders and along the walls. Our study reveals that 67% of growers windrowed their litter. Beetles were exposed for a sublethal amount of time (LT_{20}) to various insecticide formulations. The fecundity and hatched larvae were counted over 30 d beginning 3 wk after sublethal insecticide exposure. Mean number of eggs by treatment was highest in the first 6 d of the experiment. No insecticide treatment had less eggs or larvae than the control in this experiment, and some treatments had significantly greater numbers of eggs and larvae. On days 0-6, Onslaught® (Esfenvelerate) had the greatest mean of 31.75 eggs per mating pair and control had the least with a mean of 6.94 eggs per mating pair. More research is needed to determine if the increase in fecundity observed will result in an increase in population rate.

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CHAPTER I

INTRODUCTION

The lesser mealworm, *Alphitobius diaperinus*, (Panzer) is a cosmopolitan pest of grain products and poultry facilities (Dunford and Kaufman 2006). The species is thought to have originated from sub-Saharan Africa (Geden and Hogsette 1994), possibly on fresh produce being sent through Europe (Green 1980). The heat and humidity from the habitat which the lesser mealworm originated is approximately replicated in the modern broiler house with the use of heaters, large fans, and evaporative cooling helping them to reproduce quickly.

The primary concern for broiler growers is the economic impact on their operating costs. The larvae seek out pupation sites in the insulation of the broiler house and in the process reduce its insulating abilities (Geden and Hogsette 1994, Loftin 2011). There are also concerns related to animal health and food safety with this pest. The lesser mealworm is known to be a carrier for various pathogens associated with disease in animals including viruses, fungi (Aspergillus), and bacteria such as *Salmonella*, *E. coli*, and *Streptococcus* (Axtell and Arends 1990). The beetles are readily consumed by birds inside the poultry houses and can inhibit the weight gain of the

birds as a result if the chicks replace or augment the formulated feed. The transfer of pathogens from manure to the external surface and gastro-intestinal tract (GIT) of the beetles has been demonstrated for *Salmonella* (Crippen et al. 2009, Crippen et al. 2018). The lesser mealworm could excrete *E. coli* in frass for several days and when eaten can transmit the pathogen to birds that consume contaminated beetles (McAllister et al. 1996). McAllister et al. (1994) isolated *Salmonella* from the feces of the lesser mealworm for 28 d after initial exposure. The isolation of *Salmonella* from litter can be a good indicator of contamination of carcasses when the animal goes to slaughter (Bhatia et al. 1979). Consumption of a single Salmonella infected beetle was enough to isolate *Salmonella* from the cloaca of 1 d old chicks. Recently outbreaks of a disease that causes lesions in the vertebra of chickens has been linked to the bacteria *Enterorococcus cecorum* (Armour et al. 2011). Morbidity can be high, and mortality has been recorded upwards of 10%, although 5 to 7% mortality may be more common in infected broiler houses (Devriese et al. 2002, Armour et al. 2011). With density of beetles 1000/ m^2 a typical poultry broiler house can support upwards of 2 million beetles per house (Arends 2003). The lesser mealworm can be contributing to the pathogen being able to survive between flocks of birds being raised in a broiler house, allowing the pathogen to spread from contaminated litter, and for the reinfection of birds.

E. cecorum was first identified as the causative agent enterococcus spondylitis (ES) in 2002 (Wood et al. 2002). The disease presents in chickens after approximately 3 wk with clinical signs of spinal compression and lower limb paralysis (Armour et al. 2011). Previous efforts to identify the environmental niches for *E. cecorum* inside a broiler house have been unsuccessful (Robbins et al. 2012, Borst et al. 2017.). Borst et al. 2017 identified the pathway for infection as beginning with intestinal colonization < 1 wk, followed by isolation of an identical pathogenic strain in the spleen by 1 wk. Isolation from a spinal lesion from an identical isolate between 6 to 10 wk. Current research has not elucidated the mechanism for which pathogenic *E. cecorum* strains gain entry into the bloodstream through the GIT, it has been postulated that microscopic abrasions from the lesser

mealworm's cuticle may allow entry through the GIT by bacteria if the beetle is consumed by young chicks (Dunford and Kaufman 2006).

Knowledge, attitudes, and practices (KAP) surveys can be useful tools to understand the thought process and practices of a particular group of people (WHO 2014). An extension program can use the information gathered from a KAP survey to tailor programming to address knowledge gaps and gain insight to how the surveyed approach a problem. As broiler growers are present daily and firsthand observe the impact of insect pests, such as lesser mealworms, they are important to alleviating the problems associated with these insects. The integrators however usually subcontract the control of these pests to outside groups who may apply insecticides when not needed and contribute to higher resistance levels in pest species. Closer integration between the integrator, the grower and the pesticide applicator can be an important step in a more effective pest management program.

While modern broiler houses are fully enclosed and airflow temperature are highly regulated, there still exists some variability locally inside poultry houses. Relative humidity (RH) inside a poultry house can fall between 50 to 70% (Reece and Lott 1982). Litter amendments have been used for decades to reduce ammonia emissions, and more recently, alleviate foot pad burns. Litter amendments act by reducing the pH of litter, or acidifying it, to prevent ammonia from escaping the litter. The pH is an important factor in the half-life of many insecticides and mixing tanks must be closely monitored for pH. It's not well studied how litter amendments and insecticides interact within an environment with wide variation in RH and pH.

The application of insecticides is limited to applications between periods when birds are placed inside a broiler house (Tomberlin et al. 2008). The number of chemical classes with different modes of action is limited and subsequently only limited formulations are available for use inside broiler houses. The overuse of a limited number of compounds has led to high resistance in the lesser

mealworm to compounds, especially pyrethroids. More effective treatments, or combinations of treatments, must be implemented to increase the mortality. To alleviate ammonia emissions from litter, litter amendments like sodium bisulfate, known as Poultry Litter Treatment (PLT®) have been used for decades (Blake and Hess 2001b). PLT has been shown to have insecticidal properties when populations of lesser mealworms were compared between PLT® and Control out to 34 d (McWard and Taylor 2000).

There are limited number of chemicals available for treatment of the lesser mealworm in poultry houses. Additionally, treatment is limited to when birds are not inside the houses, which is typically about every 45 d for a period of 1 to 2 wk. The limited chemical classes and overuse has led to high resistance in the lesser mealworm meaning most individuals will survive treatment. The abundance of hiding locations, inside the litter and within the insulation further limits contact of these insecticides to probably minutes or less. Insects who have developed resistance mechanisms are able to recover from contact with insecticides. Limited studies have focused on the individual and population level responses of insecticides exposed survivors. Mating frequency and increased reproductive rates have been measured in weevils and stinkbugs (Guedes et al. 2010, Haddi et al. 2016). What happens to the lesser mealworms overall population rate as a result remains to be studied.

OBJECTIVES

1. Determine if the lesser mealworm, *Alphitobius diaperinus*, can function as a host of *Enterococcus cecorum* in poultry houses

2. Establish a baseline of knowledge and practices by Oklahoma broiler growers related to waste management and litter beetle management

3. Determine if insecticide and litter amendment interactions affect resistance in susceptible and field populations of lesser mealworm and identify ovicidal responses in field populations from sublethal insecticide exposure

CHAPTER II

REVIEW OF LITERATURE

The lesser mealworm, or *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), is an important cosmopolitan pest of poultry facilities The lesser mealworm disseminates pathogens, reduce weight gain of birds, and cause damages to broiler houses (Axtell and Arends 1990). High beetle density in the litter results in late-stage larvae seeking pupation sites inside the insulation of the broiler house walls. The cumulative chewing of the insulation over time by lesser mealworm larvae reduces the insulating ability of the insulation and decreases heat retention. Lesser mealworm insulation damaged broiler houses have reported energy cost 67% greater than houses without damage (Geden and Hogsette 1994, Loftin 2011). Pathogen transmission is a concern as the lesser mealworm has shown to be a competent reservoir for several pathogens. These include *Salmonella*, *Escherichia*, and the leucosis virus which causes Marek's disease. There are a few methods used to indirectly detect population density changes of lesser mealworms inside a poultry house using tube or flat traps, and sentinel insulation. Once a population of lesser mealworms is determined to be of concern for economic and flock health reasons, then cultural, biological, and chemical control methods can be used to reduce the population level. The broiler industry most often relies on chemical control to reduce lesser

Mealworms inside broiler houses, other techniques are occasionally used.

Description of Life Stages

All life stages of the lesser mealworm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) can be observed inside the litter layer (Axtell and Arends 1990). The moisture and spilled feed attract higher concentrations near the feeders and water lines inside the house.

Adult. Adult lesser mealworms are approximately 6 mm (5.8 to 6.3 mm) in length (Dunford and Kaufman 2006; Fig. 2.1). The adults are shiny in appearance varying in color from black to brownish black. The total lifespan of the lesser mealworm can approach a year (Axtell and Arends 1990), with the adult stage alone occupying 3 to 12 months (Dunford and Kaufman 2006). A single female lesser mealworm can lay up to 2000 eggs during her lifespan, but the average number of eggs laid typically is 200 to 400. Sex determination is conducted in this life stage by observing the apical tibial spurs of the metathoracic or metathoracic legs (Esquivel et al. 2012). Males have a crossed orientation and females have parallel spurs (Fig. 2.2).

Egg. The eggs are approximately 1.5 mm in length with variation in color from white to tan (Dunford and Kaufman 2006; Fig. 2.3). Eggs are laid by the female in groups or “clusters” (Axtell and Arends 1990). At 25°C, a female will lay approximately 4 to 7 eggs daily (Rueda and Axtell 1996). After 4 to 7 days the larvae will emerge from the eggs (Dunford and Kaufman 2006).

Larva. Freshly emerged larvae will be milky in color (Francisco and Prado 2001, Dunford and Kaufman 2006). After the third instar the larvae darken through its latter stages (Francisco and Prado 2001). The pale color will return during ecdysis but go back to a darker tone within a day. The larvae resemble wireworms (*Tenebrio sp.*), with 3 sets of legs and an abdominal segment that

tapers to the rear (Dunford and Kaufman 2006). The instars vary in length depending on instar from 7 mm to 11 mm (Fig. 2.4). Larval development usually occurs over 5 to 8 wk with the number of instars ranging from 6 to 10 or more (Axtell and Arends 1990); however, it has been noted to take nearly 100 d to complete the larval stage dependent on temperature and food quality (Dunford and Kaufman 2006). Ideal development temperature is between 30 and 33°C at around 90% RH (Rueda and Axtell 1996).

Pupa. The pupae range in size from 6 to 8 mm with similar variation in color as the eggs from white to tan (Dunford and Kaufman 2006; Fig. 2.5). The legs are tucked into the body and not used to move during this stage of development. The pupal stage will last anywhere from 7 to 12 d (Axtell and Arends 1990). Late instar larvae may pupate in the soil but can also go into the poultry house walls and pupate in the insulation (Axtell and Arends 1990). The movement of the larvae and adults away from the litter has been observed as larvae crawl up unpainted wood support posts (Kaufman et al. 2008). This occurs almost exclusively during hours of darkness by the late instar larvae and adults (Geden and Axtell 1987). The population density of lesser mealworms in the litter and lack of availability of soil pupation sites determines whether the late-stage immature will climb into the walls of the houses to pupate.

Biology. The lesser mealworm is thought to originate from Sub-Saharan Africa (Geden and Hogsette 1994, Lambkin 2001). Lesser mealworms naturally occur in nests of birds and bats feeding on droppings and animal parts, such as feathers and on the whole carcass (Lambkin 2001). The hot and humid climate that lesser mealworms originated from is replicated in poultry houses throughout the world. Lesser mealworm beetles are also common pests of grains and grain products, animal bones, and oilseed products (Green 1980).

Distribution. Lesser mealworms have been observed in poultry buildings in the United States, Britain, Denmark, and Australia (Green 1980, Lambkin 2005). Global shipping trade is likely responsible for the worldwide dispersal of this grain product pest (Green 1980). British authorities in 1954 discovered live specimens of a related species, *Alphitobius viator* Molsant and Goldart in ship cargoes originating from Africa. Other specimens were discovered in a ship from Sierra Leone containing ginger, and inside a bag of chilies from Lagos, Nigeria.

Economic Damage and Injury. Economic losses occur in the broiler industry through increased energy costs associated to physical damage and increased costs for medical care, death losses, and decreased productivity of the flock. As the density of beetles in the litter increases, greater numbers of adults will climb the walls and pupate in the insulation of the poultry house (Axtell and Arends 1990). Holes resembling adult emergence holes can be seen regularly inside insulation of beetle infested houses (Fig. 2.6.) Over time the insulation rating decreases leading to energy cost increases. Reports of up to 67% higher energy costs have been noted (Geden and Hogsette 1994, Loftin 2011). Despins and Axtell (1994) observed reductions in weight gain and increased mortality in poults that fed on larvae infected with enteritis. This study and others by Despins and Axtell (1995) and Axtell and Arends (1990) concluded that poultry readily consume lesser mealworms in the litter. A less thought of, but important, economic concern is civil liability when large numbers of lesser mealworms disperse from broiler house litter being applied to fields for agricultural fertilizer (Miller 1997). Beetles have been observed leaving litter spread on a farm field for up to 7 d after (Calibeo-Hayes et al. 2005). Efforts to curtail this mass exodus of adult lesser mealworms is necessary to prevent negative public perception and lawsuits, but also preventing the spread of disease-causing pathogens to nearby poultry facilities.

The lesser mealworm has been reported to be a reservoir for several pathogens (Axtell and Arends 1990, Despins and Axtell 1994, Goodwin and Waltman 1996, Bates et al. 2004,

Crippen et al. 2009). Of particular threat to the poultry industry are *Salmonella* and *Escherichia* but also fungi and leucosis viruses (Axtell and Arends 1990). Larvae and adults can transmit *Camplobacter spp.* and *Salmonella spp.* between flocks in a house (Hazeleger et al. 2008). Adults can shed *Escherichia coli* in frass for 10 d after exposure and larvae for 6 d after inoculation (McAllister et al. 1996). The lesser mealworm is reported to be a carrier for coccidia inside a poultry house (Reyna et al. 1983).

Spondylolisthesis ("Kinky Back") and Spondylitis Disease

Spondylolisthesis disease has been reported in Canada (Riddell and Howell 1972), Australia (Kelly 1971), Belgium (De Herdt et al. 2009), and the United States (Aziz and Barnes 2007, Armour et al. 2011). Spondylitis, also known as Enterococcus Spondylitis (ES), disease is caused by a bacterial infection of the thoracic vertebra usually affecting birds 6 to 10 wk of age (Aziz and Barnes 2007). The birds inflicted with bacteria related arthritis will exhibit a posture where inflicted birds will sit on the hocks with the legs up and back arched (Armour et al. 2011; Fig. 2.9). Kinky back disease causes symptoms in birds mimicking that of Spondylitis, however its cause is rooted in a skeleton disorder or possibly too heavy of a breast weight for ligaments to support (Payne 2013). Postmortem investigations of Spondylitis affected broilers will reveal lesions on the thoracic vertebra (Aziz and Barnes 2007, Armour et al. 2011; Fig. 2.10 and Fig. 2.11). These lesions reduce the space between the last 2 ribs that will eventually cause a collapse of the vertebra (Aitchison et al. 2014). *Enteroccocus cecorum* was cultured from the lesions from ES positive birds in several studies (Aziz and Barnes 2007, Armour et al. 2011, Aitchison et al. 2014.) Kinky back, Enterococcus Spondylitis, and scoliosis will have similar clinical presentations therefore requiring post mortem investigation for proper identification and subsequent corrective action (Payne 2013).

The sixth vertebra (T6) is the only vertebra that is freely articulating in poultry (Jung et al. 2018). The notarium is composed of vertebrae that are fused and support the upper portion of

the animal, and the synsancrum is composed of fused vertebra supporting the lower portion. The Free Thoracic Vertebra (FTV) is the pivot point for the notarium and synsacrum and therefore is subject to repeated stress from various directions which can eventually create microfractures (Wideman Jr 2016).

Males appear to be impacted more by the disease, likely because of the increased weight of male sexed birds on the spine according to Armour et al. (2011). The authors determined that mortality associated with this disease was 5 to 7%, whereas another study in North Carolina measured 5% mortality (Aziz and Barnes 2007). Histamine, one of many chemicals associated with elevated stress levels, has been observed to have greater effects on male broilers and may be a factor in the sex related association of the disease (Džaja et al. 1996).

Enterococcus cecorum, originally placed in the *Streptococcus* genus, was isolated from chickens in 1983 (Devriese et al. 1983). The bacteria are a common inhabitant in the gastro intestinal tract of poultry (Armour et al. 2011). By the age of 12 wk, *E. cecorum* is the most common bacteria recorded in the intestine, however it is non-existent at day one (Devriese et al. 1991). But by 3-4 weeks of age *E. cecorum* accounts for 7% of the gut fauna. Initially the 2 dominating species in the gut fauna are *E. faecalis* and *E. faecium*, but by 3-4 wk *E. faecalis* and *E. faecium* make up only 3 and 46 percent, respectively. Further investigation through the use of Pulsed Field Gel Electrophoresis (PFGE) has identified pathogenic strains that are responsible for the lesions on the vertebra (Borst et al. 2012). A study of 113 isolates from 22 epidemiologically different outbreaks of disease reported that all strains were > 72% similar suggesting clonal strains were responsible for the outbreaks. Boerlin et al. (2012) hypothesized the increase in *E. cecorum* infections was the results of changes in host or environmental factors, or an emergence of individual clones with greater pathogenicity which aligns with Borst et al. (2012). When Borst et al. (2012) compared the genotypes taken from lesions and in the caeca, the *E. cecorum* isolates rarely were similar. Of the samples taken from the caeca, the diversity was much greater than that recorded in isolates taken from lesions.

Research into 113 isolates of *E. cecorum* from Canadian cases determined birds with clinical symptoms were all from a single clonal group (Boerlin et al. 2012). Non-infected birds had more diversity in *E. cecorum* isolates taken within the same bird and between birds on the same farm or different farms. More studies on *E. cecorum* strains needs to be conducted to see if certain strains have more pathogenicity. This will aid in detection, treatment, and management of the disease in broiler flocks.

There are several management techniques that can help reduce the prevalence of this disease (Gingerich 2009). These include disinfecting the houses, composting litter between flocks, and cleaning the water lines. Starting with the first week of life and continuing regularly throughout the grow-out period the administration of either amoxicillin and/or tylosin stopped subsequent groups of birds in the broiler house from getting ES (De Herdt et al. 2009). Before the antibiotic treatments, 9 of the 10 houses in the experiment had continual problems with the disease for up to 1.5 years.

Enterococcus faecalis and *E. faecium* also account for the majority of bacteria isolates collected from box liners, feed, and litter from a study in Georgia (1,341 out of 1,478 total; Debnam et al. 2005). *E. cecorum* was not isolated on any surfaces tested in the study. The authors, however, did not test the beetles inside these houses for pathogens that the beetles may be carrying. The cuticle from the beetles can cause microscopic abrasions that may permit pathogens a pathway to enter the bird's system (Dunford and Kaufman 2006). With an estimated density of 1000 beetles/ m^2 each broiler house could have populations exceeding 3 million. Th provide a reservoir for *E. cecorum* during grow-out and between flocks. Research needs to be conducted on whether the lesser mealworm can internalize *E. cecorum* into the GIT, and then expel the bacteria in beetle frass thus making it a competent vector for this pathogen.

Sampling and Scouting Procedures

There are few options when sampling broiler houses for lesser mealworms. These include

tube traps, sentinel insulation, and flat traps (Safrit and Axtell 1984, Geden and Hogsette 1994). Tube traps consist of a polyvinyl chloride (PVC) tube with rolled corrugated cardboard inside (Safrit and Axtell 1984; Fig. 2.7). Placing the tube on the litter for a given timeframe, a week for instance, allows lesser mealworms of all life stages accumulate inside the cardboard. Then the tubes are then collected and counts of the different life stages can be recorded. Sentinel insulation is another method for monitoring. This method uses a sheet of insulation attached to the inside post of the broiler house not enclosed within the wall (Geden and Hogsette 1994). This method allows easy visual tracking of lesser mealworm populations by observing increasing or decreasing numbers of entry holes into the insulation. The third common method uses flat traps of corrugated cardboard attached to walls or beams of the broiler house and beetles are counted on a regular basis. These traps assess the lesser mealworm population in an indirect manner. The higher density will increase the dispersal numbers of immatures and adults into the walls of the house (Geden and Axtell 1987) and subsequently increase numbers entering the traps placed throughout the house. It is important that a regular schedule for monitoring the traps be followed for counts be used to detect population density changes more reliably inside each house. The placement of the traps must be uniform throughout the broiler house as well. Strother and Steelman (2001) used geographic information system (GIS) to monitor the spread of the beetles as several monitored flocks grew out over several weeks. Methods such as these we can only estimate the population density. Arends (2003) estimates place beetle densities as high as 1000 m^2. An estimation based off removing 1% of litter from an 120,000-bird poultry house estimated the total lesser mealworm population at around 19 million (Kaufman et al. 2005). Most broiler houses in the United States, however only have approximately 20,000 birds and this would be greater than expected for a singular broiler house.

Management of the Lesser Mealworm

Cultural Control. One method to control the lesser mealworm would necessitate removal of the litter between each flock, but this is not economically feasible, and is only done usually every 4 or 5 flocks (Axtell 1999). Another method involves leaving the house unheated during winter months (Axtell and Arends 1990). The supercooling point (SCP), where all movement ceases due to cold temperature, had a mean of -12.3 ± 2.5 °C for both sexes of the lesser mealworm (Salin et al. 1998). For non-cold acclimated beetles, a mean SCP was recorded to be -8.9 ± 1.6°C (Renault et al. 2004). Another study recorded that a constant temperature of 0°C would cause mortality to half the population in 5-6 d (Lalouette et al. 2007). Similar results by Renault et al. (2004) revealed that at a constant 0°C non-cold acclimated beetles survived 5 d and the acclimated survived 10 d. At a constant 5°C, non-acclimated beetles experienced 100% mortality in 10 d and cold acclimated beetles were all dead by 14 d. The effectiveness of this control strategy depends on how cold the house will get and how long those temperatures can be sustained, but in colder climates these SCP can be obtained during winter months and if the doors are opened to the broiler house for approximately 2 wk. This strategy is hindered by the ability of the beetle to seek refuge in various habitats in the poultry house to stay warm. Salin et al. (1998) noted that larvae and adults were observed within the first 10 cm of the soil layer possibly taking refuge when the ambient temperatures in the poultry house dropped. This behavior may limit the effectiveness of this strategy.

Another method of controlling the lesser mealworm is the use of heat (Fields 1992, Salin et al. 1998, Fleurat-Lessard and Dupuis 2010). Salin et al. (1998) noted that control of the lesser mealworm could be achieved at 48°C and higher based on their work to find the heat stupor point or point before death where the insect becomes motionless. Most stored product pests, in which the lesser mealworm falls into, will eventually die at temperatures greater than 35°C (Fields 1992), due to enzyme imbalances that occur at high temperatures.

Ultraviolet (UV) radiation has been reported to cause death or various sublethal effects

when used on the larval stage (Faruki et al. 2005). As the larval instars increased in age, the larvae became less susceptible to UV treatment. 50% of the second instar larvae succumb by 72 h with ~24 min of UV exposure. Third instar larvae took ~215 min. of exposure to achieve 50% mortality. Sublethal exposure, with 4 minutes of exposure, reduced the mean fecundity in adults by 55.95% when exposed as second instars and 69.89% when exposed as third instars.

Pheromone research has been successfully used to lure adults and larvae to pitfall traps with aggregation pheromones (Singh and Johnson 2012). Bartelt et al. (2009) used gas chromatographic-mass spectrometric analysis of volatiles given off by feeding males and females to identify male specific aggregation pheromones [(R)-(+)-limonene, (E)- β-ocimene, (S)-(+)-linalool, (R)-(+)-duacene, and 2-nonanone]. Specifically, research has indicated that the 3 compounds: (E)- β-ocimene, (R)-(+)-duacene, 2-nonanone appear to elicit a behavioral response from lesser mealworms (Singh and Johnson 2012, Cossé and Zilkowski 2015). Interestingly the same composition proved non effective without the addition of a sixth component, (E,E)- α-farnesene suggesting that validation of pheromone profile effectiveness on a local population is required before being used for traps (Hassemer et al. 2016).

Biological Control. The use of living organisms to control *A. diaperinus* has been met with limited success. After studying protozoan infections in lesser mealworms inside poultry houses in North Carolina, Apuya et al. (1994) noted that the larval stages were more susceptible than adults and suggested using protozoans to control lesser mealworm populations. The author noted that management practices involving litter removal and environmental temperature changes in the period between when birds are placed in a broiler house may affect the prevalence of infection. Strains of *Beauveria bassiana* (Moniliales: Moniliaceae) were tested against the lesser mealworm to gauge its effectiveness as a control agent (Crawford et al. 1998, Geden et al. 1998). Two *B. bassiana* strains were taken from the field and tested against larvae and adults in both used and new litter (Crawford et al. 1998). The authors reported higher than 90% mortality in larvae at the

highest application rate (2.37 X 10^{11} conidia per square meter) after 2 wk in new litter. Used litter limited the effectiveness of the strains against the larvae to 84%. With the adults, the highest application rate achieved 60% mortality in new litter, but the mortality dropped to 33% in used litter. Another study reported that application of the conidia to the soil required 1/10th the dosage as would be required in the litter for complete larvae control (Geden et al. 1998). The young immatures were 1000 times more susceptible than the adults in their bioassays. There were concerns by the authors that the use of disinfectants between flocks to sanitize the houses, and high temperatures would have negative impacts on this control strategy. Geden et al. (1987) noted reductions in *Steinernema feltiae* (Nematoda: Steinernernatidae) persistence in the field after several weeks and subsequently percent mortality of the lesser mealworm went from an average of 79% in wk 1 post treatment to 11% by week 9.

Chemical Control. With limited cultural control successes and even fewer biological control techniques available, chemical control has become the most widely used form of lesser mealworm control in broiler houses. There are limitations to this control method as well. As Table 2.1 below highlights, there are relatively few chemical classes and compounds labeled specifically for the control of the lesser mealworm, and treatments can usually only be applied in between flocks (Tomberlin et al. 2008). Insecticides can be applied along the cracks and crevices or under the feed and water lines along the length of the house. After the broiler house has been prepared for new birds, sprayer trucks will apply insecticide usually in a broadcast spray from wall to wall (Fig. 2.8). Resistance has become an issue for the industry as some chemicals, such as permethrin, have been overused. In Texas, lesser mealworm individuals from 6 farms were exposed to 4 insecticides (Tomberlin et al. 2008). Four h after exposure to Befenthrin (Talstar WP™; FMC Corp., Philadelphia, PA) treated filter paper; mortality varied from 18.3 ± 4.9 to 100 ± 0 %, and by 24 h mortality was 8.00 ± 1.00 to 95.6 ± 4.3 %. Since mortality decreased between

the 4h and 24h observations, the authors suggest knockdown rather than mortality is a better descriptor when mortality is assessed within 24h since resistance leads to high recovery. At 24 h, Bifentrhin (Talstar Pro®, FMC Corp., Philadelphia, PA) exhibited similar resistance variation (6.7 to 100 % mortality). Lesser mealworm adults tested from several locations along the eastern USA were measured to be between ~2 to 10 % more resistant than the susceptible colony from Denmark (Hamm et al. 2006). Because of previous chemical use at a facility, subsequent generations may have differences in susceptibility. (Steelman 2008). With the lesser mealworms tested from Arkansas poultry facilities, topical insecticide bioassays indicated that the adults were more susceptible than the eighth instar larvae. However, residual studies of both life stages measured no discernable differences in susceptibility.

Insecticide classes that have been available for lesser mealworm control include neonicotinoids, pyrethroids, organophosphates, and spinosyns with 9 active ingredients spread across the classes (Elanco 2012). The different modes of action afforded by the different classes will help slow down resistance if a rotation is carried out periodically (Adams 2003, Tomberlin et al. 2008). Overuse of fenitrothion in Australia has led to high levels of resistance (Lambkin 2005). The resistance levels correlated to how long fenitrothion has been used on the broiler farms. The highest levels of resistance were as high as 79-fold and as low as 25-fold. A study of Australian broiler farms tested resistance build up to cyfluthrin beginning in 2000 and repeated in 2001, 2004, and 2005 (Lambkin and Rice 2006). The resistance levels increased throughout the years and were correlated to the continued use of cyfluthrin insecticides. Also, disinfectants should not be sprayed at the same time as the insecticide to avoid destroying the chemical property and effectiveness of either the insecticide or disinfectant.

If the litter is removed from the house, treating with insecticide before the litter is replaced, or treating the litter itself before placement inside the houses has been shown to be an effective method for controlling lesser mealworm larvae (Weaver 1996). Treatment of the soil layer if accessible is also an effective location to apply insecticide (Axtell 1999). Integrating

chemical control more with cultural control techniques such as windrowing may increase the effectiveness of both if used separately.

Conclusions and Future Directions

Limited availability of insecticidal compounds coupled with restricted access to applications has led to insecticide resistance issues that are common with the lesser mealworm in poultry facilities around the world. With primarily only 4 chemical classes used for lesser mealworm control, proper rotation of chemical classes is important to prolong the effective lifespan of available compounds until novel classes come to market.

Exploration of other combinations of cultural and chemical control techniques may offer promise in more productive ways of controlling beetle populations in facilities and should be the focus of future research. For example, commonly used litter acidifiers can reduce lesser mealworm populations (McWard and Taylor 2000), but more research of the interactions between insecticides and acidifiers is needed to understand how the use of these compounds fit into a pest management plan.

FIGURES

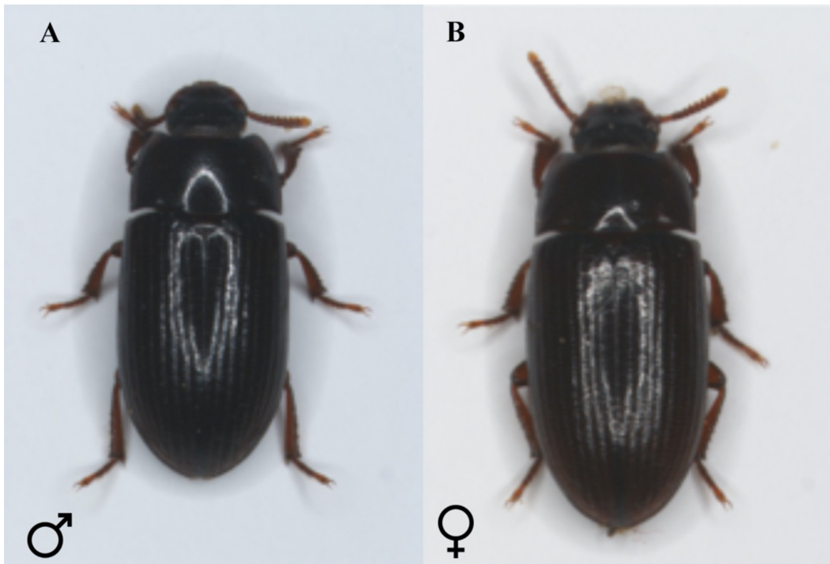


Fig.. 2.1. Adult lesser mealworm. A. Male B. Female. 10X zoom (Photographs by R. Grantham).

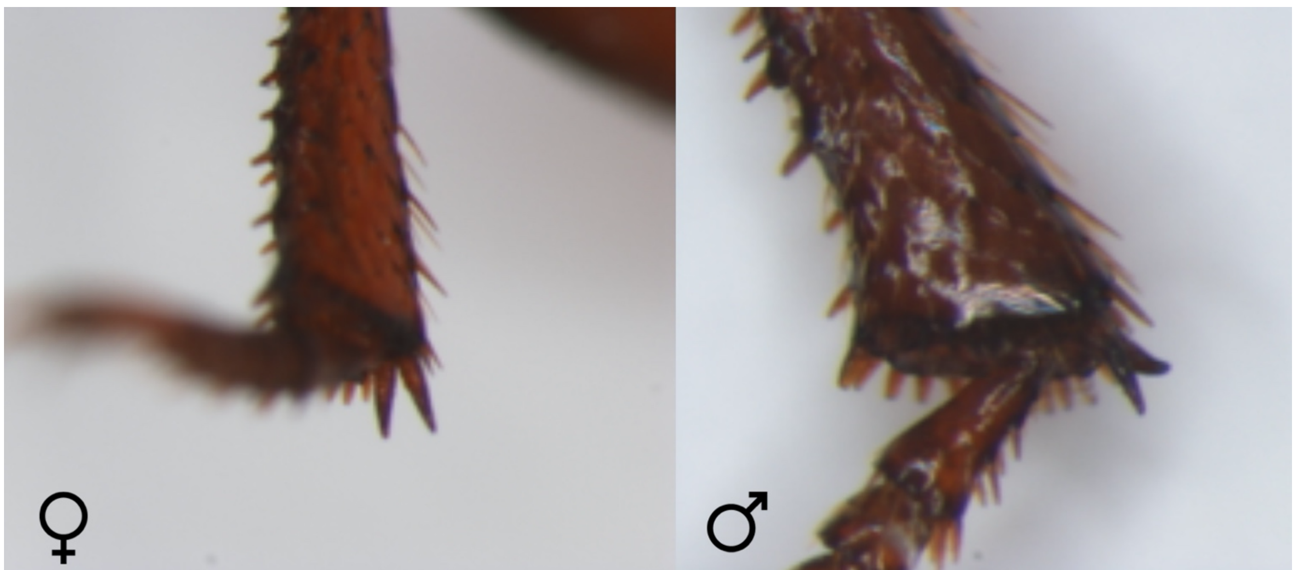


Fig.. 2.2. Apical tibial spurs used for sex determination in adult lesser mealworm A. Parallel orientation (female). Curved orientation (male) (Photographs by R. Grantham).

Fig.. 2.3. Lesser mealworm eggs (photographs by R. Grantham).

Fig.. 2.4. Several instars of the lesser mealworm. 7X zoom (photograph by R. Grantham).

Fig.. 2.5. Pupa of lesser mealworm. 7X zoom (photograph by R. Grantham).

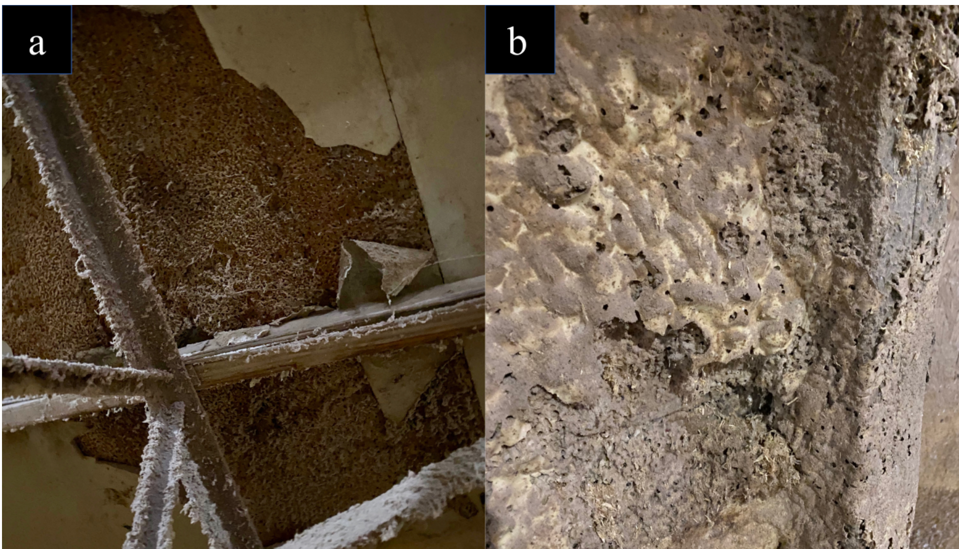


Fig.. 2.6. Rigid insulation panel damage (a) and spray foam insulation damage (b) caused by *Alphitobius diaperinus* larvae seeking pupation sites inside a broiler house. (photograph by B. Lyons).

Fig.. 2.7. *Alphitobius diaperinus* larvae emerging from rolled corrugated cardboard in Oklahoma State University colony (photograph by B. Lyons)

Fig. 2.8. Chemical sprayer truck applying insecticide inside a poultry house. (photograph by J. Talley)

Fig. 2.9. Lower limb paralysis caused by infection of *Enterococcus cecorum* lesions on the vertebra. Taken from (Armour et al. 2011)

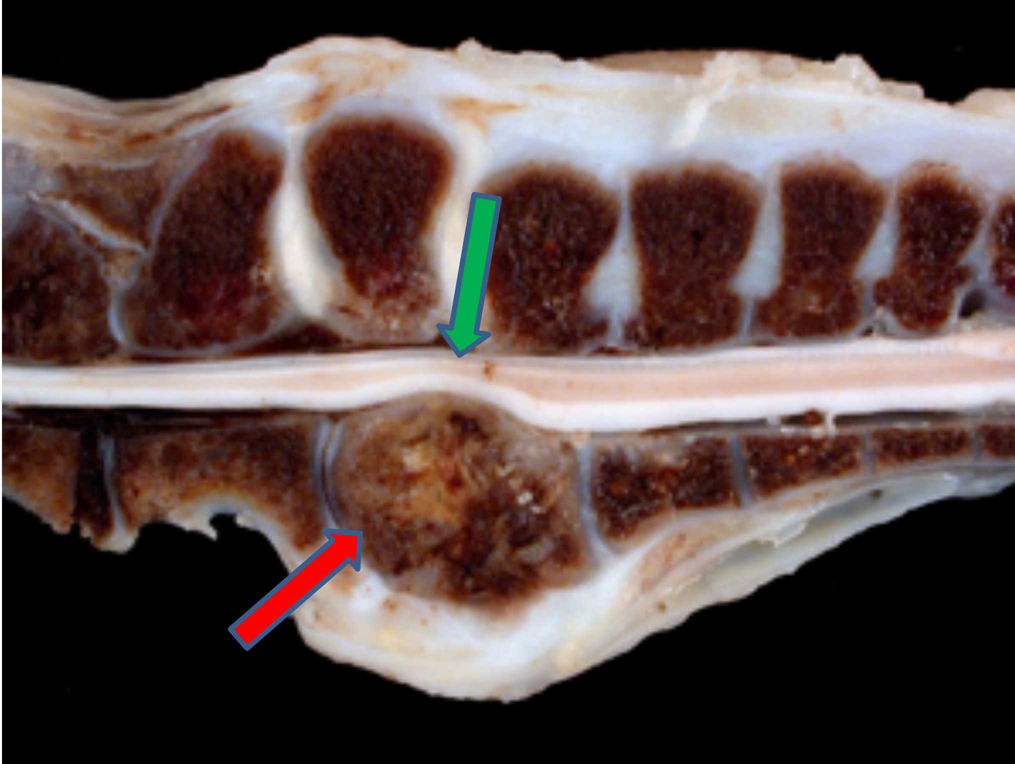
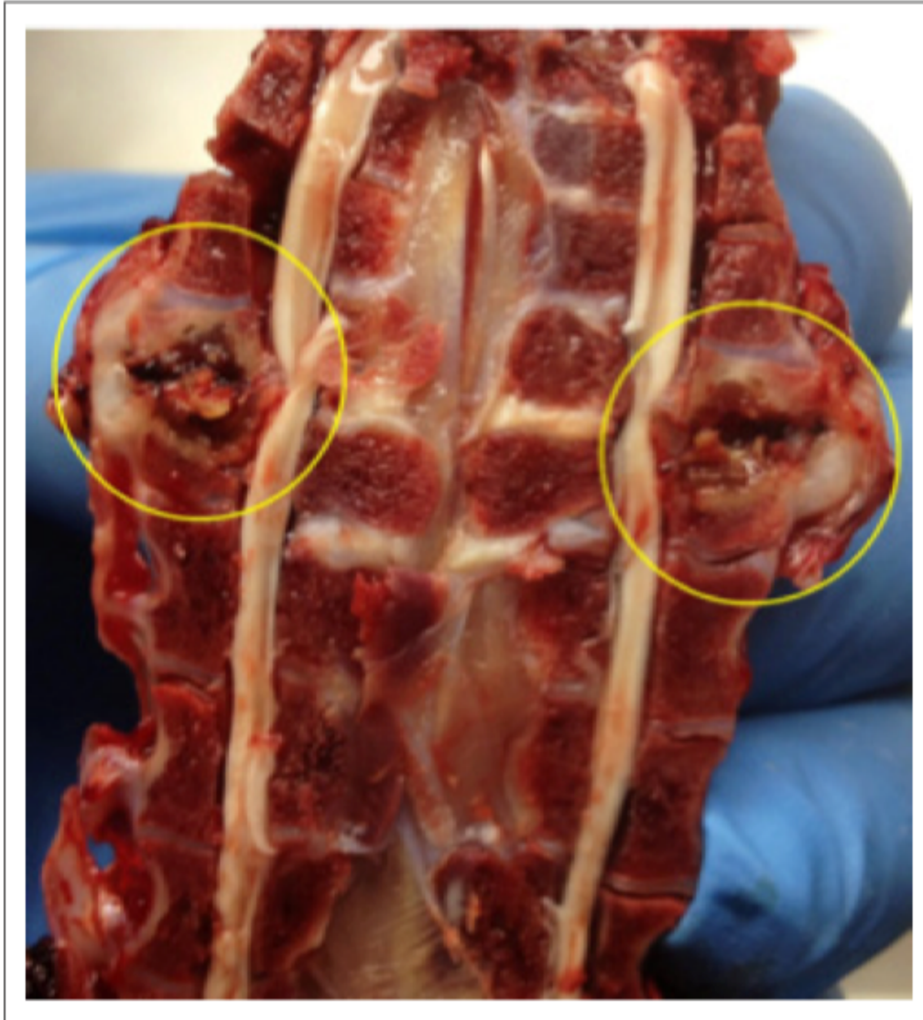


Fig. 2.10. Inflammation of seventh vertebra (shown with red arrow) causing compression of spinal column (green arrow). Image taken from (Gingerich 2009).



Source: Photo taken by Dr Petrus Poolman

Fig. 2.11. Cross section along the length of the vertebra column. Highlighted by the circles is the abscess causing inflammation and compression of the spinal canal. Taken from (Aitchison et al. 2014)

TABLES

Table 2.1. Insecticides used in the control of the lesser mealworm and various studies conducted evaluating the effectiveness of these compounds

	IRAC		
Category	Class	Insecticide	Reference(s)
1A	Carbamates	Carbaryl	Vaughan and Turner Jr (1984), Steelman (2008)
1B	Organophosphates	Chlorpyrifos	Hickmann et al. (2018)
	Organophosphates	Dichlorvos	Chernaki-Leffer et al. (2011)
	Organophosphates	Fenitrothion	Lambkin (2005)
	Organophosphates	Tetrachlorvinphos	Vaughan and Turner Jr (1984), Hamm et al. (2006), Kaufman et al. (2008), Steelman (2008), Singh and Johnson (2015)
3A	Pyrethroids	*Beta*-Cyfluthrin	Tomberlin et al. (2008), Lambkin and Furlong (2011)
	Pyrethroids	Bifenthrin	Tomberlin et al. (2008)
	Pyrethroids	Cyfluthrin	Hamm et al. (2006), Lambkin and Rice (2006), Kaufman et al. (2008), Steelman (2008), Lambkin and Furlong (2011), Singh and Johnson (2015)
	Pyrethroids	Cypermethrin	Steelman (2008), Chernaki-Leffer et al. (2011), Hickmann et al. (2018)
	Pyrethroids	Deltamethrin	Lambkin and Furlong (2011)
	Pyrethroids	*gamma*- Cyhalothrin	Lambkin and Furlong (2011)
	Pyrethrins	Permethrin	Vaughan and Turner Jr (1984), Steelman (2008), Tomberlin et al. (2008)
3B	DDT[a]	DDT	Steelman (2008)
	Methoxychlor[a]	Methoxychlor	Steelman (2008)
4A	Neonicotinoids	Imidacloprid	Singh and Johnson (2015)
5	Spinosyns	Spinosad	Lambkin and Rice (2007), Lambkin and Furlong (2014), Singh and Johnson (2015)
7C	Pyriproxyfen	Pyriproxyfen	Zorzetti et al. (2015)
13	Chlorfenapyr	Chlorfenapyr	Singh and Johnson (2015)
15	Benzoylureas	Triflumuron	Chernaki-Leffer et al. (2011)
3A/15	Pyrethroid/Benzoylureas	Pyrethroid/Triflumuron	Salin et al. (2003)

CHAPTER III

MECHANICAL TRANSMISSION BY *ALPHITOBIUS DIAPERINUS* (COLEOPTERA: TENEBRIONIDAE) AND PRIMER DESIGN FOR THE DETECTION OF *ENTEROCOCCUS CECORUM*

INTRODUCTION

Alphitobius diaperinus, (Panzer) (Coleoptera: Tenebrionidae) commonly known as the lesser mealworm is considered an indirect mechanical vector of bacterial pathogens. This beetle feeds partly on the grain intended for the birds, but also feeds on the feces expelled from the birds. As the beetles walk on feces contaminated with bacteria, the beetles spread those pathogens to the bird's feed and other surfaces in the poultry house.

Fecal samples to measure *Salmonella* contamination taken at the last week of grow out were 5% positive, yolk sac from day old chicks were 9% positive (Jones et al. 1991). A variety of serotypes of *Salmonella* has been isolated from various environments throughout the broiler production system would require a comprehensive control strategy if it were to be controlled. Of

all the locations in the broiler production system, the researchers revealed that feed mills to have the highest positive rate of *Salmonella* of the samples taken from their study. Included in their study was trapping and testing various insects including the lesser mealworm. *Salmonella* was isolated from the lesser mealworm in 4.5% of the samples (Jones et al. 1991). After a 20 mg/kg chlorine rinse of the lesser mealworm, the rate of *Salmonella* isolated decreased suggesting surface contamination is an important source of bacteria transmission in this system. The researchers concluded that insects are primarily mechanical carriers of *Salmonella*.

Despins and Axtell (1994) provided further evidence of the lesser mealworms functioning as mechanical vectors for transmission for pathogens is in turkey poults. Beetle larvae were exposed to droppings from commercial flocks exhibiting symptoms of acute enteritis. Then hatchling poults were each fed about 360 previously exposed beetle larvae or surface sterilized larvae over a period of 19 hours. Symptoms of enteritis began 2 d post exposure for those poults fed non sterilized beetle larvae. Enteroviruses were detected from the feces of the poults on day 2 and onward.

The horizontal transmission of *Salmonella* was demonstrated by Crippen et al. (2018) between the lesser mealworm and poultry manure. A 10^3 cfu/ml concentration was sufficient to transfer the bacteria from the lesser mealworm to the manure and then back to an uninfected lesser mealworm beetle. A 10^7 cfu/ml concentration on a beetle was able to spread high loads of *Salmonella* onto manure. The levels of *Salmonella* internally and externally did not significantly change over the course of 24 hours. This long duration of contamination may provide reinfection opportunities to other lesser mealworms and may also provide a way for the bacteria to persist inside a poultry facility as beetles walk on contaminated surfaces and consumes contaminated manure (Crippen et al. 2012, Zheng et al. 2012).

Mechanical transmission is commonly associated with pathogens carried on the surface of the host, but also includes the passage of bacteria through the gut without multiplication (Foil and Gorham 2004). The lesser mealworm has been observed to mechanically transmit *Salmonella typhimurium* (McAllister et al. 1995). External and internal transmission of *Escherichia coli* has been demonstrated by the lesser mealworm (McAllister et al. 1996). *Escherichia coli* was expelled in the feces for 6 d by larvae and 10 d by adults. Larvae were more likely to cause positive cloacal swabs for Congo red-binding *E. coli* than adults in 1 d old chicks.

Enterococcus cecorum, originally described as *Streptococcus cecorum* in 1983, was first isolated from poultry cecal flora (Devriese et al. 1983). Once thought to be strictly a commensal species, pathogenic strains that cause bacterial disease in poultry have recently been identified (Dolka et al. 2016). The bacteria have been linked to lesions on the vertebra between the 5th and 7th vertebra causing inflammation and paralysis of lower limbs and eventual death of the bird (Armour et al. 2011). Mortality was estimated to be between 5 to 7% of the flock, affecting primarily males. An outbreak of this disease in a Dutch broiler farm had higher mortality approaching 10% with the first lameness cases seen around 19 days of age (Devriese et al. 2002). This outbreak spread through all of the houses on this particular broiler farm and was primarily affecting healthy birds. One objective of this study was to determine if the lesser mealworm, *Alphitobius diaperinus,* can be a potential host of *Enterococcus cecorum* in poultry houses.

The ability to specifically detect pathogenic strains from both healthy and morbid birds is vital for biosecurity protocols and developing a primer set is vital to determine the presence of this pathogen in flocks. Quantitative Polymerase Chain Reaction (PCR) requires a properly designed set of primers to be successful (Thornton and Basu 2011). Key elements for highly specific primers include primer length (18-24 bp), proper melting and annealing temperatures, product size (80-150 bp), appropriate Mg++ concentration for the dye used and repeats less than 4 di-nucleotides (Thornton and Basu 2011). The melting temperature should fall within the 59-

68°C range, however optimal would be considered between 63-64°C. Each of the primer pairs should be within 1°C of one another as well. The annealing temperature for the PCR should be 59-60°C. The optimal annealing temperature should be 5-8°C under the melting temperature of the primers (Ochoa Corona 2014). The second objective of this study was to develop a quantitative primer set to detect the *E. cecorum* pathogen in environmental samples.

Materials and Methods

Bacterial Strain. The *E. cecorum* strain utilized for this study was isolated from an Arkansas poultry broiler house with active cases of Enterococcus Spondylitis. Samples were sent to Oklahoma Animal Disease Diagnostic Lab (OADDL) and identified as *E. cecorum* in 2015. The colony was subcultured onto Columbia CNA blood agar (Hardy Diagnostics, Santa Maria, CA) and stored at 37 °C at the Institute for Biosecurity and Microbial Forensics at Oklahoma State University.

Primer Design. Primer design protocol was adapted from Thornton and Basu (2011) and Ochoa Corona (2014). PCR protocol was adapted from Palumbi (1991). *Enterococcus cecorum* 16s2 gene sequences were obtained from National Center for Biotechnology Information (NCBI) and designed using Primer3 Software (Untergasser et al. 2012).

Primer Analysis. Primer performance was analyzed using mFold software (Zuker 2003) and Primer3. The PCR product subjected to NCBI nucleotide BLAST (Blastn) (NCBI, Bethesda, MD) to identify potential products.

Primers. 16s2 left and right primers were obtained from Invitrogen (Waltham, MA) and reconstituted for PCR gene amplification.

Left (forward): CTCGTGTCGTGAGATGTTGG.right (reverse): ACGTGTGTAGCCCAGGTCA

Beetles. Beetles were originally obtained from a poultry farm in eastern Oklahoma and kept in colony at Oklahoma State University. Beetles were reared at 27°C 16:8 light:dark cycle and fed Dog Food (Kibble's 'n Bits, Big Heart Pet Brands, Orrville, OH), 5 Grain Scratch (Red River Commodities, Fargo, ND). Fish Meal (Omega Protein Inc., Reedville, VA), Wheat bran (Siemer, Teutopolis, IL) and red apples sliced 2-4mm thick fed *ad libitum*.

Beetle Transmission

Exposure Plates. Liquid suspension with 1mL of peptone water was made from *E. cecorum* plates in colony. Then 100 µl of suspension was plated onto blood agar and incubated for 24 h at 37°C. This was repeated for a total of three *E. cecorum* exposure plates. For acquisition control 100 microliters of Phosphate Buffered Saline (1xPBS) was lawned on five blood agar plates. 100 microliters of peptone water were plated onto 5 blood agar plates for sterilization validation. Two colonies of bacteria were cultured for each peptone rinse and incubated for 24 h at 37 °C.

Beetles were surface sterilized using methods modified from Crippen and Sheffield 2006. Five beetles were immersed in 1ml EtOH and vortexed for 5 seconds, and then the fluid was drained. After 5 minutes to allow for evaporation of EtOH, the beetles were immersed in Sporgon® (Decon Labs Inc., King of Prussia, PA) for 5 sec and fluid was again drained. Then the beetles were immersed in distilled water and vortexed for 5 sec and fluid was drained. The distilled water rinse was repeated and then the beetles were transferred to a sterile Petri dish with only viable beetles utilized for the bacterial exposure. Five beetles were allowed to walk on a sterile blood agar plate for 30 min acquisition control. Five adult beetles were allowed to walk on either peptone or *E. cecorum* exposed blood agar plate for 30 min, then transferred to sterile blood agar plates for another 30 min. Afterwards, beetles were transferred off dishes with sterile forceps and destroyed. These treated plates were taped shut and placed inside an incubator for 24 h at 37°C for 24 h. Petri dishes lids were taped shut. After 24 h, bacteria were visually inspected

for bacterial growth presence and description of growth. The *E. cecorum* plates were replicated three times.

Quantitative PCR. The template was made from a 100 µl suspension from two colonies of *E. cecorum and* was mixed with 20ul of RNase-Free Water (Qiagen, Hilden, DEU) 25 µl of PCR master mix with GoTaq Green®(Madison, WI), 5µl of 5µm left and right primer to make a 50µl volume for each reaction. PCR was performed with the following program: Initial denaturation at 94℃ for 5 min, followed by 40 cycles of denaturation at 94℃ for 25 sec, annealing at 57℃ for 25 sec, and elongation at 72℃ for 25 sec. A final extension followed at 72℃ for 4 min and then the product was held at 20℃ for 5 min. PCR product was electrophoresed on 2% agarose gel using TAE buffer at 80V for 100 min. The gels were loaded as follows: 5µl 1kb ladder, 1ul sample for intermediate lanes, 5ul 1kb ladder

PCR Product Purification. A Quigen DNA Purification Kit (Thermofisher Scientific, Waltham, MA) was used to purify the PCR product and was followed by nanodropper (Thermofisher Scientific, Waltham, MA) to verify purity of sample by measuring uv absorbency.

RESULTS

Beetle Exposure to plated E. cecorum Figure 3.1 shows examples of bacterial growth post exposure to inoculated beetles exposed to *E. cecorum* plates and the resulting “beetle tracks” when placed onto blood agar plates. Identification of *E. cecorum* was done phenotypically for blood agar plates using Armour et al. 2011 (See Fig. 3.2) and Dolka et al. 2016. Beetle tracks were identified as pathways of bacteria growth that transects the dishes or along the edge of the Petri dish where the lid meets the agar. These locations correlate to beetle movements observed during the experiment. Growths of bacteria identified as beetle track pathways or had opaque growths that are small and grayish with α-hemolysis or greening around the colonies, typical of growth on Columbia CNA agar with 5% sheep’s blood (Dolka et al. 2016).

The peptone rinse dishes had a milky-white lawn on two dishes and an opaque lawn. The fifth dish had no growth. Surface sterilized beetles had inoculated these dishes for 30 minutes. The growth indicates that the sterilization technique was not effective, however none of the bacterial growth phenotypically matched *E. cecorum.* The growths on the acquisition control dishes were opaque with a beetle track. Three acquisition control dishes had white and circular growths. One acquisition plate had a growth similar to a Venn-diagram shape.

All 15 dishes exposed to inoculated beetles had bacterial growths resembling *E. cecorum.* Of those dishes, 53% had *E. cecorum* growths resembling a track on the dish where the beetle was observed walking and is in line with previous observations of beetle movement on dishes. *E cecorum* A-1 had an opaque 3mm growth and white irregular growths. Dish *E cecorum* A-2 had opaque circular growths with two separated colonies. Dish *E cecorum* A-3 also had beetle track with opaque "bullseye" growth ~7mm in diameter. Dish *E cecorum* A-4 had innumerable colonies with no beetle track. Dish *E cecorum* A-5 had innumerable opaque growths and a beetle track. Dish *E cecorum* B-1, B-2, and B-3 all had opaque growths but only dish *E. cecorum* B-2 had a beetle track. Dish *E cecorum* B-4 had ~6x7 mm "egg shaped" growths and a beetle track. Dish *E cecorum* B-5 had opaque colonies with a contaminant colony that had cleared the blood from the agar. This dish also had beetle tracks. Dish *E cecorum* C-1 had opaque innumerable colonies with a contaminant colony that had cleared the blood from the agar as well. Dish *E cecorum* C-2 had a 6 x 7mm growth area with a beetle track. Dish *E cecorum* C-3, *E cecorum* C-4, and *E. cecorum* C-5 had opaque innumerable colonies, except dish 25 did not have a beetle track.

Primer Design and Analysis. For primers designed for detection of *E. cecorum* and their characteristics please refer to Table 3.2. The PCR product with these primers should be 163 bp in length. The forward primer is 20bp in length and 19bp for the reverse. The melting point is ~60°C for both primers and the reverse primer has 4 secondary structures whereas the forward

has 3. The quality analysis from the nanodropcan be seen in Table 3.3. According to Thermofischer's technical bulletin (2012) to have sufficient nucleic acids the concentration should exceed 10. Our results indicate the concentration of nucleic acids in our PCR product was 10.9 ng/µl. The 260/280 ratio of 2.07 is above 1.8 which is considered a pure sample of DNA but a wavelength inaccuracy reading by the machine can eschew the ratio by 0.4 which my account for this high ratio. Overall, the quality and concentration of the PCR product was good.

mFold estimates that around 60°C, the melting temperature of the primers, that there is both ssDNA and bacteria bound together. It's at this point where 50% of the DNA is dissociated at this temperature. At 3°C to 5°C below that temperature is the annealing temperature, where half the primer is expected to be binding the target.   As both the annealing and melting temperature are similar, the sensitivity of the PCR is expected to be good and produce good yield of product.

Primer blast results show 16S ribosomal RNA genes of 29 *E. cecorum* strains. Blast n returned four results with 99% identity match to *E.* cecorum 16S ribosomal RNA gene. Two were complete sequences and two were partial. Umelt estimates the PCR product will have a melting curve peak at 87.5°C. Using high resolution melting point analysis to measure the fluorescence you would be expected to find a single peak at 87.5°C to verify the product is pure *E. cecorum*.

Quantitative PCR. Successful demonstration of *E. cecorum* detection using 16s2 primers is in Fig 4.3. The DNA bands labeled Colony PCR are clear and are approximately 163bp showing the primers were able to amplify DNA taken from two separate colonies (Fig. 4.3).

Discussion

The beetle surface sterilization technique was modified from Crippen and Sheffield (2006). In their paper, the researchers noted the exoskeleton of the lesser mealworm was difficult

to sterilize due to the irregularity of the surface. Their technique was focused on removing surface contaminants for studies of internalized bacteria, whereas this mechanical transmission experiment required living individuals. To completely sterilize individual beetles, Crippen and Sheffield (2006) used ethanol followed by hydrogen peroxide or hydrogen peroxide/paracetic acid rinses. The surface sterilization technique used in this study reduced sonication from 2 min to 5 sec intervals was not sufficient to get rid of all surface bacteria, however resulting colonies on the control dishes did not resemble *E. cecorum* colony morphology. Further modification of the surface sterilization technique will be required for studies that need the survival of individuals and result in a completely sterilized surface of the beetle.

PCR confirmation of *E. cecorum* from the exposure experiment was not possible using primers outlined in Jackson et al. (2004). Subsequently, we designed a new primer set and verified with PCR and gel electrophoresis that we could identify *E. cecorum*. For this study however we only had to rely on phenotypic identification. The colonies resembled those of confirmed *Enterococcus spp.* but genetic confirmation is necessary.

Adult lesser mealworm beetles can live for a year and each female can oviposit hundreds of eggs (Axtell and Arends 1990). The population density within a single broiler house has been estimated to be around 1000/ m^2 (Bates et al. 2004). In a badly infested poultry house at this rate a large poultry house may have upwards of 3 million beetles. The beetles are attracted and readily feed on the bacteria laden feces from poultry (Despins and Axtell 1994). Transmission of *Salmonella* to broilers from contaminated adults and larvae has been demonstrated by Roche et al. (2009).

Another tenebrionidae stored product pest, the red flour beetle, was recorded to acquire *Enterococcus faecalis* and transfer it to sterile poultry feed and cattle feed over the course of several days post infestation (Channaiah et al. 2010). Previous studies have also recorded the

presence of *Enterococcus* on the surface of lesser mealworms captured from feed mills (Larson et al. 2008). The lesser mealworm accounted for just four of the approximately 300 beetles tested, but the beetles all had positive growth of bacteria on m-Enterococcus® agar (Remel; Lenexa, KS) . The blood agar experiment demonstrated that a lesser mealworm beetle having a 30-minute contact with a contaminated surface could disseminate the bacteria in the litter as it moves about the house. The moisture, pH, temperature, dust, and other environmental conditions may affect pathogen spread in field conditions.

Mechanical transmission by beetles can be a method of spreading *E. cecorum* and other pathogens inside a poultry house. Further research is needed to explore trophic transmission of *E. cecorum* inside the lesser mealworm beetle to see how long the pathogen can survive inside the beetle and how long it can be expelled in its frass and infect other beetles and birds. Much research has already been conducted on the common poultry associated bacteria, *Salmonella*, where the lesser mealworm was able to maintain the bacteria in the gut and it was able to persist through metamorphosis (Crippen et al. 2009, Crippen et al. 2012). *Salmonella* and *Camplyobacter* were determined to be transmittable by lesser mealworm larvae and adults from one flock to the next (Hazeleger et al. 2008). A previous study by this group of researchers discovered a correlation between positive *Campylobacter* beetles and positive *Campylobacter* flocks (Skov et al. 2004). Using pulse field gel electrophoresis (PFGE) the researchers connected *Salmonella indiana* strains between two consecutive bird flocks and *S. indiana* contaminated beetles. Re-exposure of birds throughout the grow out period, and if the lesser mealworm is a reservoir of *E. cecorum* can help maintain the pathogen inside the broiler house between flocks. Even with windrowing or other methods to sterilize the surface of the litter and house, the beetles inside the walls can lead to recontamination of the next flock.

When developing our primer design the primer length was 20 and 19, which is well within the ideal range between 18 and 24. The number of secondary structures the primer can

undergo is 3 and 4, which is acceptable. Taq Polymerase used in the master mix for PCR attaches to linear strands of primer DNA. When it is in a secondary structure Taq will not bind and will remain in the solution. The melting temperature of the primer falls within the acceptable range but is not in the ideal 63-64℃ range. The melting temperature is around 60℃ which makes the annealing temperature between 55℃ and 57℃. The annealing temperature is important as it indicates when half of the primer is binding to the DNA. The primers are within 1℃ of each other which is ideal. The Nanodropper indicates the PCR product sample is of good quality and concentration.

The testing conducted on various computer programs indicates that the PCR product should be *E. cecorum*, however further verification is needed by analyzing the sample using a high-resolution melting (HRM) technique (Rotorgene, Qiagen, Hilden, DE). uMelt indicates that the sample should have a melting temperature at 87.5℃. If there was a mutation or if the sample was another species, the high sensitivity of the HRM should indicate a different melting temperature. Impurities would lead to a second, sometimes smaller, peak in the output.

FIGURES

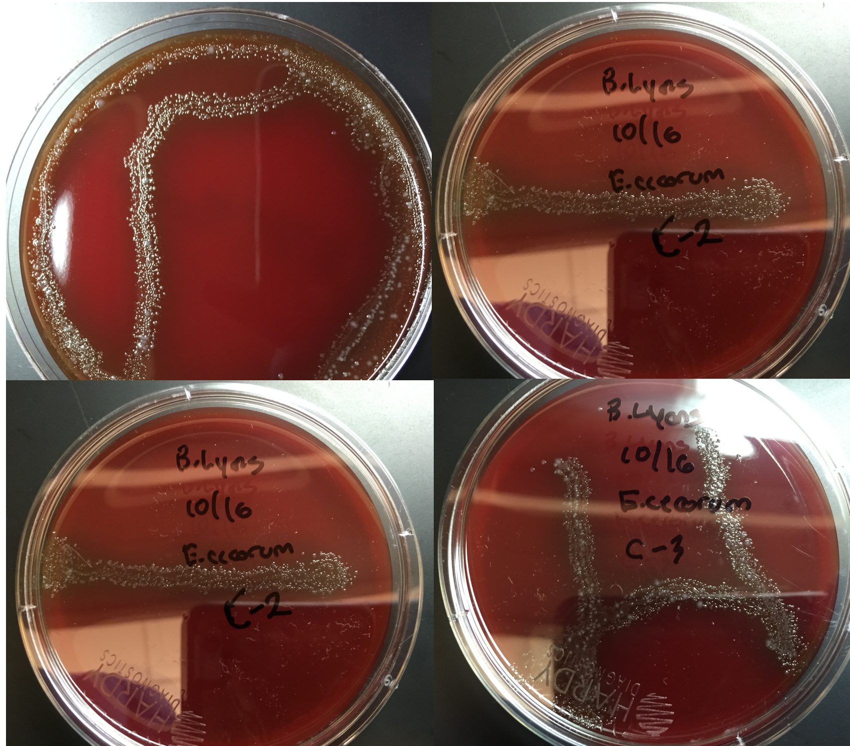


Fig. 3.1. *E. cecorum* bacteria growth along walking path of the lesser mealworm (photographs by B. Lyons)

Fig. 3.2. Pure culture of *Enterococcus cecorum* on blood agar. The greening of the agar surrounding the colonies is highlighting the hemolysis characteristic of the bacteria on this medium. Taken from (Armour et al. 2011).

Fig. 3.3. Colony PCR gel electrophoresis. Colony PCR bands indicate primers amplified bacterial DNA that was approximately 163bp. Lane 1-1kb maker, lanes 2-10 unrelated samples, lanes 11-12 *Enterococcus. cecorum* colonies.

TABLES

Table 3.1. Bacterial growth by beetle acquisition treatment

Treatment	Growth	Description of Growth	Beetle Track
Peptone Rinse	Yes	Milky-white lawn	
Peptone Rinse	Yes	Milky-white lawn	
Peptone Rinse	Yes	Opaque lawn, except one quadrant where mixed lawn and dispersed	
Peptone Rinse	Yes	Opaque lawn	
Peptone Rinse	No		
Acquisition Control	Yes	Opaque lawn	Yes
Acquisition Control	Yes	white circular ~3-4mm diameter	
Acquisition Control	Yes	white circular ~1mm growth x 5	
Acquisition Control	Yes	2 x (2-3mm) white circular growth with filaments	
Acquisition Control	Yes	Venn diagram shaped growth	
E. cecorum A-1	Yes	2 x white irregular growth ~ 1mm; 2 x white irregular growth < 1mm; Opaque ~3mm circular growth; Opaque dispersed	
E. cecorum A-2	Yes	Opaque circular growth ~1mm; Opaque circular < 1mm multiple colonies; 2 dispersed colonies	
E. cecorum A-3	Yes	Opaque "bullseye" ~7mm diameter	Yes
E. cecorum A-4	Yes	< 1mm, innumerable colonies.	
E. cecorum A-5	Yes	Opaque irregular less than 1mm innumerable. One circle of growth	Yes
E. cecorum B-1	Yes	Opaque innumerable colonies	
E. cecorum B-2	Yes	Opaque growth	Yes
E. cecorum B-3	Yes	Opaque, innumerable colonies	
E. cecorum B-4	Yes	~6mm x 7 mm "egg shaped" growth with opaque multiple colonies	Yes
E. cecorum B-5	Yes	Opaque, innumerable colonies; 3 contaminant colonies that have cleared blood tint from agar with largest contaminant ~3-4mm circular shaped	Yes
E. cecorum C-1	Yes	Opaque, innumerable colonies; 1 contaminant that has cleared blood from agar	
E. cecorum C-2	Yes	6-7mm circular growth area	Yes
E. cecorum C-3	Yes	Opaque, innumerable colonies	Yes
E. cecorum C-4	Yes	Opaque, innumerable colonies	Yes
E. cecorum C-5	Yes	Opaque innumerable colonies	

Table 3.2. Primer sequences for detection of *Enterococcus cecorum*

Primer	Sequence (5'-3')	Length	Start	Stop	Tm (℃)	GC%	any
Forward	CTCGTGTCGTGAGATGTTGG	20	1019	1038	60.31	55	3
Reverse	ACGTGTGTAGCCCAGGTCA	19	1181	1163	60.17	57.89	4

Table 3.3. Nanodrop quality assessment of PCR product.

Nucleic Acid (ng/μl)	A260 (Abs)	A280 (Abs)	260/280	260/230
10.9	0.219	0.106	2.07	1.46

CHAPTER IV

KNOWLEDGE, ATTITUDES, AND PRACTICES OF OKLAHOMA POULTRY GROWERS TOWARDS INSECT PESTS, BIRD MORTALITY MANAGEMENT AND LITTER MANAGEMENT

INTRODUCTION

A knowledge, attitudes, and practices (KAP) survey are given to gain insight on a particular topic (WHO 2014). Through a structured questionnaire, or survey, researchers can understand what a group of individuals knows, what the surveyed are thinking, and what is done in response to a topic. A KAP survey can help generate data that can identify any knowledge gaps or behavior that can be a barrier to implementation of a program. It can establish a baseline from which you can measure any changes to a program and can find points of communication that can make a program more effective. It can also help set priorities for program decisions. A survey was developed to identify a baseline for Oklahoma poultry growers on waste management, insecticide treatments, and of the particular poultry pest, *Alphitobius diaperinus.* A short 28 question survey was given to the growers attending the Oklahoma Poultry Waste Management Education course, hereby referred to as PWME.

The survey was designed to be taken over the course of 10 to 15 minutes. Basic questions were asked about their poultry operation, general pests of the poultry industry, litter beetle (*A. diaperinus*), insecticide program, litter management and waste management. With the results, a baseline can be established from which future extension programming can target the shortfalls in knowledge or practices by the growers and help improve communication between university, extension, and industry members on how to alleviate pest problems inside poultry houses in the state of Oklahoma.

Materials and Methods

KAP Survey. KAP survey distributed to broiler growers in Ottawa County, OK March 2020 Poultry Waste Management Education Meeting (Appendix). The Oklahoma State University Institutional Review Board (IRB) determined the study does not qualify as human subject's research. IRB-20-87 was approved on February 18, 2020.

PWME Pre/Post Knowledge Exam. As part of the course a pre and post test was given to all participants in the course to help gauge knowledge before and right after an educational program. Three questions were given as part of the questionnaire that were related to the litter beetle.

Results

Poultry Operation. Unless otherwise noted, responses to the survey are in Table 4.1. There were a total of 12 respondents. Fifty-eight percent of the growers that were surveyed manage between one and two million birds individually in their houses annually. The remaining growers raised from 8,000 to 600,000 birds annually. Of the growers surveyed, 58% of growers had 7 annual grow-outs. Eight percent of growers had one grow-out or eight grow-outs. Seventeen percent had 5 annual grow-outs.

Pests of Poultry Industry. Of the surveyed growers, house flies (*Musca domestica* [L.]) and litter beetles (*A. diaperinus*) were each observed by 83%. Mites were observed by 33% of the growers and no observations of ticks were reported. Insect damage was observed in almost half the surveyed growers (42%), and most the observed damage type was insulation damage.

Litter Beetle Knowledge. Growers were asked if they thought litter beetles could spread pathogens and 58% responded in the affirmative. The remaining 42% surveyed growers answered, "Don't Know". Growers were then asked if litter beetles consumed by the birds can lead to problems and 58% responded "Don't Know" but 42% answered in the affirmative. Growers were asked what litter beetles consume inside a poultry house and 58% responded bird feed, 33% responded bird feces, 42% responded insulation, 25% responded wood shavings, 17% responded grain, and finally sick and dead birds each got an 8% response (Fig. 4.1). Seventy-five percent of the growers surveyed reported that a high population of litter beetles would impact energy costs. Seventeen percent of the growers responded, "Don't Know" and 8% responded "No."

Insecticide Program. Ninety-two percent of the growers surveyed monitor populations of insect pests in their own houses, while 8% responded "No Monitoring." Fifty-eight percent of the respondents do not use alternatives to chemical control to control pests in their poultry houses. Most growers (58%) target beetles for their insecticide applications. Other pests like flies were targeted by 25% of the growers and 9% of the growers targeted mice. Of the growers that responded, 64% of the growers rotate pesticides on their poultry farm,18% replied "No" and 18% answered "Don't Know". Fifty-eight percent of the growers target their insecticides to specific locations inside the poultry house, whereas the remaining apply their insecticides as a broadcast spray. Of the growers that target their applications to certain areas inside the poultry house, most (67%) sprayed under feeders and along the walls (50%) (Fig. 4.2). Other areas of application included under water lines (42%), along the concrete footing (25%) and other areas, along cracks

or by the compost bin. When growers were asked what insecticides, the growers have used in the past two years 17% responded Lime, and 25% responded fly spray.

Litter Management. Most growers (67%) surveyed in northeast Oklahoma windrow their litter. Of the ten growers that windrowed their litter 10% waited to turn the windrow after 5 days, 10% windrowed 4 to 5 days, 50% waited 4 days, and 10% waited for 3 days. Twenty percent of these growers answered "Other" (Fig. 4.3).

When asked about bedding material used inside their poultry houses, rice hulls (43%) and pine shavings (38%) were the most cited litter bedding substrates (Fig. 4.4). Poplar (5%), mixed (5%), and other woods (9%) accounted for the other litter bedding materials used by growers in Ottawa County. Half of the ten growers that responded fully replaced their litter, whereas 33% will retain a portion of the old litter in what is called a partial cleanout. Thirty-seven percent of the growers surveyed had an average litter depth of 4 inches (10 cm). Thirteen percent of growers had an average litter depth of 12 inches (30 cm), 13% had 6 to 10 inches (15 cm to 25 cm), and 13% had 6 to 8 in (15 cm to 20 cm). Twelve percent of growers had an average depth of 6 in (15 cm), and 12% had an average of 4 to 6 in (10 cm to 15 cm). (Fig. 4.5) Seventy-five percent of growers surveyed composted their bird mortalities on their farm. Two growers used an incinerator, one used an Eco-drum and one listed "other" (Fig. 4.6).

Thirty-three percent of the respondents listed they "decake" their litter once a year, and another 33% listed "Other" (Fig. 4.7). The growers that windrowed their litter were asked for their layout times (Fig. 4.8). The responses were that 38% waited more than 12 d, 25% windrowed 12 d, and the remaining 37% waited less than 12 d. Sixty-three percent of the growers that windrowed monitored its moisture content during the layout process. Forty-three percent of the growers that windrowed either waited every 5 d or every 3 to 4 d to turn the windrows (Fig. 4.9).

PWME Pre and Post Tests. Questions and responses to both the pre- and post-test at the PWME meeting for all meeting attendees are in Table 4.2. There was a total of 21 respondents. The first question asking if darkling beetles can increase energy costs was answered true by 95% of the workshop participants and 100% of respondents answered true in the post test. The second question asked was targeted to get a better understanding of producer knowledge related to litter beetle abundance. When producers were asked what population level of litter beetles a typical broiler house support the majority (43%) answered 1 million when asked before the course. The post-test showed 19 million garnering an 81% response and 14% answering 1 million. The third question asked the best location to target an insecticide application inside a poultry house. Seventy-one percent responded under a feed or water line, and along the walls. The post-test raised the correct response to 20 respondents (95%) and the other response was under the feed/water line only.

Discussion

Based on the number of birds annually raised, most of the growers would have 8 to 10 houses on their farm for a total of about 160,000 birds at a time with an average of two weeks between flocks.

The most common pests encountered by the growers on their farms was either the house fly, *Musca domestica* or the lesser mealworm, *Alphitobius diaperinus,* which is commonly known as the litter beetle by broiler growers. Almost half the respondents have found damage related to the litter beetle in the form of insulation damage. This is a common complaint with the litter beetle as the larvae remove insulation to create a pupation site (Axtell and Arends 1990). House flies are pests encountered in caged layer houses quite frequently but are observed in broiler houses less frequently as the litter is too dry to be utilized as a breeding substrate. Litter moisture content was recorded in the range from 21 to 88% within broiler houses (Stafford III and Bay

1987). This study revealed that 85.9% of third instar house flies were recovered when poultry litter moisture ranged between 60% and 79%. Since around 75% moisture content is considered ideal for house fly development there are mechanical controls such having sufficient airflow that can help keep the litter moisture content low enough to hinder their development in the litter (Ward and Lachance 2015). Additionally, keeping the water lines from dripping into the litter can help. As the problem of urban encroachment on poultry facilities occurs it is important for growers to be mindful of the pest populations, especially house flies as the flies can migrate to nearby neighborhoods and cause complaints.

Fifty-eight percent of growers surveyed answered litter beetles could spread pathogens and 42% believed that consumption of the beetles could lead to problems with the birds. *Camplobacter* and *Salmonella* inoculated larvae and adult litter beetles were able to transmit these pathogens to chicks with a single feeding (Hazeleger et al. 2008). *Camplobacter* positive samples increased for 5 to 9 d after feeding on inoculated insects. While *Salmonella* also persisted for days, its positivity rate decrease decreased over time. One study revealed that consumption of 5 inoculated litter beetle larvae were sufficient to cause positive *E. coli* cloacal tests in birds 48 h later (McAllister et al. 1996). In another study four *Salmonella* inoculated litter beetles were fed to chicks to measure how long the cecal samples would remain positive for those birds and birds housed with them (Roche et al. 2009). Three weeks after feeding the birds were tested and *Salmonella* was not present in inoculated birds penned alone however those that were housed with other birds had positive cecal samples at 3 and 6 wk. This highlights the importance of the litter beetle as a reservoir pest in broiler houses. The litter beetle help pathogens persist in a flock and maintain them when the houses are empty between flocks as Skov et al. (2004) revealed when testing successive flocks for *Salmonella*. Of the growers surveyed 75% answered that the litter beetle could cause higher energy costs. Energy costs have been reported to increase by upwards of 67% resulting from litter beetle damage costing Georgia growers $4000 per

infested house in 1990 dollars (Geden and Hogsette 1994). When the growers were asked what the litter beetle consumes inside the poultry house, seven answered bird feed and the next common answers was bird feces. The knowledge of the growers towards the litter beetle is well rounded as they have an idea or where the beetles are inside the house, what they primarily feed on, and know that the beetles can impact the bird's health and the economics of their operation through the insulation damage. A spatial analysis study was conducted using six sites in a broiler house over the course of five grow-outs was conducted by Strother and Steelman (2001). Their predictive model was 86.7% in agreement with their sampling and can be used to target insecticides more accurately. The litter beetle density spreads from the east and center of the houses to the west end of the houses as the weeks progress during the grow-out. The day-old chicks are confined to the east end to keep them closer to the supplemental heaters added for their early development. The beetles would be close to the flock utilizing their heat and consuming their feed and feces. Once the middle barrier is removed to allow full access to the broiler house is when we see the push towards the west end of the house by the beetles. Although the houses were positioned from east to west the spatial pattern of litter beetles within a house is related to the where the chicks are placed initially at the beginning of the grow-out period regardless of the positional direction the house is oriented. Geden and Axtell (1987) has observed the movement of late-stage larvae and adults up the walls primarily in the evening hours when the density of beetles is too high for finding suitable pupations sites in the litter. As these beetles move into the insulation for pupation, the beetles will form holes in all types of insulation including fiberglass and polystyrene panels (Dunford and Kaufman 2006) (Fig. 4.10). As the beetle density would be high throughout the whole house towards the end of the grow-out period, damage would not be isolated to a single wall.

Of the growers surveyed, 92% responded that they monitor their houses for insect pests. This practice is in agreement with sound integrated pest management practices that utilize

monitoring as a basis to initiate control measures. Insecticide treatment is the most common and sole method to treat pest problems as reflected in the 58% response. Sixty four percent of growers rotate pesticides to alleviate resistance issues. All growers responded when asked where they apply insecticides mostly responded (67%) that they target the sprays under the feed lines and 50% targeted along the walls. It is a common industry practice for the integrator who owns the birds to contract out to another company the pesticide applications on behalf of the grower. It is unclear if these responses reflect what is done on their farm by others or by themselves. To keep the survey short to incentivize responses more detail on this topic was eliminated when drafting the survey originally.

Insecticide treatment is usually sprayed on top of litter before bird placement (Weaver 1996) This limits treatment of insecticides to about every 7 weeks also relying on a relatively limited number of insecticide classes (Tomberlin et al. 2008). Overuse of chemicals such as pyrethroids has led to resistance issues. The different surface types commonly utilized within a poultry house can also contribute to lower mortality with permethrin and synthetic pyrethroids (Lyons et al. 2017).

Considering the importance of litter type and age of litter can impact litter beetles it was interesting to see that 50% of growers fully replaced the bedding inside the poultry house regularly. Growers usually clean out the litter every 8 to 10 flocks (Sistani et al. 2003) however total cleanout is not common due to costs. The type of bedding materials utilized by broiler operators is usually limited to what is commonly available in their region. The growers in Oklahoma primarily utilize rice hulls as the most common material for litter (43%) but pine shavings were also commonly utilized (38%). These two materials are readily available to broiler operators in Oklahoma with rice production in nearby Arkansas and timber production in eastern Oklahoma. Broiler operators will most likely utilize both materials throughout the year and not just rely on one material due to availability. Aged litter is reused rather than replaced because of

costs and availability issues (Dittoe et al. 2018). Reusing litter can cause Nitrogen (N), Phosphorus (P), and Potassium (K) values to increase in the litter (Coufal et al. 2006). After seven consecutive grow-outs, N values went from 2.08% to 3.53% on a dry matter basis. Phosphorus went from 0.85% to 1.92% and K went from 1.97% to 3.55% on a dry matter basis. These grow-outs were in Texas during the summer months and would likely mimic conditions in much of Oklahoma and therefore similar results would be expected. The temperature and moisture in the litter factors into the volatilization of N in the form of ammonia (Elliott and Collins 1982, Carr et al. 1990).

Windrowing of litter is a management technique that can lessen the buildup of these nutrients, reduces ammonia, and can kill certain microorganisms. Windrowing decreases anaerobic bacteria populations by more than 78% (Lavergne et al. 2006). The process of windrowing involves using equipment to form long rows of litter piled up along the length of the poultry house. The metabolism of the bacteria will cause a composting process to occur (Tabler et al. 2019). A minimum of 12 to 14 days is required if turning of the windrows is done according to Tabler et al. (2019). Of the Oklahoma broiler operators surveyed, 63% of those that windrowed waited 12 days or longer for their windrows to compost. According to Tabler (2019) at Mississippi State Extension, the windrow should be undisturbed for 3 days before turning the windrow. Forty-three percent of the growers that windrowed waited 3 to 4 days before turning with another 43% preferring to wait 5 days. Moisture content of the windrow is a concern for many growers, as the moisture content of litter must fall between 32% to 35% for windrowing to be considered effective (Barker et al. 2011). Of the growers that responded, 62% monitored the moisture content of their windrows. Growers appeared to have good practices for turning their windrows and monitoring their moisture levels. The next best thing to full litter replacement is windrowing to minimize pathogens to improve the health of their birds.

A third of the growers decake their litter once a year. "Decaking" is a recent development that involves removing the fresh manure that has combined with bedding and spilled feed to form a "cake". It forms on the surface and is usually 5 to 10 cm thick (Sistani et al. 2003). After each flock the cake is collected usually with an implement that separates the cake from the bedding materials. A grower would then add enough fresh bedding to accommodate the removed material in the form of cake. Approximately 57% of the litter should remain after the decaking process and provides cost-effective litter management strategy compared to complete removal after several flocks.

Composting of bird mortalities on the farm was common (75%) with other mortality management techniques that a grower utilizes are an incinerator, have a rendering service collect mortalities on a schedule for off-site handling. One grower used an Ecodrum® (Ecodrum, Ozark, AR), which is an in-vessel composting apparatus for bird mortalities. Proper handling of mortalities is important for limiting pathogen spread, and not attracting insects to their carcasses.

As a whole, the KAP survey highlighted a good base knowledge of the most common pest that affects the broiler industry. Growers surveyed in this county were consistently monitoring their broiler houses for pest populations, usually with each flock (92%). Sampling is one of the most important components of a pest management program and important for gauging management decisions (Bohmont 2000). Damage resulting from the litter beetle leads to a reduction in energy efficiency often manifests itself as a 67% increase in energy costs (Geden and Hogsette 1994, Loftin 2011). This would lead to higher overhead costs to run the facility and reduce the profit margin. Additionally, the pest can impact the health of the bird leading to more sickness, and death as well as reduced weight gain for those birds that consume the beetles in place of feed (Axtell and Arends 1990, Despins and Axtell 1994, Despins and Axtell 1995). It is in the best interest of the growers to have a more active role in reducing the beetle population with insecticide and non-insecticide approaches integrated in an economically feasible manner.

Unfortunately, growers and pesticide applicators are limited by when they can apply chemicals to when birds are not present which is between flocks and this time can be strict in the number of days which would limit pest management practices that require longer time to reduce the beetle population such as biological or cultural control techniques. Insecticides are typically targeted in locations that are common for litter beetles but 42% of the growers reported a broadcast spray is conducted through their houses. When asked specifically which insecticide only two responses for lime and three for fly spray were recorded. This suggests lack of direct knowledge of products used throughout their houses. As it is common practice for the companies that own the birds to contract the insecticide treatments to those other than the growers this is likely the case for most if not all the growers in this survey. Forty-two percent of growers answered that they use methods other than chemicals to control for pests in their broiler houses. It is unclear what specifically is being done but it is common throughout the industry to open the doors to the cold weather in winter months between flocks and is likely one of the techniques referred to by their responses. Future surveys may want to consider elucidating a detailed response.

Finally, the survey was permitted in February 2020 to be given to the PWME meeting beginning in March 2020 to continue throughout the rest of 2020. After the first meeting, the Covid-19 pandemic forced the cancellation of the remaining PWME meetings for the remainder of the year. An online format was given to the PWME group for dispersal to its members however the response rate was only 1.

FIGURES

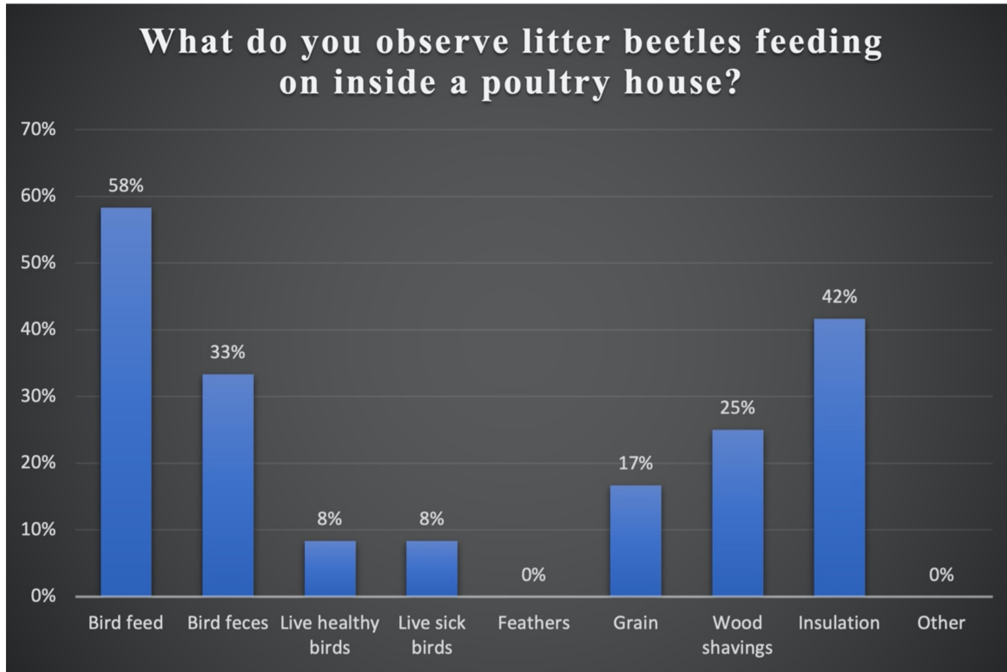


Fig. 4.1 Knowledge of Northeast Oklahoma broiler operators of litter beetle feeding behavior.

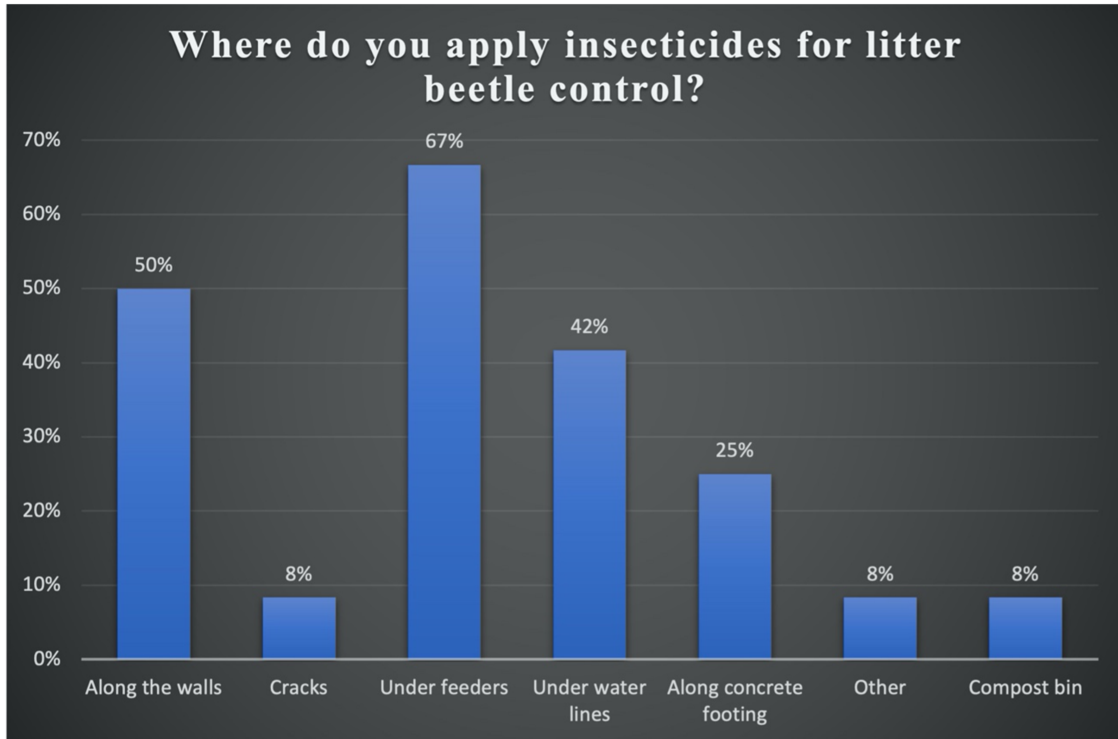


Fig. 4.2. Practices of Northeast Oklahoma broiler operators of where insecticide applications should be applied for litter beetle control.

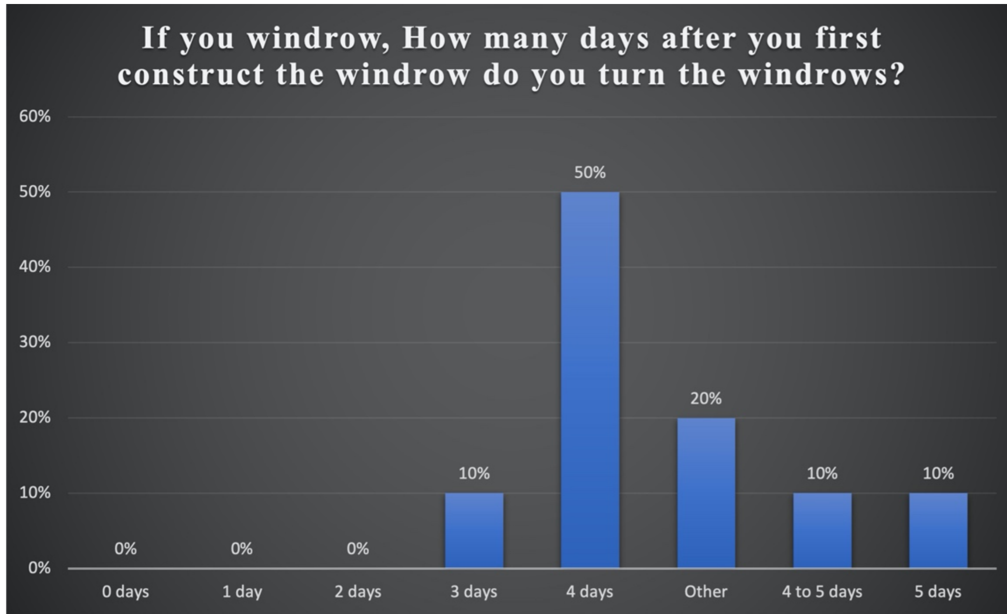


Fig. 4.3. Practices of Northeast Oklahoma broiler operators on the frequency they turn their windrows.

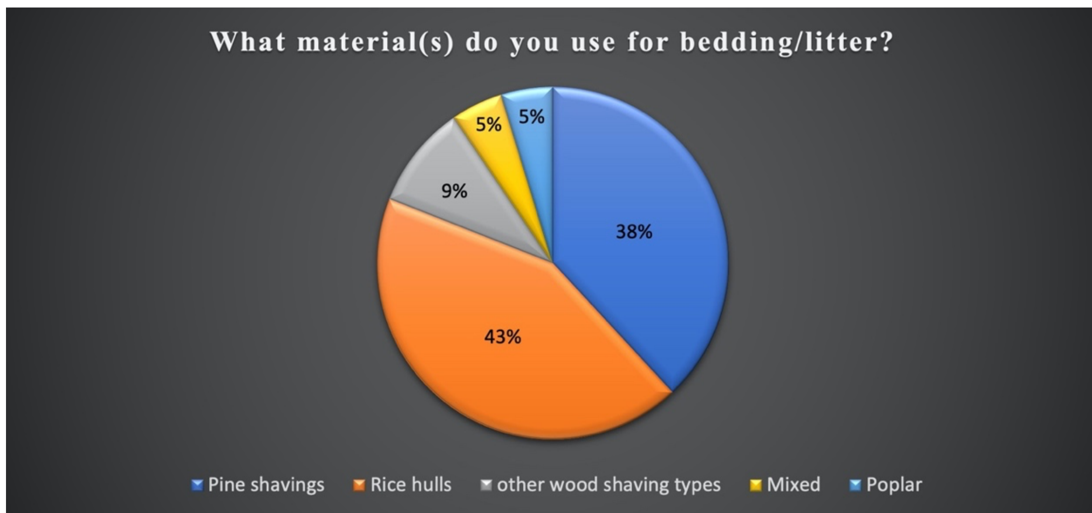


Fig. 4.4. Practices of Northeast Oklahoma broiler operators related to the type of litter material utilized within their operation.

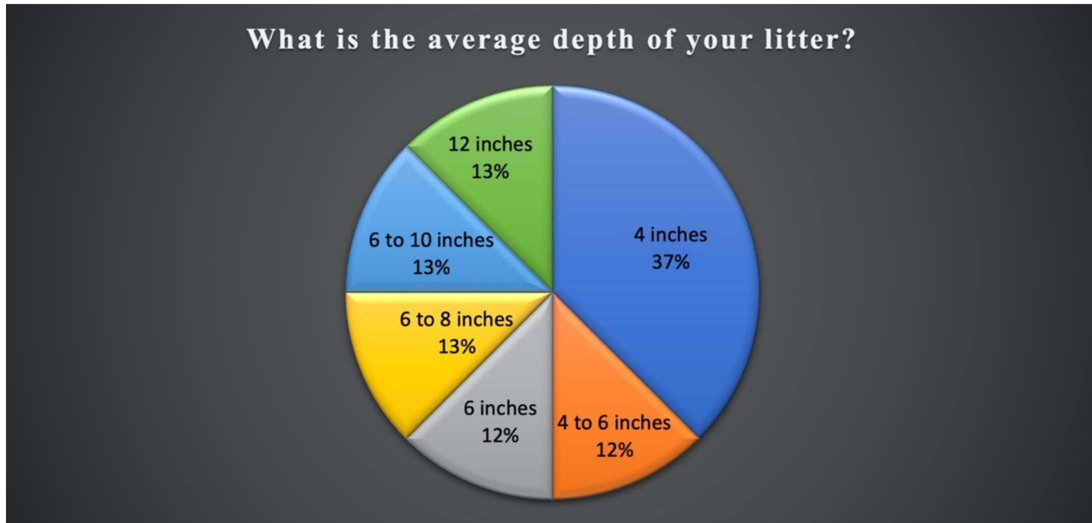


Fig. 4.5. Practices of Northeast Oklahoma broiler operators related to the average litter depth within their operation.

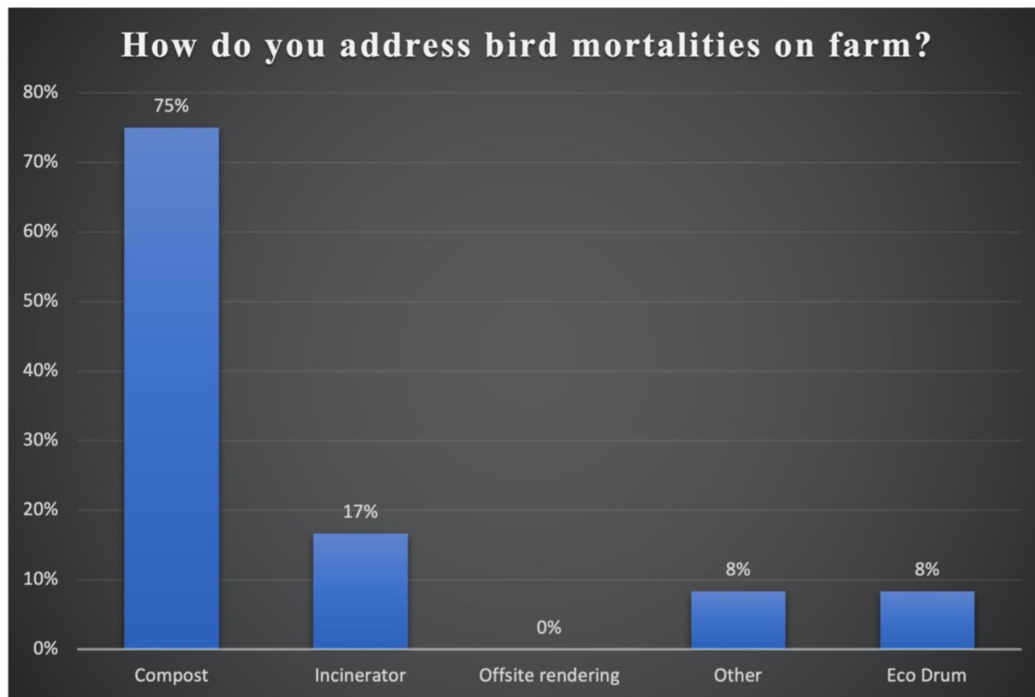


Fig. 4.6. Practices of Northeast Oklahoma broiler operators related to animal mortality management on farm.

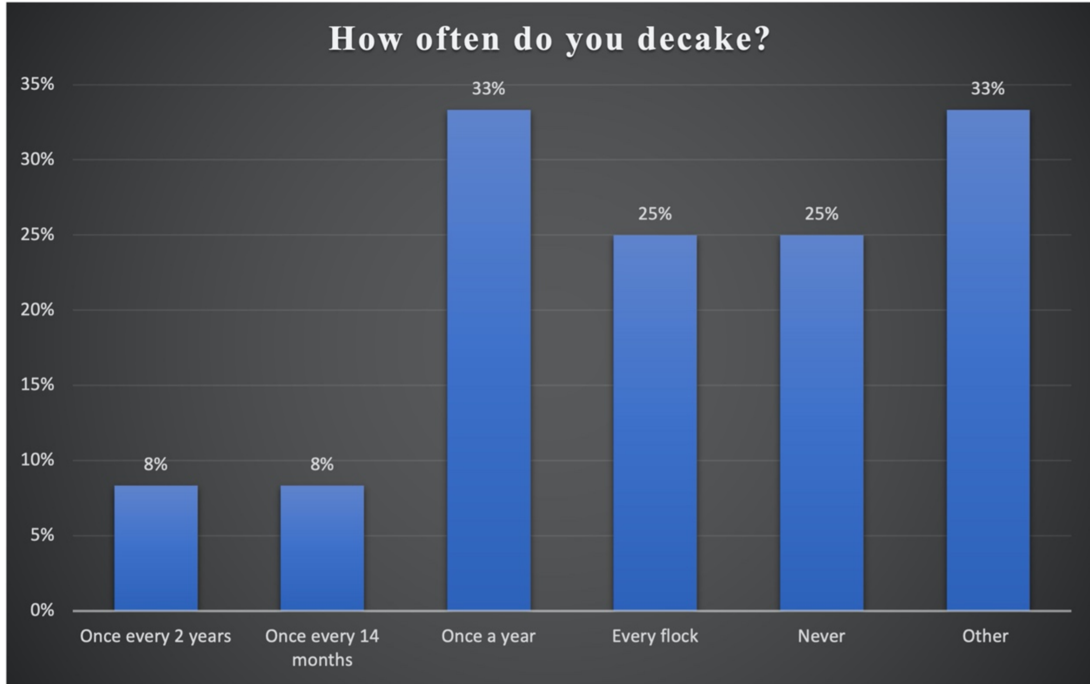


Fig. 4.7. Practices of Northeast Oklahoma broiler operators related to the frequency of decaking events on their operations.

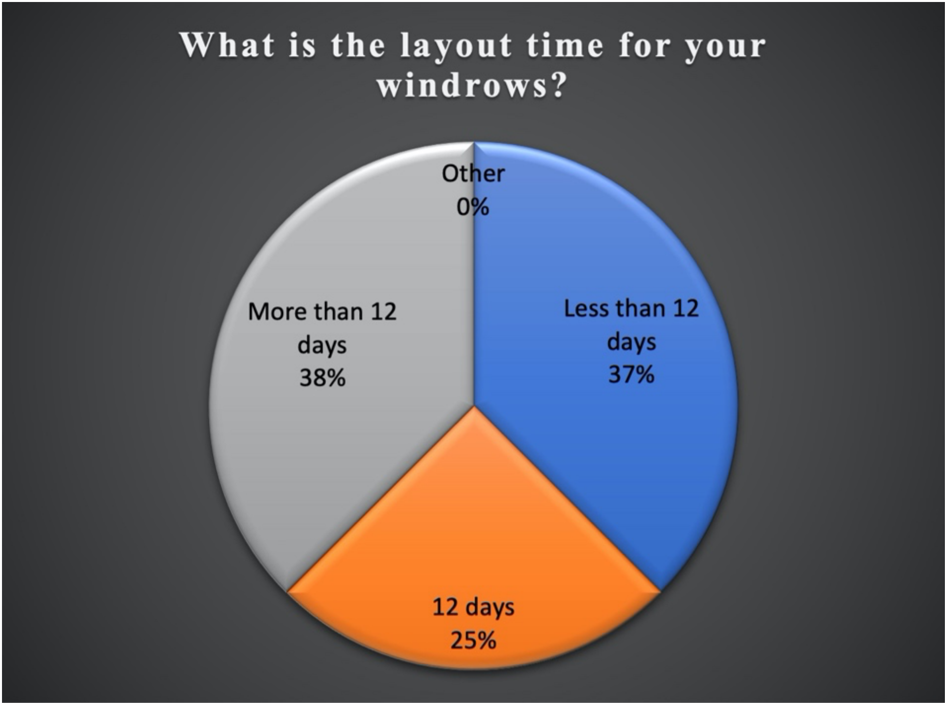


Fig. 4.8. Practices of Northeast Oklahoma broiler operators associated with the period of time windrows are in a layout period.

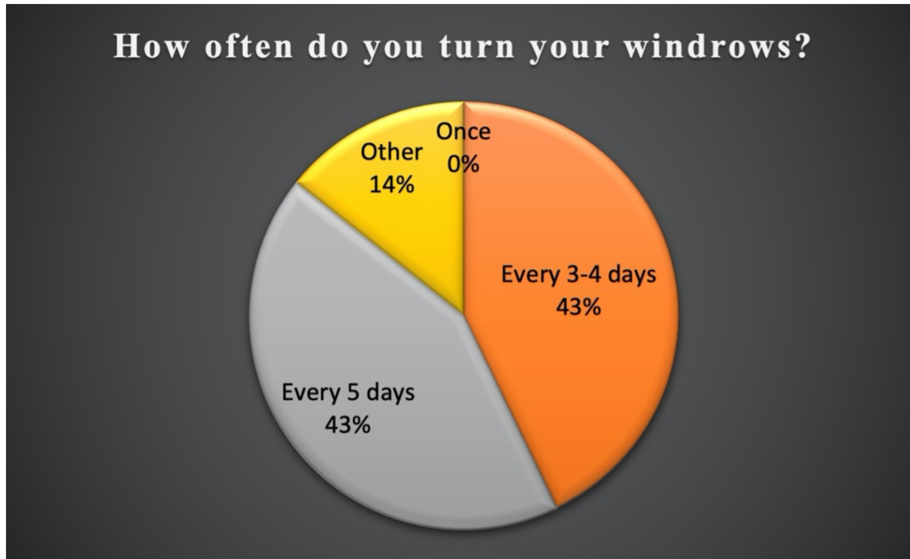


Fig. 4.9. Practices by Northeast Oklahoma broiler operators related to windrow management.

Fig. 4.10. Insulation damage to spray foam insulation and insulation panels inside a broiler house. Orange circle is highlighting an adult lesser mealworm, *Alphitobius diaperinus,* inside the damaged insulation. Images taken by B. Lyons

TABLES

Table 4.1. KAP Survey responses from NE Oklahoma broiler operators on key questions related to overall litter management and pest management within broiler houses

Question	Response	Percent Response (%)
Poultry Operation		
How many grow outs annually?	1 grow-out	8.33
	5 grow-outs	16.67
	7 grow-outs	58.33
	6 to 7 grow-outs	8.33
	8 grow-outs	8.33
Pests of Poultry Industry (General)		
Observed pests?	Ticks	0.00
	Litter beetles	83.33
	Mites	33.33
	House flies	83.33
Have you seen any insect damage?	Yes	41.67
	If yes, then where: Insulation	33.33
	No	58.33
Litter Beetle Knowledge		
Are litter beetles capable of spreading pathogens?	Yes	58.33
	No	0.00
	Don't Know	41.67
Can the consumption of litter beetles by birds lead to problems?	Yes	41.67
	No	0.00
	Don't Know	58.33
Would a high population of litter beetles impact energy costs?	Yes	75.00
	No	8.33
	Don't Know	16.67
Insecticide Program		
How often do you monitor populations?	Each Flock	91.67
	Rarely	0.00
	Don't Know	0.00
	Other	0.00
	No Monitoring	8.33
Do you use methods other than insecticides to control pests	Yes	42.00
	No	58.00
What was your target pest(s) for your insecticide applications?	Beetles	63.63
	Flies	27.27
	Mice	9.09
Do you rotate pesticides?	Yes	63.36
	No	18.18
	Don't Know	18.18
How do you apply your insecticides?	Targeted	58.33
	Broadcast	41.67
What insecticides have you used in the past two years?	Lime	16.67
	Fly spray	25.00
Litter Management		
Do you windrow you litter?	Yes	66.67
	No	33.33
Do you fully replace or do a partial cleanout?	Fully Replace	50.00
	Partial Clean-out	33.33
Do you check the windrow for moisture content?	Yes	62.50
	No	37.50

Table 4.2. NE Oklahoma PWME pre and post test beetle responses.

Question	Pre-test Response (%)	Post-test Response (%)
Darkling beetle infested houses will have energy costs greater than 60% compared to a house with little or no beetles?	True: (95%) False: (5%)	True: (100%) False (0%)
Estimate the darkling beetle population inside a broiler house?	19 million (29%) 1 million (43%), 500,000 (14%) 250,000 (5%) NR (9%)	19 million (81%) 1 million (14%) 500,000 (5%)
Where should you apply insecticides targeting darkling beetles?	Along walls, Under feed/water lines (71%) Under feed/water lines (10%) Along edges (14%) Center of broiler house (0%) No response (5%)	Along walls, Under feed/water lines (95%) Under feed/water lines (5%) Along edges (0%) Center of broiler house (0%) No response (0%)

* NR is non response

CHAPTER V

POULTRY LITTER ACIDIFIER AMENDMENTS, INSECTICIDE INTERACTIONS AND SUBLETHAL EFFECTS OF INSECTICIDES ON *ALPHITOBIUS DIAPERINUS* (COLEOPETRA: TENEBRIONIDAE)

Introduction

Lesser mealworm *Alphitobius diaperinus* (Panzer) resistance to insecticides is a problem affecting the broiler industry throughout the world. In a study of eastern United States broiler houses, resistance at LC_{50}, where half the population was dead, to the pyrethroid Cyfluthrin with adult lesser mealworms ranged from 1.8 to 4.0-fold greater than the susceptible colony from Denmark (Hamm et al. 2006). In the same study adult lesser mealworm resistance at LC_{50} to the organophosphate tetrachlorvinphos ranged from 1.6-fold to 16-fold greater than the susceptible population. Lambkin and Rice (2006) measured cyfluthrin resistance buildup on Australian broiler farms beginning in 2000 and repeated 3 times until 2005. The researchers correlated increased resistance with increased applications of cyfluthrin products. One farm between the years 2000 and 2005 increased population resistance by 9.98-fold. Application of insecticides is

generally limited to intervals between grow-outs once the birds are not present within the house. This occurs approximately every 6 to 7 weeks. Additionally, the number of chemical classes labeled for use against the lesser mealworms inside broiler houses is limited and has resulted in high levels of resistance in field strains (Hamm et al. 2006, Tomberlin et al. 2008, Lyons et al. 2017). Tomberlin et al. (2008) concluded that while susceptible populations exist, these populations of lesser mealworms are difficult to identify. Lyons et al. (2017) recorded high levels of recovery of adult lesser mealworms within 48 h when *ß*-cyfluthrin was treated on various surfaces commonly used in broiler house construction, such as concrete, inside a broiler house.

As the population density of lesser mealworms gets too high, late-stage larvae will seek pupation sites in the walls of the broiler house, specifically in the insulation (Geden and Hogsette 1994). The larval feeding will cause voids in the insulation for their pupation site, and over time the insulation loses its insulation abilities leading to higher costs as it takes more energy to maintain the temperature for the birds. The economic losses from insulation damage can be costly, but the lesser mealworms infesting chicken houses has also been implicated in the transmission of pathogens such as *Salmonella* (Crippen et al. 2009), *Eimeria* (causative agent of coccidiosis; Goodwin and Waltman 1996), and the virus implicated as the causative agent of Marek's Disease in chickens (Axtell and Arends 1990). The higher veterinary costs and increased mortality from disease will impact the profit margins of the broiler growers.

Since the 1970's, a shift in building construction has occurred where broiler houses are typically fully enclosed (Malone 2005). This industry shift has created a new problem related to ammonia (NH_3) gas build up. NH_3 volatilization occurs when microorganisms break down the nitrogenous wastes (uric acid) from poultry feces (Blake and Hess 2001a). Curtain sided houses were able to provide sufficient ventilation by raising the curtains along the length of the broiler house (Malone 2005). To accomplish this with modern building standards, large ventilation fans must circulate air from one end of the building to the opposite end. Modern broiler houses end up

trapping NH_3 gasses inside the broiler house despite the ventilation fans. While 25 mg/kg is the goal for NH_3 inside a broiler house, as little as 10 mg/kg can lead to respiratory issues in birds that can allow pathogen entry (Blake and Hess 2001a). Reduced feed efficiency can accompany health issues leading to economic losses from additional vet and feed costs. Additionally, recent foreign market trends have greatly increased the value of the chicken's foot pads thus further incentivizing broiler growers to control litter quality (Tabler et al. 2019). Various factors contribute to the buildup of NH_3 in a poultry house such as humidity, ventilation, moisture content, and type of litter substrate (Choi and Moore 2008). However, one of the most important factors is high litter pH. Since the 1980's several NH_3 control products have been developed and used, with litter amendments such as poultry litter treatment (PLT®) being one of the most common in use within broiler houses (Blake and Hess 2001b, Malone 2005). Each litter amendment has a different active ingredient, thusly will have variation in mode of action. However, all litter amendments work on the principal of reducing the pH of the litter (Blake and Hess 2001b).

In between intervals of birds inside a broiler house are an average of 2 weeks when litter amendments and insecticides are applied to the litter of a broiler house. Little research has focused on the interaction between litter amendments that alter the pH of the litter and the insecticides that are applied to the litter over a short period of time. Additionally, the insecticides applied for lesser mealworm control are expected to be effective for approximately 45 days when the birds are inside a broiler house. Exposure to sublethal toxicity of insecticides towards the latter period of the bird placement interval, and the overall limited contact insects have to the surface of litter where these products are used will often confer sublethal insecticide exposure to lesser mealworms. The ovicidal responses to sublethal insecticide exposure has been poorly studied in the lesser mealworm. The objective of this study was to determine how the common litter amendment known as PLT® impacts insecticide efficacy of the lesser mealworm and if

sublethal effects occur on this economically important pest when insecticides are applied in conjunction with acidifying litter amendments.

Materials and Methods

Arkansas lesser mealworm field strains were collected in March 2019 from Arkansas and kept in colony at Oklahoma State University. For later experiments, *A. diaperinus* Oklahoma field strain beetles were collected from several broiler houses in eastern Oklahoma in January 2020 and brought back to Oklahoma State University in Stillwater, OK. Beetles were maintained in colony at 27℃; 16:8 Light: Dark; 80% RH and fed ad libitum dog Food (Kibble's 'n Bits, Big Heart Pet Brands, Orrville, OH), 5 Grain Scratch (Red River Commodities, Fargo, ND), Fish Meal (Omega Protein Inc., Reedville, VA), red apples sliced 2-4 mm thick and used wheat bran (Siemer, Teutopolis, IL) for substrate. Insecticide susceptible strain *A. diaperinus* were reared from a starter colony sent to Oklahoma State University in March 2019. The susceptible colony was maintained in the same environmental conditions and fed the same diet as the field strains.

A litter substrate comparison measuring adult lesser mealworm (Arkansas Field strain) mortality with combinations of two pyrethroids and two litter amendments on used litter collected from Arkansas broiler houses and wheat bran. A separate field trial in Arkansas recorded surface litter pH over the course of several days before and after PLT® treatment was applied inside a broiler house with birds placed inside. Arkansas field strain lesser mealworm larvae were also tested at two relative humidity's based on PLT® activation using two pyrethroids, an imidacloprid, and an organophosphate. A colony from the University of California Riverside represented the insecticide susceptible colony used to compare the Oklahoma population of lesser mealworms by comparing lethal time mortality to calculate resistance ratios. A preliminary lethal time bioassay was conducted with adult lesser mealworms taken from Oklahoma broiler houses to determine sublethal exposure times for the ovicidal response study. Individual late-stage

larvae were collected from the Oklahoma strain colony and reared individually until pupation. At pupation the individual lesser mealworms were sexed and separated until 1 to 2 months of age and then the adults were treated for LT_{20} exposure and kept separate for 3 wk. One male and female were randomly chosen within a treatment group to be paired together in a Petri dish. Number of eggs were counted every 3 d and separated into individual Petri dishes for a total of 30 d. The emerged larvae were then counted every 3 d for a total of 12 d.

The insecticide formulations used in this study are all commercially available and labelled for use on lesser mealworms in broiler houses (Table 5.1). *β*-Cyfluthrin (Tempo SC Ultra®, Bayer Healthcare LLC, Shawnee, KS) and Esfenvelerate (Onslaught Microencapsulate® MGK Co., Minneapolis, MN) are within the pyrethroid class. A neonicotinoid, Imidacloprid (Credo SC®, Bayer Healthcare LLC, Shawnee, KS) and the organophosphate Chlorpyrifos (Durashield CS®, BASF, Florham Park, NJ; Table 5.1) were also used. All treatments applied at high label dose rate onto 9 cm diameter cellulose filter paper. Poultry Litter Treatment® (PLT®; Jones Hamilton Co., Walbridge, OH) was applied at the labeled rate of 45.36 kg/92.90m^2, and Al^+ Clear A7® (Chemtrade Logistics, Toronto, CAN) was applied at 95 L / 92.90 m^2. Distilled H_2O was used for control.

Surface pH of Litter

Surface litter pH from an active broiler house was measured by mixing 25g of litter with 75 ml of distilled water and measured with a portable pH meter. pH was recorded in the brood chamber of broiler house in Arkansas when birds were present beginning on day -3 from PLT® treatment until 6 d after.

Substrate (pH) Treatment Comparison

Used litter taken previously from Arkansas broiler houses but used for colony substrate and wheat bran pH were measured with a calibrated Extech pH meter (Extech Instruments Corporation;

Nashua, NH) following protocols established by Coufal et al. (2006). Used poultry litter was a mix of rice hull and soil collected from Arkansas poultry houses previously and used as litter substrate for the Arkansas strain of lesser mealworms. To determine the effect of pH on the interaction of litter amendments and insecticide two litter amendments were used (sodium bisulfate PLT® and acidified aluminum sulfate A7®) and two different commonly used pyrethroids Tempo SC Ultra® (β-Cyfluthrin) and Onslaught Microencapsulate® (Esfenvelerate). Wheat bran represented the more neutral environment with a consistent pH of 6.74 and used poultry litter with a pH of 7.65. Insecticides were applied with a calibrated chemical sprayer at high dose label rate scaled for 56.7 cm^2 Petri dish. Control treatments used distilled water applied at equivalent amount of water for 56.7 cm^2 at rate of 1 gallon/1000sq. ft. (3.78 liters/ 929030 cm^2). PLT® applied at equivalent rate of 100lbs/1000sq. ft. (45 kg/929030 cm^2). A7® applied at equivalent of 25lbs/1000 ft^2 (94.63liters/929030 cm^2) in a 56.7 cm^2 Petri dish. Beetles were provided with 0.5 mL fish meal and a cotton ball moistened (remoistened every 2-3 days) was placed on insecticide exposure day. Each litter amendment, insecticide, and combination of each litter amendment with each insecticide was replicated 3X with 30 beetles per treatment dish. For each Petri dish 3.0 g of wheat bran was spread evenly inside before litter amendment or insecticide application. A 2-wk application schedule was used to mimic the application schedule in a typical broiler house, or period where birds are not inside the broiler houses. Insecticides were applied on d 0 and then Petri dishes placed inside growth chamber (27°C; 16:8 Light: Dark). As per the recommended label A7® was applied 24 h later to represent the time from bird placement and A7® was applied evenly to each applicable Petri dish using a pipette and dishes were then replaced inside growth chamber (27°C; 16:8 Light: Dark). On day 5 PLT® granules were spread evenly on applicable Petri dishes and then replaced inside growth chamber (27°C; 16:8 Light: Dark). On day 7 ~30 adult lesser mealworm beetles were exposed to treatment dishes and placed again inside growth chamber (27°C; 16:8 Light: Dark). Rubber bands were used around treatment dishes to prevent escapes. On day 8 and day 14 mortality was assessed.

Mortality assessment typically follows Lambkin (2005) definition: "walk straight in a forward motion using all 6 legs with no jerky movements."

Larval RH Bioassay

Two trials were conducted to assess the mortality of unsexed late-stage larvae to various treatments of insecticides and PLT®.

Trial 1 was conducted with RH at 70% with a constant temperature of 27°C and a photoperiod of 16:8 Light: Dark. Insecticides were applied with a calibrated pesticide sprayer (Chaplin International Inc.; Batavia, NY). Twenty Arkansas colony lesser mealworm larvae were used for each treatment dish and replicated 3 times. Mortality was observed after 60 minutes and observations were made for 240 minutes at 10-minute intervals. Morality was assessed using Lambkin (2005).

Trial 2 was conducted with RH at 60% with the same constant temperature of 27°C and a photoperiod of 16:8 Light: Dark. Insecticides were applied with a calibrated sprayer gun at high dose label rate scaled for 56.7 cm^2 Petri dish. Control treatments used distilled Water applied at equivalent amount of water for 56.7 cm^2 at rate of 3.78 liters/ 929030 cm^2. Ten Arkansas colony lesser mealworm larvae were used for insecticide treatments and 3 replicates of 10 larvae were used for insecticide and litter amendment treatments. Mortality was measured using Lambkin (2005). Observations were made at 10-minute intervals for 180 minutes.

At the beginning of the experiment clean filter papers were placed in each treatment dish. PLT® applied to applicable Petri dishes and evenly spread by hand on d 0. Dishes were placed inside growth chamber for 22 h. Insecticides were then applied as described above. Petri dishes were then replaced inside the growth chamber for an additional 2 h. Afterwards, lesser mealworm larvae were exposed by dumping all individuals at once onto treated filter papers. Mortality was assessed according to Lambkin's (2005).

Preliminary Bioassay to Determine Lethal Time of Compounds

The litter amendment, PLT® was measured on a level scale and spread evenly by hand onto clean 90 mm filter papers (Global Life Sciences Solutions; Marlborough, MA) and placed inside growth chamber for 24 h. Insecticides were applied onto either clean 90 mm filter papers or onto the PLT® treated filter papers. Insecticide treated filter papers were allowed to dry for 1h prior to 20 adult lesser mealworm beetles being exposed by dumping unsexed individuals onto each treatment Petri dish. Mortality was assessed over the first four hours to determine the LT_{20} of the population for sublethal insecticide exposure fecundity study. LT_{20} refers to the amount of time it takes to cause 20% mortality of the population according to Lamkin's (2005).

Resistance Ratio

Ninety adult lesser mealworm beetles were collected from the field colony and susceptible colony (UCR) and exposed to insecticides and PLT® applied at high label rate for insecticides at a rate of 45.36 kg/92903m^2 rate for PLT®. The insecticides included the pyrethroids, noenicitinoid and organophosphate mentioned previously. Mortality was assessed within the first 4 h post exposure. Using temporal measures of mortality for each populations a resistance ratio was calculated.

Sublethal Insecticide Exposure Fecundity Study

Late-stage larvae taken from the field colony were reared individually in 118 ml minicups (Walmart, Bentonville, AR) with approximately 0.1 g wheat bran, 0.1 g fish meal, 1.0 g bird seed, green dog food (Kibble's 'n Bits; Big Heart Pet Brands, Orrville, OH) and ~2 mm^2 red apple slice every 3 d. Larval minicups were checked weekly for pupae. When pupae were observed, the pupae were sexed using morphological traits on the terminal abdominal segment following the method described by Esquivel et al. (2012). Male and female pupae were kept separately in minicups until molting. Once the males and females molted adults were transferred to 100 mm X

15 mm Petri dishes, maintaining the separation of sexes. Adults were fed red apples (~4 mm^2), fish meal, dog food, and bird seed ad libitum for 1-2 months. The treatments were prepared as listed for the preliminary bioassay to determine lethal time of compounds. Approximately 25 males and 25 female adult lesser mealworms were randomly chosen for each treatment. Exposure times for each treatment varied based on the LT_{20} estimated from the probit analysis on the preliminary bioassay (Table 5.2). PLT® treated individuals were exposed for 6 min 5 s; Tempo® for 36 min and 47s; Tempo® x PLT® for 3 min 29s; Onslaught® 21 min 7 s; Onslaught® x PLT® for 4 min 28s; Credo® for 40 min 7 s; Credo® x PLT® for 6 min 48s; Durashield® for 55 min; and Durashield® x PLT® 5 min 69s. Three adults were transferred off from the treatment dish and kept separate based on sex and placed inside a clean 100 mm Petri dish placed inside an 887 ml meal preparation container (Rubbermaid; Atlanta, GA). Adults were fed apples, fish meal, dog food, and bird seed ad libitum for 3 wk. One male and one female were randomly assigned to a 60 mm clean Petri dish (Celltreat; Pepperell, MA) with a 56 mm clean filter paper (Stonylab; Nesconset, NY) and fed 0.1g wheat bran, 0.1g fish meal, ~2.0 mm^2 red apple slice (every 3 days). A rubber band was used to prevent escapes. An egg packet (3 sheets of ~12 mm x 12 mm black construction paper (Pacon; Appleton, WI) was stapled on one side and slightly fanned out) to encourage egg deposition in the folds of the paper. The number of eggs were counted every 3 d for a total of 30 d. The egg packet was then transferred to a clean Petri dish and the number of emerged larvae were counted from the egg packet every 3 d for a total of 12 d. After the egg packet was removed from a mating pair's Petri dish it was replaced with another egg packet prepared as described above for egg deposition for the next 3 d.

Egg and Emerged Larvae Multiplier of Treatments Compared to Control

The Percent Reproductive Control (PRC) formula was adapted from Rizvi et al. (1980) and Percent Ovicidal Activity (POA) from Arivoli and Tennyson (2013). PRC and POA was calculated by taking the total weekly number of eggs for each treatment and comparing it to the

control with the formulas below. Few values were a positive percentage, so the values were converted by taking the absolute value then subtracted from 1 and divided over 100 to calculate the proportion multiplier for each treatment compared to the control.

$$PRC = \frac{(number\ of\ eggs\ laid\ by\ control - number\ of\ eggs\ laid\ by\ treated)}{number\ of\ eggs\ laid\ by\ control}\ x\ 100$$

$$POA = \frac{(number\ of\ eggs\ hatched\ by\ control - number\ of\ eggs\ hatched\ by\ treated)}{number\ of\ eggs\ hatched\ by\ control}\ x\ 100$$

Statistical Analysis

Probit Analysis. Using mortality over time, a probit analysis was conducted with JMP Pro 15 (SAS; Cary, NC). An inverse prediction was done with the modeled fit line to estimate LT_{20} which was used for the temporal treatment in the sublethal effects assay.

Nominal Logistic Model. A Nominal logistic fit model with target level 1 was conducted with the data transformed to binomial (Yes for eggs=1/No eggs=0) using JMP Pro 15 (SAS; Cary, NC). Treatment, time, and treatment*time were added to the model effects to determine if treatment had an effect. All mating pairs were analyzed in the model. Treatment*time created instability in the model's parameters, possibly a result of the model calculating a parameter as being theoretically infinite, so it was removed to calculate odds ratios. Tempo x PLT and Time (24-30) was used as the baseline parameter for generating the model. The general equation for the logistic regression model: $(p)=b_0 + b_1x_1 + b_2x_2 + \ldots b_nx_n$, where p is the probability of a mating pair producing eggs, b_0 is the intercept, $(x_1 \ldots x_n)$ estimates for the explanatory effects.

One-way ANOVA for Substrate (pH) Treatment Comparison. Mean percent mortality was compared within a substrate. Mean percent mortality of treatments were also compared between substrates within a time interval. If the model indicated significant difference between means,

then a post hoc analysis was conducted using Fisher's Least Significant Difference (LSD) at α = 0.05

One-way ANOVA for Fecundity. Mating pairs that had no eggs or larvae were excluded from the one-way analysis of variance (ANOVA) using JMP Pro 15 (SAS; Cary, NC). The number of eggs and emerged larvae by treatment were compared with time as a fixed effect to test difference in means within a treatment across time intervals. Subject and subject*time were modeled as random effects. If the model indicated significant difference between means, then a post hoc analysis was conducted using Fisher's Least Significant Difference (LSD) at α = 0.05.

Mating pairs that had no eggs or larvae were excluded from the ANOVA using JMP Pro 15 (SAS; Cary, NC). The number of eggs and larvae by time were compared with treatment as fixed effect to compare differences in means between treatments within a time period. Treatment was nested within subject for the model to test if there is a significant difference between means. If the model indicated significant difference between means, then a post hoc analysis was conducted using Fisher's Least Significant Difference (LSD) at α = 0.05.

Results

Surface pH of litter

The surface pH of broiler litter inside an active poultry house is given in Fig. 5.1. Two days before litter amendment application the surface pH was approximately 8. Subsequently, 24 h after PLT® application the pH decreased to approximately 2 pH. Then 48 h after litter amendment application the surface pH increased to approximately 2.5 pH. Three days after amendment application, the pH was recorded at 4. The surface pH continued to rise up to 7 4 d after PLT® application. The surface pH of the broiler litter stabilized around 6 on d 5 and d 6.

Substrate (pH) Treatment Comparison

Used poultry litter was measured to have a pH of 7.65 and wheat bran 6.74. Mean percent mortality at 24 h was greater for treatments applied on wheat bran (Table 5.3). On used poultry litter at 24 h, individuals treated with Onslaught® (9.44% ± 2.00) , PLT® (4.96 ± 1.20), and A7® (3.35% ± 1.21) had similar mean percent mortality as Control (3.33% ± 2.28) and had a mean percent mortality that was significantly less than the remaining treatments (F= 24, df= 8,18, P< 0.0001). On wheat bran substrate at 24 h, individuals treated with Tempo® X A7® (100.00% ± 0.00), A7® (100.00% ± 0.00), and Onslaught® X A7® (100.00% ± 0.00) all had the greatest mean percent mortality (F=93, df= 9,20, P< 0.0001).

PLT® mean percent mortality on used poultry litter (4.96% ± 1.20) was significantly lower than on wheat bran (78.26 ± 5.13; F=93.56, df=1,4, P=0.0006) at 24 h. At 24 h individuals treated with A7® had a mean percent mortality that was significantly lower on used poultry litter (3.35% ± 1.21) than wheat bran (78.26 ± 5.13; F=1936, df=1,4, P<0.0001). Tempo® treated individuals had a mean percent mortality on used poultry litter (39.44% ± 6.87) that was significantly lower than the mean percent mortality on wheat bran (82.89% ± 4.08; F=11, df=1,4, P=0.0304). Tempo® mean percent mortality on wheat bran was greater when used with A7® (100.00% ± 0.00) than PLT® (89.52% ± 7.85).

The mean percent mortality for individuals treated with Tempo X A7® (27.22% ± 8.71) was about 18% less than Tempo X PLT® (45.09% ± 5.93). Mean percent mortality on used poultry litter for Onslaught® (9.44% ± 2.00) was less than mean percent mortality of both Onslaught® X PLT® (25.00% ± 7.97) and Onslaught X A7® (25.54% ± 10.54). On wheat bran mean percent mortality for Onslaught® (81.86 ± 4.06) and Onslaught® X PLT® (80.94% ± 5.98) was less than the mean percent mortality of Onslaught X A7® (100.00% ± 0.00). The greatest mean percent mortality on used litter was Tempo x PLT® (45.09% ± 2.12) but was approximately half as effective as the least effective compound on wheat bran (Tempo®, 80.14%). The greatest

mean percent mortality on wheat bran was A7®, Tempo® X A7® and Onslaught X A7® all with 100% mortality.

Mean percent mortality on used litter and wheat bran at 168 h exposure is compared in Table 5.4. For treatments on used litter observed at 168 h, individuals treated with Tempo® X PLT® had the greatest mean percent mortality followed by Tempo® then Onslaught® ($F=26$, $df=8,18$, $P< 0.0001$). Onslaught® X A7® treated individuals had significantly less mean percent mortality compared to Onslaught®, Tempo®, and Tempo® X PLT® but similar to the other treatments ($F=26$, $df=8,18$, $P< 0.0001$). At the 168 h observation, Control had the least mean percent mortality of the treatments ($F= 206$, $df= 8,18$, $P<0.0001$). Tempo® and Onslaught® had significantly greater mean percent mortality than Control but was significantly less than the remaining treatments ($F= 206$, $df= 8,18$, $P<0.0001$).

Individuals treated with PLT® had a mean percent mortality on used litter (7.18% ± 0.51) that was significantly lower than on wheat bran by 168 h (100.00% ± 0.00; $F=1738$, $df= 1,4$, $P < 0.0001$). A7® mean percent mortality on used litter was significantly lower (4.48 ± 1.09) than mean percent mortality on wheat bran (100.00% ± 0.00; $F=7645$, $df=1,4$, $P<0.0001$). Tempo® mean percent mortality on used litter (28.53% ± 4.28) was significantly lower than the mean percent mortality on wheat bran (80.14% ± 5.14; $F=59$, $df=1,4$, $P= 0.0015$). Mean percent mortality on wheat bran was 100.00% for both Tempo® X PLT® and Tempo X A7® treated individuals, which was greater than the mean percent mortality for Tempo® X A7® (8.89% ± 3.31) and Tempo x PLT® (41.83 ± 2.62) on used litter. Mean percent mortality for Onslaught® (10.00% ± 1.92) applied to used litter was slightly greater than mean percent mortality of both Onslaught® X PLT® (7.78% ± 1.11) but 5-fold greater than Onslaught X A7® (2.19% ± 1.09).

However, performance of these compounds was greater on wheat bran at 168 h. The mean percent mortality for Onslaught® (82.44% ± 3.95) was approximately 18% less than the

mean percent mortality of both Onslaught® X PLT® (100.00% ± 0.00) and Onslaught X A7® (100.00% ± 100.00). The greatest mean percent mortality on used litter after 168 h was Tempo X PLT® (41.83% ± 2.62) but was almost half of the efficacy of the lowest mean mortality on wheat bran, which was Tempo® (80.14% ± 5.19).

On used litter some recovery was observed after 168h with litter amendments and insecticide formulations (Table 5.3 and Table 5.4). On used litter the mean percent morality was significantly greater at 24 h for Onslaught® X A7® (F= 222, df= 1,4, P<0.0001), Onslaught® X PLT® (F=57, df=1,4, P= 0.0017), and Tempo® X A7® (F=31, df=1,4, P= 0.0051). On used litter the mean percent morality was significantly less at 24 h for PLT® (F= 8, df= 1,4, P= 0.0450). On wheat bran substrate after 168 h mean percent mortality was significantly less at 24 h for Onslaught® X PLT® (F= 41, df= 1,4, P = 0.0031), PLT® (F= 74, df= 1,4, P = 0.0010).

On used litter the mean percent mortality was both similar at 24h for Onslaught X A7® (25.54% ± 10.54) and Onslaught® X PLT® (25.00% ± 7.97) but mortality at 168h was 2.19% ± 1.09 for Onslaught® X A7® and 7.78% ± 1.11 for Onslaught® X PLT®. Onslaught® had about the same mortality between the two observations (10.00% vs 9.44). At 24h Tempo® X A7® (27.22% ± 8.71) mortality decreased when the compound was treated on used litter down to 8.89 % (± 3.31). On wheat bran PLT® increased mortality from 78.26% ± 5.13 to 100.00% ± 0.00 by 168 h. Similarly, Tempo® X PLT® increased its mean percent mortality over time from 89.52% ± 7.85 to 100.00 % ± 0.00 from 24 h to 168 h.

Larval RH Bioassay

The LT_{50} for larvae exposed to various treatments at RH~60% and RH~70% is in Table 5.5. Durashield® treated larvae did not have high enough mortality to estimate LT_{50} when the RH was around 70% but took 339.86 min to kill 50% of the population when the RH was around 60%. Durashield® X PLT ® had an LT_{50} of 216.81 min at around 70% RH and to approximately 40

more min to reach LT_{50} (256.93 min) at ~60% RH. Larvae treated with PLT® (72.13%) at RH ~70% took approximately 4-fold less time to kill half the population as it took at ~60% RH (280.80 min). Tempo® $LT_{50.}$ at RH ~60% (104.01 min) was about ten min less than at ~70% RH (113.11 min). The LT_{50} of Tempo® X PLT® was about the same for both ~60% RH (139.22 min) and ~70%RH (134.64 min.). Credo took approximately 50 minutes longer to achieve LT_{50} at RH ~70% (265.27 min) than at RH ~60% (211.11 min). When larvae treated with Credo® X PLT® were exposed at RH ~60% (194.50 min) the LT_{50} is approximately 60 minutes longer than at RH ~70% (132.72 min).

At RH ~60%, the combination of PLT® to a pyrethroid increased the resistance by increasing the LT_{50} (Table 5.5). Tempo® (104.01 min) took 35 minutes longer to achieve the same mortality when PLT® was also used (139.22 min). Onslaught® (92.95 min) saw a greater increase to 210.68 min to achieve LT_{50}. The opposite trend was observed with Credo (211.11 min) and Credo X PLT® (194.50 min). This decrease was also seen with Durashield® (339.86 min) and Durashield® X PLT® (256.93 min).

Preliminary Bioassay to Determine Lethal Time of Compounds

The exposure times for various treatments used for the subsequent fecundity study are shown in Table 5.2. The LT_{20} was the longest for Durashield® at 55 min followed by Credo® at 40.11 min and shortest for Tempo® X PLT® at 3.49 minutes. Durashield® did not obtain high enough mortality to calculate a probit but achieved 20% mortality at approximately 55 min. Tempo® took 36.78 min and Onslaught® 21.12 min to kill a fifth of the population. PLT® (6.09) and Credo® X PLT® (6.80) took approximately the same amount of time to achieve LT_{20}.

Resistance Ratio

Resistance ratios were calculated for the susceptible colony (UCR) and a field strain collected from eastern Oklahoma (Table 5.6). Confidence intervals did not overlap for any treatments

between the 2 strains indicating both colonies were statistically different. The probits for each strain and compound are in Fig. 5.2. Control and PLT® had essentially no lesser mealworm mortality. Individuals treated with Tempo® X PLT® (RR_{50} = 11.01) had greater resistance in relation to time needed to reach 50% mortality (LT_{50}) than those treated with Tempo® alone (RR_{50} =5.96). Similarly, field strain individuals treated with Onslaught® X PLT® (RR_{50} =7.14) had greater resistance than those treated with Onslaught alone (RR_{50} =3.77). Credo® X PLT® (RR_{50} =4.23) had lower resistance (by a factor of 5) than those treated with Credo® alone (RR_{50} =19.62). Durashield® was left out of analysis due to lack of mortality. Other than Durashield®, the field strain was most resistant to Credo SC Ultra®. The RR_{50} (19.62) for Credo® was the greatest for all the compounds analyzed and was 1.78-fold greater than the next highest RR (Tempo® X PLT®). The level of resistance for the field strain exposed to Credo® was greater at LT_{50} than LT_{90}, but all treatments had greater resistance at RR_{90} than RR_{50}.

Susceptible individuals treated with Tempo® took 13.65 min to reach LT_{50} but resistant individuals took 81.37 min to reach the same level of mortality. PLT® was demonstrated no insecticidal properties for this bioassay. Susceptible individuals treated with Tempo® X PLT® took 12.10 min to reach LT_{50} whereas it took resistant individuals 133.20 min. Ninety percent of the susceptible strain was dead by 18.88 min, but it took 256.16 min for the resistant colony. Onslaught® treated susceptible populations took 14.17 min to kill half the population and 90% in 18.63 min. For the resistant strain it took 53.37 min for LT_{50} and 80.33 min for $LT_{90.}$ Onslaught® X PLT® increased the susceptible strain's LT_{50} (19.56 min) and LT_{90} (32.09 min) and LT_{50} (139.61 min) and LT_{90} (263.39 min) of the resistant population compared to just Onslaught®. When the susceptible strain was treated with Credo® the LT_{50} (15.66 min) and LT_{90} (23.86 min) was less than the LT_{50} (42.13 min) and LT_{90} (65.59 min) for Credo® X PLT®. Credo® took 504.47 min to kill 90% of the resistant field strain. To kill half the population of the field strain it took 307.13 min.

The use of PLT with pyrethroids on the field strain increased the LT_{50} but decreased when used with the imidacloprid (Table 5.6). The LT_{50} of Onslaught® (53.37 min) increased almost 87 min when PLT® was applied with Onslaught® (139.61 min). Similarly with Tempo® (81.37) it took 52 min longer when PLT® was applied with Tempo® (133.20 min). The use of PLT® reduced the resistance (decreased LT_{50}) when PLT® was used with Credo® (307.13 min). Credo® X PLT® took 178.17 min, a reduction of approximately 2 h, to kill half the population.

Sublethal Insecticide Exposure Fecundity Study

Nominal regression model of all mating pairs was calculated to evaluate the relationship between the likelihood of egg deposition (eggs = 1, no eggs = 0), and treatment with time (24-30) and Tempo® X PLT® used as reference parameters for the model (Table 5.7). When time*treatment was modeled, it added instability in the estimates and was therefore removed from the model. In the model both time (df=4, L-R ChiSq=108.60, P <0.0001) and treatment (df=9, L-R ChiSq=43.21, P <0.0001) were significant.

The parameter time (0-6 days; 1.05) had the greatest positive effect in the model for time and had almost twice the effect on whether mated pairs produced eggs compared to the interval 6-12 d (0.46). The model indicated that the effect for producing eggs for all treatments was greatest in the first 6 d and decreased throughout the experiment. The interval (18-24 d) had the strongest negative effect relative to the other time parameters (-0.84), which indicates that this interval had the lowest likelihood of mating pairs laying eggs.

Credo® X PLT® (-0.54, P = 0.0196) and Durashield® (-0.74, P = 0.0034) had a negative effect on the egg produced by mating pairs in this model (Table 5.7). Durashield® X PLT® (0.20), Onslaught® X PLT® (0.02), and PLT® (0.11) had a slightly positive effect on the egg production relative to Tempo® X PLT® but not significantly different. Onslaught® (1.08) had the greatest positive effect on eggs being laid relative to Tempo x PLT (P <0.0001; Table 5.7).

The odds ratios for treatment are listed in Tables 5.8 and 5.9. An odds ratio (OR) > 1 is a greater association whereas OR<1 is less likely association. Mating pairs treated with Onslaught® were more likely to produce eggs than the other treatments in the study (Table 5.8). Mated pairs treated with Onslaught® were 4.5-fold more likely to have eggs than Control (OR=4.53), three times more than Credo® (OR=3.05), and about 5 times than those treated with Credo® X PLT® (OR=5.06). Onslaught was about 6 times as likely to produce eggs than Durashield® (OR=6.19) and more likely than Durashield® X PLT® (OR=2.42). However, when PLT® was used in conjunction with Onslaught® the likelihood of having eggs was not different than just using Credo® (P =0.8668), Control (P =0.1726), and Credo® X PLT® (P =0.0985; Table 5.8). Onslaught® X PLT® did not significantly increase the likelihood of having eggs compared to control, Credo®, and Credo® X PLT®.

When comparing the two pyrethroid formulations used in this study, Tempo® treatments were almost a third as likely as Onslaught® (OR=0.29, P = 0.0003) to have eggs (Table 5.8). Tempo® treated mated pairs were less likely to produce eggs than Onslaught® (OR=0.29). Onslaught® treated mated pairs were almost three times more likely to produce eggs (OR=2.88) than those mated pairs treated with both Onslaught® and PLT®. Tempo® X PLT® treated mated pairs were over 2.5 times as likely to have eggs than control (OR=2.53), and similarly more likely than Credo® X PLT® (OR=2.82) to produce eggs. Onslaught® X PLT® treatments were more likely to increase fecundity than Durashield® (OR=2.14).

When mated pairs were treated with only PLT®, only those treated with Durashield® (OR=2.35, P = 0.0184) was more likely to produce eggs. Conversely, only Onslaught® (OR=0.38, P = 0.0045) treated mated pairs were less likely to produce eggs than PLT® (Table 5.8). When PLT® was used with insecticides, it had a stimulatory effect on two treatments in our study. Lesser mealworms treated with Durashield® X PLT® were 2.5 times more likely to produce eggs than those treated with just Durashield ® (OR=2.56). Durashield® X PLT® treated

pairs were also more likely to produce eggs than the control (OR=1.87). Mated pairs treated with Credo® and PLT® were more likely to produce eggs than those treated with just Credo® (OR=0.60, P = 0.1386; Table 5.8).

Durashield® was less likely to produce eggs than Credo (OR=0.49). When PLT was used in conjunction with Durashield®, the likelihood of eggs being produced was significantly increased when compared to the control (OR=1.87, P = <0.0001) and Credo x PLT (OR=2.09, P = 0.0223; Table 5.8). Egg likelihood did not increase for Durashield® X PLT® when compared to Credo® (P = 0.4660; Table 5.8). Mating pairs treated with Durashield® have the same likelihood as Control to produce eggs (P = 0.3843).

The odds ratios for treatment time are given in Table 5.9. Our model of the five time intervals indicates mating pairs are more likely to lay eggs in the earlier time intervals and the likelihood decreases with each subsequent time interval. Treated mated pairs were almost twice as likely (OR=1.80) to produce eggs in the first interval (d 0-6) than in the following interval (d 6-12). The likelihood of having eggs was about a tenth as likely (OR=0.13, P = <0.0001) when comparing the period between 24 d to 30 d and 0 and 6 d (Table 5.9). The likelihood of eggs being laid between 6-12 d and 12-18 d were similar (OR=1.12, P = 0.591), as was the comparison between 18-24 d and 24-30 d (OR=1.19, P = 5517). The likelihood of having eggs for treated pairs was less likely on days 6-12 d compared to days 0-6 (OR=0.56, P = 0.0052; Table 5.9).

One-way ANOVA's were used to compare treatments within a time period (Table 5.10). On the first 6 d of the experiment, Onslaught® had the highest mean number of eggs per mating pair (31.75 ± 4.92) and was significantly higher than all other treatments except for PLT® (21.00 ± 4.49) (F= 2.8, df= 9,117, P =0.0051). The lowest mean number of eggs per mating pair were observed in the Control (6.94 ± 3.89) and Tempo® (8.75 ± 3.10) groups. Onslaught® X PLT® (8.70 ± 3.37), Tempo X PLT (12.93 ± 4.02), Credo® (13.50 ± 4.49), Credo® X PLT® (10.23 ±

4.31), Durashield® (15.40 ± 4.92), and Durashield® X PLT® (15.31 ± 4.31) had similar mean number of eggs to one another and were not statistically different than the Control group (6.94 ± 3.89).

On d 6-12; Durashield® X PLT® (16.85 ± 3.51) had a greater mean number of eggs per mating pair than all other treatments ($F=2.0$, $df=9,117$, $P = 0.0444$; Table 5.10). Credo® (13.50 ± 3.66), Onslaught® (14.58 ± 3.59), Tempo® X PLT® (9.20 ± 3.27) and Tempo (12.42 ± 3.66) had a similar mean number of eggs per mating pair as Durashield® X PLT®. Durashield® (2.00 ± 3.51) had the lowest mean number of eggs per mating pair in this time frame. PLT® (5.79 ± 3.39), Control (4.63 ± 3.17), Onslaught® x PLT® (3.80 ± 3.66) and Credo® X PLT® (5.85 ± 3.51) had a similar mean to Durashield® (Table 5.10).

On d 12-18 ($F=0.7$, $df=9,117$, $P= 0.7403$) and Days 18-24 ($F=0.6$, $df=9,117$, $P = 0.7808$) no significant differences were observed in the mean number of eggs per mating pair between the treatments within those time frames. On d 24-30; mated pairs treated with Durashield® X PLT® (7.23 ± 1.35) had the greatest mean number of eggs of all the other treatments (p=0.0077; Table 5.10). Onslaught® (3.08 ± 1.41), Onslaught® X PLT® (1.00 ± 1.54), Credo® (0.82 ± 1.41), Durashield® (2.40 ± 1.54), Control (0.25 ± 1.22), PLT® (0.00 ± 1.30), Tempo® (0.33 ± 1.41) and Tempo® X PLT® (1.13 ± 1.26) all had less mean number of eggs per mating pair compared to Durashield® X PLT®.

One-way ANOVA's were conducted within each treatment to compare the number of eggs across the 30 days. All means were similar for Control across the entirety of the experiment ($F=2.5$, $df=4,60$, $P=0.0555$; Table 5.11). The means for Control ranged from 6.94 (± 1.58) on d 0-6 to 0.25 (± 1.58) on d 24-30. PLT® had a significantly higher number of eggs per mating pair in the first 6 d (21.00 ± 2.90) compared to the other 24 d (p= 0.0001). On d 6-12, PLT® had 5.79

eggs per mating pair (± 3.20) and decreased egg production throughout the remainder of the experiment until the mean for d 24-30 was 0.00 (Table 5.11).

Mated pairs treated with Tempo® had the greatest mean number of eggs on d 6-12 (12.42 ± 2.90) and lowest on d 24-30 (0.33 ± 2.90; F=3.2, df=4,44, P = 0.0205; Table 5.11). Tempo® on d 0-6 (8.75 ± 2.90) and days 12-18 (6.33 ± 2.90) had similar mean number of eggs as days 6-12. On d 12-18 (6.33 ± 2.90) and 18-24 (2.58 ± 2.90) had a similar mean to d 24-30 (0.33 ± 2.90). Mated pairs treated with Tempo® X PLT® had the greatest mean number of eggs on d 0-6 (12.93 ± 2.75) and d 6-12 (9.20 ± 2.75) and lowest on d 18-24 (1.40 ± 2.75) and d 24-30 (1.13 ± 2.75). Mated pairs treated with Tempo® x PLT® had a similar mean number of eggs per mating pair on d 12-18 (8.13 ± 2.75) as the days preceded.

Onslaught® treated mated pairs had a near 50% reduction in mean number of eggs per mating pair from the first six days (31.75 ± 3.59) to the following six days (14.58 ± 3.59). On d 18-24 (1.42 ± 3.59) and d 24-30 (3.08 ± 3.59) had a similar number of eggs that were significantly less than d 0-6 (F=13.2, df=4,44, P < 0.0001). On d 12-18 (8.33 ± 3.59) had a similar mean number of eggs as all other days (Table 5.11)

Onslaught® X PLT® treated mated pairs had a similar mean number of eggs on d 12-18 (11.10 ± 2.77) and d 0-6 (8.70 ± 2.77) that was significantly greater than d 18-24 (1.30 ± 2.77) and d 24-30 (1.10 ± 2.77; F=4.1, df=4,36, P = 0.0074). Days 6-12 (3.80 ± 2.77) was similar to d 0-6 and d 18-30 (Table 5.11).

Mated pairs treated with Credo® had significantly more mean number of eggs per mating pair on d 0-6 (13.50 ± 2.74) and d 6-12 (13.50 ± 2.77) compared to d 12-18 (5.00 ± 2.74), d 18-24 (1.17 ± 2.74) and d 24-30 (0.75 ± 2.74) (F= 5.7, df=4,44, ,P= 0.0009) (Table 5.11). Credo® X PLT® treated mated pairs had significantly more mean number of eggs per mating pair on days 0-6 (10.23 ± 2.16) compared to d 12-18 (3.54 ± 2.16), d 18-24 (0.38 ± 2.16) and d 24-30 (0.23 ±

2.16) (F=4.5, df=4,48, P=0.0038; Table 5.11). Mated pairs treated with Credo® X PLT® had a similar number of eggs per mating pair on d 6-12 (5.85 ± 2.16) as the other days (Table 5.11).

Durashield® treated mated pairs had significantly higher mean number of eggs on d 0-6 (15.40 ± 3.03) compared to the remaining days of the experiment (F=3.8, DF=4,36, P= 0.0110) (Table 5.11). The mean on d 12-18 (3.70 ± 3.03) was similar to d 6-12 (2.00 ± 3.03), d 18-24 (0.50 ± 3.03) and d 24-30 (2.40 ± 3.03) (Table 5.11). Mated pairs treated with Durashield® X PLT® had the greatest mean number of eggs on d 6-12 (16.85 ± 4.23) and the least on d 24-30 (7.23 ± 4.23) (F=3.1, DF= 4,48, P= 0.0245; Table 5.11). On d 0-6 (15.31 ± 4.23) the mean was similar to d 6-12 (16.85 ± 4.23). The mean on d 12-18 (7.62 ± 4.23) and d 18-24 (3.85 ± 4.23) was similar to d 24-30 (7.23 ± 4.23; Table 5.11).

The mean number of larvae that emerged from mated pairs were compared between treatments within a time frame using one way ANOVA's (Table 5.12). Within d 0-6, mated pairs treated with Onslaught® had the highest mean number of emerged larvae of all the treatments (21.09 ± 3.23; F= 2.2, df= 9, 129, P=0.0265; Table 5.12). Control (6.44 ± 2.68) treated mated pairs had the lowest mean number of emerged larvae but was similar to those treated with PLT® (12.36 ± 2.87), Tempo® (7.31 ± 2.97), Tempo® X PLT® (10.63 ± 2.68), Durashield® (8.14 Onslaught® X PLT® (7.60 ± 3.39) and Durashield® X PLT® (12.38 ± 2.97). Onslaught® X PLT® (7.60 ± 3.39) had less mean number of emerged larvae than just Onslaught® (21.09 ± 3.23) (Table 5.12).

On d 6-12, Onslaught® (10.55 ± 2.18) and Durashield® X PLT® (8.46 ± 2.01) treated pairs had the highest mean number of emerged larvae of all the treatments (F= 2.1, df=9, 129, P= 0.0363; Table 5.12). Onslaught® treated pairs had a mean (10.55 ± 2.18) that was approximately half of (21.09 ± 2.68) the previous 6 d mean emerged larvae for each mated pair. Durashield® X PLT® (1.29 ± 1.94) had the lowest mean number of emerged larvae. Control (3.75 ± 1.81), PLT®

(3.50 ± 1.94), Onslaught® X PLT® (3.40 ± 2.29) had the same mean and was similar to Credo® (6.14 ± 1.94), Tempo® X PLT® (5.13 ± 1.81) and Tempo® (7.62 ± 2.01; Table 5.12).

On d 12-18 (F= 1.5, df= 9, 129, P= 0.1428) and d 18-24 (F=1.4, df=9, 129, P= .2068), mated pairs all had similar number of emerged larvae regardless of the treatment within their respective intervals (Table 5.12). On d 24-30, mated pairs treated with Durashield® X PLT® (4.50 ± 0.90) had the highest mean number of emerged larvae compared to those treated with the other treatments (F= 2.0, df= 9,129, P= 0.0406) but was similar to Onslaught® (2.91 ± 0.98) and Tempo® (2.00 ± 0.90). Credo® X PLT® (0.39 ± 0.77) had the lowest mean number of emerged larvae. Control (0.69 ± 0.81), PLT® (0.43 ± 0.88), Tempo® X PLT® (2.00 ± 0.81), Onslaught® X PLT® (1.30 ± 1.03) and Credo® (1.29 ± 0.87) all had the same mean number of emerged larvae (Table 5.12).

The mean number of emerged larvae per treatment across the 30 d of the experiment, broken down to 5-time stages, is compared in Table 5.13 using one way ANOVA's. On d 0-6 the mean for Control (6.44 ± 1.19) was greater than the mean on d 12-18 (1.56 ± 1.19), d 18-24 (0.75 ± 1.19) and d 24-30 (0.69 ± 1.19) and similar to d 6-12 (3.75 ± 1.19) (F= 4.8, df= 4, 60, P=0.0021; Table 5.13). Mated pairs treated with PLT on d 0-6 (12.36 ± 2.04) had the greatest mean number of emerged larvae compared to the other intervals (F= 5.7, df= 4, 52, P= 0.0007). The mean number of larvae was reduced significantly to 6.43 ± 2.04 on d 12-18 and reduced significantly again on d 24-30 (0.43 ± 2.04). On d 6-12 (3.50 ± 2.04) was similar as d 18-24 (1.29 ± 2.04) and was similar to d 12-18 (6.43 ± 2.04) and d 24-30 (0.43 ± 2.04).

Mated pairs treated with Tempo® had a similar number of emerged larvae throughout the whole experiment (F= 1.9, df= 4, 48, P= 0.1289). Mated pairs treated with Tempo® X PLT® had the greatest mean number of emerged larvae on days 0-6 (10.63 ± 1.71). On d 6-12 (5.13 ± 1.71),

d 12-18 (4.75 ± 1.71), d 18-24 (1.13 ± 1.71) and d 24-30 (2.00 ± 1.71) all had similarly lower number of emerged larvae than d 0-6 (F= 5.4, df= 4, 60, P= 0.0009; Table 5.13).

Mated pairs treated with Onslaught® had the highest mean number of emerged larvae on d 0-6 (21.09 ± 3.37) and lowest on d 18-24 (3.27 ± 3.37) and d 24-30 (2.91 ± 3.37) (p< 0.0001) (Table 5.13). On d 6-12 (10.55 ± 3.37) had a similar mean number of emerged larvae as d 12-18 (7.09 ± 3.37). The mean number of emerged larvae from Onslaught mated pairs decreased significantly by 10.54 between d 0-6 (21.09 ± 3.37) and d 6-12 (10.55 ± 3.37; (F= 9.9, df= 4, 40, P < 0.0001). Onslaught® X PLT® was not significantly different between d 0 and 30 (F= 2.1, df= 4, 36, P < 0.0001) in the number of emerged larvae (Table 5.13).

Credo® treated mating pairs had a higher mean number of emerged larvae on days 0-6 (6.64 ± 1.47), d 12-18 (2.71 ± 1.47) and d 18-24 (1.43 ± 1.47) than d 24-30 (1.29 ± 1.47) and days 6-12 (6.14 ± 1.47) (F= 4.2, df= 4, 52, P = 0.0052). Credo® X PLT® treated pairs had the highest number of emerged larvae on d 0-6 (6.50 ± 1.27) and was significantly higher than the remaining 24 days (F= 4.2, df= 4, 68, P= 0.0043). The reduction between d 0-6 and 6-12 was 3.89 emerged larvae per mating pair (Table 5.13).

Durashield® had its greatest mean number of emerged larvae produced by a mating pair on days 0-6 (8.14 ± 1.53) compared to the remaining 24 d (F= 4.2, df= 4, 52, P = 0.0005) (Table 5.13). The reduction between d 0-6 (8.14 ± 1.53) and d 6-12 (1.29 ± 1.53) was 6.85 emerged larvae per mating pair. Durashield® X PLT® treated mated pairs also had the greatest mean number of emerged larvae on d 0-6 (12.38 ± 2.68) and the lowest on d 18-24 (3.38 ± 2.68) (F= 3.3, df= 4, 48,P = 0.0184). On d 24-30 (4.46 ± 2.68) had the same number of eggs as d 12-18 (6.38 ± 2.68) and d 18-24 (2.71 ± 2.89). On d 6-12 (8.46 ± 2.68) was similar to d 0-6 (12.38 ± 2.68) and d 12-30.

Egg and Emerged Larvae Multiplier of Treatments Compared to Control

The percentage multiplier of percent reproductive control (PRC) in number of eggs between control and various insecticide treatments by day is in Table 5.14. The stimulatory nature of many of the treatments created negative percentages with the PRC, so a percentage multiplier was calculated from PRC. Numbers greater than 1 represent a stimulatory effect in number of eggs. On d 0-6 there was a reduction in number of eggs with Tempo® (0.95) and Onslaught® X PLT® (0.78). Onslaught® (3.43) and PLT® (2.65) produced the most eggs per mating pair compared to Control. On days 6-12, Onslaught® X PLT® (0.51) produced less eggs than control, by approximately half. Durashield® (0.27) had approximately a third as many eggs as control. Durashield® X PLT® (2.96) treated mated pairs produced almost 3 times as many eggs as control and Onslaught® (2.36) produced almost 2.5 times as many eggs as control. On d 12-18, Credo® X PLT® (0.71) and Durashield® (0.57) produced less eggs than Control. Tempo® X PLT® (1.88) treated mated pairs produced almost twice as much as control and was the highest producer of eggs in this time frame. On d 18-24, only Tempo® (1.09) and Durashield® X PLT® (1.47) treated mated pairs laid more eggs than the control treatment. On d 24-30, PLT® treated mated pairs had no eggs and Tempo® (1.00) had equal number of eggs per mating pair as those in Control. Only Credo® X PLT® (0.75) and PLT® (0.00) produced less eggs than the Control treatment. All other treatments had mated pairs ≥ Control (Table 5.14). On the last interval, d 24-30, several treatments had significantly more eggs produced than the Control treated mated pairs. Durashield® X PLT® had 23.50-fold increase in number of eggs and Onslaught® had 9.25 fold increase in egg production. Durashield® (6.00), Tempo® X PLT® (4.50), Onslaught® X PLT® (2.75) and Credo® (2.25) also had increased fecundity at the end of our study

The stimulatory nature of many of the treatments created negative percentages with the percent ovicidal activity (POA), so a percentage multiplier table was made (Table 5.15). Numbers greater than 1 represent a stimulatory effect in number of eggs. The POA as described by Arivoli and Tennyson (2013) calculates the magnifier of difference between the number of

eggs hatched by treatment and control. On d 0-6, Onslaught® X PLT® (0.78), Tempo (0.96) and Credo® (0.95) had less eggs hatch than those treated with distilled water (Control). In this time frame, Onslaught® (2.36) had slightly over 2X as many eggs hatch as the Control treatment. Mated pairs treated with Durashield® X PLT® (1.66), PLT® (1.78) and Tempo® x PLT® (1.74) had over 1.5X as many eggs hatch compared to the Control treatment. On d 6-12, PLT® (0.82), Credo® x PLT® (0.77), Durashield® (0.30) and Onslaught® X PLT® (0.57) all exhibited fewer emerged larvae than Control. Onslaught® (1.93) treated mating pairs had nearly 2 times as many emerged larvae when compared to the Control. On d 12-18, only Durashield® (0.90) exhibited less emerged larvae than Control, but decreased by 10%. Mated pairs treated with PLT® (4.50) exhibited four and a half times as many emerged larvae as the Control in this timeframe. Mated pairs treated with Tempo® (3.40), Tempo® x PLT® (3.50), Durashield® X PLT® (4.15), and Onslaught® (3.80) exhibited over three times as many emerged larvae as Control on d 12-18. Credo® (1.85) and Credo® X PLT® (1.35) treated mated pairs showed approximately one and half times as many emerged larvae as the Control treatment. On d 18-24, Onslaught® (0.60), Tempo® X PLT® (0.90), Credo® (0.70), Credo® X PLT® (0.40), and Durashield® (0.30) treated mating pairs had less emerged larvae than Control. PLT (1.60), Durashield® X PLT® (2.90), Onslaught® X PLT® (1.30), and Tempo (2.30) treated mated pairs exhibited more emerged larvae than Control. On d 24-30, PLT® (0.00), Credo® (0.00), and Credo® X PLT® (0.00) had no emerged larvae and were the only treatments to have less hatched eggs than the control (n=2). Durashield® X PLT® (26.00) had twenty-six times as many emerged larvae as Control. Tempo® X PLT® (2.50) and Onslaught® X PLT® (2.50) treated mated pairs each had two and a half times as many emerged larvae as the Control treatment. Durashield® (5.00) had 5X and Credo® (3.00) had 3X more emerged larvae than the Control group.

Discussion

Most pesticides have a slightly acidic or neutral pH and when placed in an alkaline solution will degrade to a form that is either less effective or non-effective against the pest organism (Tharp and Sigler 2013). Additionally, the basic solution will reduce the half-life of the pesticide depending on the alkalinity. Carbaryl, (Sevin®) at a solution pH of 6 has a half-life of 125 d but the half-life decreases to 24 d at pH 7 and down to 1 d at pH of 9. When insecticides are mixed with alkaline water (pH > 7), a process known as hydrolysis occurs. Hydrolysis breaks down the larger pesticide molecules into smaller, simpler compounds and molecules. These simpler compounds are degraded to forms that are generally less effective against the target pests as the chemical may not be absorbed properly by the pest or become inert in their action (Whitford 2009). This phenomenon also occurs to pesticides applied on soil that are high in alkalinity (Yu 2015) with the specific insecticide classes organophosphates and pyrethroids degrading rapidly in alkaline soils. Conversely, research has discovered imidacloprid has greater persistence in higher pH soils (Sarkar et al. 2001). The longest persistence within the Sarkar et al. (2001) study was in soils at a pH of 8.5 (43.7 d) and least at pH 5.2 (36.4 d). Our study with used poultry litter (7.65 pH) and what bran (6.74 pH) had a 0.91 pH difference between the substrates. This pH difference may account for the much higher observed mortality on wheat bran. Wheat bran was closer to optimum pH for insecticides and less likely to result in chemical degradation and decreased efficacy in the lesser mealworm. Our used poultry litter had a pH lower than what is expected from an active broiler house. A broiler house that has had several flocks without fully replacing the used litter would be expected to have a pH around 8.5, while new rice hull litter has been reported to be around 7.05 pH (Coufal et al. 2006). Other studies have noted that pH of used litter should be between 8.4 and 8.8 (Singh et al. 2004). The age of the litter used in our study was unknown and contained soil mixed in, which may have affected the pH as well.

The microencapsulated insecticides used in this study, Onslaught® and Durashield® may demonstrate a decrease in mortality when the moisture (RH), temperature, and pH of the substrate are not in optimal ranges for the insecticides. The polymeric matrix that coats the insecticides of controlled release, or encapsulated, products limits the diffusion rate of insecticide into the environment (Yu 2015, Ashitha and Mathew 2020). A rate of diffusion can be determined by the manufacturer in certain ideal conditions, but environmental parameters like moisture can break down the physical and chemical bonds. Enzymes and microorganisms present in the litter can also break down the coating. The high RH and temperature in poultry houses (27 °C, RH=80%) could have implications on the efficacy of these compounds if the rate of diffusion become altered. One of the most important features of microcapsules is the large surface area to volume ratios (Dubey 2009). The increased surface area can allow increased surface area for diffusion and interactions with chemicals and light. This increased surface area allows more contact with other chemicals, which can be slightly acidic that could result in prematurely degrading the outer matrix allowing more insecticide compounds to release than intended (Tharp and Sigler 2013).

Insecticides used in this study applied to an acidic surface created by PLT® may have had altered performance as a result. PLT® is a proprietary blend of sodium bisulfate and various other ingredients (Blake and Hess 2001a). PLT® is hygroscopic and dissociates when introduced to water in the air into sodium (Na^+), hydrogen (H^+) and sulfate ($SO4_2^-$; USDA 2015b). The hydrogen reacts with NH_3 to form ammonium (NH_4). The NH_4 then reacts with the sulfate to form ammonium sulfate ($(NH_4)_2(SO_4)$). Ammonium sulfate continues to bind the NH_4 and stay in the litter. The H^+ ions will react with other compounds to form water and other stable compounds. Sodium bisulfate is a hydrogen producing compound that acidifies the litter (Blake and Hess 2001a). According to Jones-Hamilton, the surface pH can lower to around 1.8-2.0 after PLT® has activated (Jones-Hamilton 2021). In experiments conducted by Choi and Moore (2008), the pH for their first experiment went from a control level of 8.56 to 7.80 with 4g PLT®/100g

litter. In the second experiment with the same application rate the pH went from 8.00 (control) to 7.07. The researchers noted that in a field setting with additional water and feces being deposited continually will cover the treated liter and causes the pH to increase at a higher rate because of the titration of acidic litter. The effectiveness of the PLT® amendment would therefore be suspect and reapplication would likely be necessary. The surface pH Fig. 5.1 presented in our study shows that the drop in surface pH to 2 is short and the pH will increase to ~6 by 4 days after application.

Our study has shown that an application of PLT® to the surface of broiler litter can lower the surface pH from ~8 to ~2 in 24 h. According to the USDA (2015b) report on sodium bisulfate, the active ingredient of PLT® and the strong acid it forms is used for decreasing the pH in swimming pools and for cleaning metals. The strong acidic nature has also been demonstrated to lyse cells and to be an effective sanitizer in a food processing environment (Weerarathne et al. 2021). The litter amendment A7® also acidifies the litter, however its mode of action is different. Aluminum sulfate reacts with water and generates sulfuric acid to lower the pH of the litter (USDA 2015a). It is possible the strongly acidic nature of PLT® and A7® can break down the polymeric shell that surrounds the pesticide allowing it to rapidly diffuse into the environment. Insecticides are generally weakly acidic or neutral and work best when the pH is between 4 and 7 (Tharp and Sigler 2013). When PLT® drops the pH to 2, the surface is 100,000-fold more acidic than neutral pH (pH=7). In our study we observed increased time to cause mortality (LT_{50}) for the field strain when pyrethroids were applied with PLT®, but Credo SC® become more effective (lower LT_{50}) in the low pH environment.

A poultry house should maintain RH between 50% and 70% for drying the poultry excretions and maintaining proper litter moisture (Reece and Lott 1982). This is also the recommended activation RH for the product as outlined by the manufacturer of PLT® (Jones-Hamilton 2021). In our experiment the RH was 60% and the granules were mostly intact

suggesting not much of the chemical breakdown has occurred converting sodium bisulfate to ammonium sulfate and water. In our experiments with RH 70% more granules have liquified as more reaction between PLT® and the water in the air has occurred. As more granules liquify, higher and more rapid mortality of the lesser mealworm occurs (unpublished). Future studies should focus on using PLT® at slightly higher RH that may have a greater effect on egg and larval counts for those compounds that are used in conjunction with PLT®. Despite the low mortality of the lesser mealworms exposed to PLT® at RH=60%, the mean number of eggs was about 3 times greater within the first 6 days, and still higher for the following twelve days. On days 18-30 PLT® exposed mating pairs produced less eggs than the untreated control. The number of emerged larvae was twice the control within the first six days and then was the same as the control group for the remaining 24 d of the experiment. Future research should address the higher humidity ranges which may occur in summer months in poultry houses. It may however be difficult to find separations between populations and compounds when mortality comes quickly at the higher RH.

Room humidity has been demonstrated to affect the drying rate of the pesticide and its fugacity, or equilibrium of the compound in a gas and liquid form, when a broadcast spraying method is used (Matoba et al. 1995). The aerial concentration of pesticide varies with humidity. Their study revealed that at lower humidity the amounts of compound on the wall and ceiling of their test was slightly greater. Temperature affects the application by influencing the volume in the droplets and on surfaces like walls and ceilings which was also dependent on whether an aerosolized insecticide, electric vaporizer or a broadcast sprayer was used.

Our study is in agreement with published studies on the level of resistance in field populations to cyfluthrin insecticides. A 2006 study measured RR's ranging from 1.8-4.0 at RR_{50} and 1.7-9.5 at RR_{90} when comparing five field strains to a susceptible population exposed to cyfluthrin (pyrethroid) (Hamm et al. 2006). Their study used technical grade active ingredient at

various doses as opposed to a commercial formulation and lethal time which may account for some differences in RR's however our study revealed that Tempo®, a cyfluthrin pyrethroid, had RR's of 5.96 and 7.90 for RR_{50} and RR_{90} respectively which has similar levels of resistance as several eastern US strains. A study conducted by Lambkin and Rice (2006) continually retested beetle colonies with topical doses of cyfluthrin over several years and revealed resistance to vary from ~2 to 22 fold greater than the susceptible population. Each method of bioassay used to calculate RR may affect the ratio and it has been observed that the use of lethal time to estimate RR may underestimate the level of resistance present but provides a more realistic field scenario than the other methods (Collins 1975). It is possible that if a dose response bioassay was conducted that the resistance levels would be higher than we measured, but lethal time aligns more closely with the goal of this study.

The increase in egg stimulation was greater compared to the number of emerged larvae resulting in a lower hatch rate for several treatments. For example, the eggs from Onslaught® treated adults had a higher proportion of eggs that did not emerge as larvae than those in the Control group. Synthetic insecticides all appear to affect reproductive potential in the form of egg fertility or development of offspring (Moriarty 1969). Onslaught treated mated pairs had a mean of 31.75 eggs on d 0-6, but only 21.09 larvae from those eggs. In the same period, Control had a mean 6.94 eggs per mating pairs and a mean of 6.44 larvae for the same time period. PLT® treated mated pairs similarly had a mean of 21.00 eggs but only 12.36 larvae hatched from those eggs. This suggests a physiological response is occurring to those treated with Onslaught® and other treatments with significantly greater fecundity than Control. The design of this experiment didn't allow for direct correlation between eggs and larvae to calculate percent emergence however emerged larvae value represents greater than 50% of the larvae emerging from counted laid eggs. Our study suggests lower percent emergence from insecticide treated mated pairs compared to Control, but the treatments such as Onslaught® had greater emergence than PLT®.

This phenomenon has been observed previously and include variations in development times, fecundity, and behavioral changes resulting from sub lethal exposure to pesticides and is often described as either hormesis or hormoligosis (Desneux et al. 2007). Hormesis occurs when the compound used on the pest species is considered toxic to that species being studied (Cohen 2006, Guedes et al. 2009). Hormoligosis requires that the effect to be a stressor, which can include temperature, pH, etc. Hormetic response could be missed in a traditional mortality bioassay as measurements are typically only taken at the toxic phase or too late after overcompensation has occurred (Calabrese and Baldwin 2001). Hormesis occurs when low exposure or dose levels of a stressor resulting in physiological fitness improvements. When the biological equilibrium is disrupted, the body overcompensates to try to reestablish homeostasis (Calabrese and Baldwin 2001). Hormesis has also been defined formerly as an evolutionary adaptation to help maintain fitness and results in energy trade-offs (Forbes 2000). Hormesis is a dose response by a stressor where a low dose generally is stimulatory and high doses are inhibitory (Jager et al. 2013). Jager et al. (2013) has proposed three categories of hormesis. The first is acquisition, where the performance increase that an organism exposed to a stressor can be caused by an increase in uptake of energy from the environment likely in the form of increased food consumption. The second is allocation, where the stressor causes a reallocation of resources in response to the stressor. The reallocation of resources could lead to less energy invested in the offspring leading to greater number of offspring, but with smaller size. The final explanation given by Jager et al. (2013) is medication. Some toxicants may be elements that the organism is deficient in, therefore the organism could use them as a resource to make up the deficiency. The category that best explains the hermetic response observed in this study is reallocation. The lesser mealworm may be reallocating energy towards increasing reproductive rates, but the definition suggests there may be a trade off in size of offspring or in the longevity of the adults.

The trade-offs from hormetic response may favor fecundity at the expense of the survivability of the parents. The process of detoxification is costly for the organism with regards to metabolic resources and energy (Calow 1991). The level of resistance in our study was the highest for Durashield® so the individuals exposed to this compound would have the most metabolic resistance requiring a lot of resources thus likely leading to decreased lifespan of these individuals. Durashield® x PLT® treated mated pairs had 23.50-fold greater number of eggs compared to Control at the last interval of our study. Onslaught® had 3.43 times as many eggs as Control in the first interval. We observed that mortality of these insecticide treatments was greater than Control. However, by Day 30, Durashield® (50%) and Durashield® x PLT® (58%) exhibited lower mortality rates when compared to Control 70%. Another explanation given by Jager et al. (2013) is that the resource allocation increases reproductive capacity but affects the likelihood of survivability. In our study we saw Durashield® (50%) have greater mortality than Control (69.57%) on day 30 of the experiment. Durashield® and Durashield® x PLT® had the lowest survivability at 6 days as well. In a literature review of published hormesis studies, Forbes (2000) revealed only 18 studies where a toxicant increased individual performance, however only one study reported an increase in overall fitness from a low dose toxicant. Forbes (2000) concluded that while some performance might be stimulated, the trade-offs of energy affect different traits to varying degrees making it difficult to determine if overall fitness is increased. The need to understand what impact insecticides have on the neurohormonal system that regulates insect reproduction (Lee 2000) is vital to determine the sublethal impacts on different pests. In response to sublethal exposure to permethrin, the spined soldier bug, *Podisus distinctus* had an increase in net reproduction rate when compared to control, however the intrinsic rate of population growth remained unchanged (Guedes et al. 2009). The authors concur that the energetic life history trade-offs are a likely explanation.

Not all studies taken into the account the temporal aspect of hormesis and can be missed in their study. As a result, insecticide induced hormesis has recently become more explored by researchers with approximately 80 publications by 2014 (Guedes and Cutler 2014). Fifty of those papers were specific to insects and mites. Many of these studies also may not have focused on examining hormesis closely. However, with some changes in experimental design, hormesis can be demonstrated (Calabrese and Baldwin 2003). Demonstration of the phenomenon can occur by introducing temporal elements of exposure for a compound or using a range of doses while also undertaking longer studies of fecundity and population growth. Hormesis studies are typically lab based, rather than field based (Cutler 2013). The research undertaken in this study is focused on field applications of commercial formulations and may be a better indicator of actual performance of products and biological responses by lesser mealworms.

Little is known about the molecular mechanism of hormesis (Kendig et al. 2010). However, gene expression, such as upregulation of certain genes, in response to a stressor may be the mechanism that leads to hormesis (Vaiserman 2010, Guedes and Cutler 2014). An example of hormetic response that has been documented is heat shock proteins (Mattson and Calabrese 2010), which are proteins that protect other proteins from damage from denaturation (Feder and Hofmann 1999, Kim et al. 2006). Heat shock proteins are induced and regulated in insects in response to stressors that include ultraviolet radiation, temperature, and pesticides (Zhao and Jones 2012). Down regulation of Hsp60, a heat shock protein, was discovered in individuals treated with sub lethal concentrations of imidacloprid (Ayyanath et al. 2014). The next generation however saw upregulation of the same gene. Genes related to stress, mitochondrial function, developmental and dispersal had either up or down regulation when two generations of the green peach aphid *Myzus persicae* were exposed at sublethal imidacloprid concentrations. While metabolic resistance mechanisms play a part in resistance of pyrethroids, there are other mechanisms that are important in detoxification in the lesser mealworm of these compounds

(Collins 1998, Lambkin and Furlong 2011). Using a synergist like Piperonyl butoxide (PBO) can inhibit detoxifying enzymes according to Moores et al. (2009) methodology, Lambkin and Furlong (2011) revealed only a fraction of the resistance could be attributed to the synergist when comparing the use of an insecticide with and without the synergist on the same population of lesser mealworms. Gene regulation modulation from a hormesis response may affect insects that rely more heavily on resistance mechanisms other than metabolic or enzymatic.

An increase in fecundity in response to low concentrations of imidacloprid from 0 mg kg^{-1} to 0.5 mg kg^{-1} was observed in *Myzus persicae* (Yu et al. 2010). The authors recorded an apparent hormesis response in Juvenile Hormone (JH) III titers from a regression analysis with imidacloprid concentration seen in the form of a U-shaped curve as dose increased. The authors also noted that pesticide induced hormesis can be a mechanism of pest resurgence. Juvenile hormone's observed function in immatures is to prevent premature metamorphosis (Yu 2015). The mechanism of JH is such that titers fall to undetectable or low levels at the end of the larval instar stages, which allows the process of metamorphosis in insects. In adults JH has been observed modulating reproduction. In *Drosophila melanogaster* exposure to a JH analog significantly increase fecundity for the first 10 days post treatment, however at the expense of a shorter lifespan (Flatt and Kawecki 2007). Literature reviews of the topic highlight that the stimulation in fecundity appears to be species dependent (Lee 2000). Other studies have revealed reductions in fecundity resulting from a pyrethroid application in *Chrysoperia carnea* (Garzón et al. 2015). Our study has observed an increase in fecundity from insecticide exposure especially in the first 6-12 d (3-5 wk post exposure). Mated pairs treated with Onslaught® are 4.53 times more likely to have eggs than those treated with Control. In the first eighteen days no treated mated pair had statistically less eggs than Control. On d 18-24 all treatments were similar and on the d 24-30 all treatments had a greater mean than Control except for PLT®, which was similar. Only Durashield® on d 6-12 had statistically less number of emerged larvae than the Control.

Future study is needed to understand any genetic mechanism, such as JH III titer upregulation, as the biological process of the observed increased fecundity by the lesser mealworm resulting from sublethal insecticide exposure.

Future studies that seek to be able to address hormesis need to change the experimental design (Calabrese and Baldwin 2003) to include a range of doses that incorporates time periods of exposure, the doses should be broad enough not to focus on the upper bounds and include measurements that consider the health of the organism other than mortality. When mortality is observed at doses including the upper and lower bounds an inverted U-shaped dose response curve may be observed indicating stimulatory response which may be a demonstration of hormesis. Hormesis has historically been associated with homeopathy and has been therefore neglected by much of the scientific community (Rozman and Doull 2003, Kendig et al. 2010). The research conducted in this study itself was not designed to observe hormesis but the stimulation in egg production by several treatments throughout the experiment highlights the importance of hormesis in the lesser mealworm and other agricultural pests. A more thorough knowledge base on the ovicidal response and mechanisms involved can help benefit future pest management programs.

Resurgence of pests after treatments is often seen and usually attributed to reduction in natural enemies, but hormesis is too often overlooked and should be a factor in future studies (Morse 1998). Depending on treatment, individuals were exposed to compounds for 3.49 to 55 minutes and in a poultry house exposure to compounds by lesser mealworms would be expected to be very short as the beetles are usually hidden within the fiberglass insulation or under the surface of the litter. Exposure to pyrethroids have been shown to cause periods of hyperexcitation in beetles which would further limit the time of contact to surface applied compounds (Tooming et al. 2014). Reapplication of compounds cannot occur inside a poultry house while birds are placed inside so reapplication would have to wait approximately 6 wk.

Future research should focus on sublethal effects of commercial formulations and active ingredients on the lesser mealworm to better understand the population impact of pesticide treatments. Our observations show that on the tail end of our observations Durashield® x PLT® treatments have a 26-fold increase in eggs hatching. If the larvae were to survive to adults the implications on the population growth could be substantial. Each female lesser mealworm can lay 200-400 eggs (Dunford and Kaufman 2006). This would be alleviated possibly as the high mortality over time of Durashield® treated individuals but the R_0, or net increase in population, may still increase. For every single extra female resulting from a treated mated pair that survives to reproduction, there might be 200 or more offspring by the F_2 generation resulting in an increase in R_0, assuming the number of deaths of the parents or F_1 does not exceed the number of offspring. For the treatments that had higher mortality like Tempo® and Onslaught®, the mean number of eggs was statistically greater than the control. The mean number of larvae was greater than the control but still similar to control statistically. A study that focuses on a temporal scale using formulations applied at field rate can elucidate the differences more thoroughly than we did in this study to determine if there is a true hormetic effect that leads to an increased rate of population growth. This invariably could impact the pest management plan for producers.

There were five observations of mites on or around eggs throughout the experiment. The mite *Acarophenax mahunkai* is an egg parasite of the lesser mealworm (Steinkraus and Cross 1993) and is the mite observed in or around the egg batches. The female of this species parasitizes egg masses and can significantly reduce the number of hatched eggs. Non parasitized egg masses had a hatch rate approaching 74% whereas parasitized eggs had approximately 30% hatched eggs. The authors noted that the mass of the egg is reduced to less chorion volume. The eggs appear round and will shrivel up after being fed upon, which was similar to what was noted during egg counts. Most feeding on eggs occurs within the first 24 h of being hatched. During sex sorting of pupae, only one mite was observed on a female adult. The first observation yielded

2 mites (Tempo® dish 1 and Credo® 16). Ten other treatments had eggs that were round and/or mushy, 5 of which were Onslaught® x PLT®. The second observation time two treatments had round eggs (Onslaught® 2 and Credo® x PLT® 5). The fourth observation, on the twelfth day of the experiment, had 2 reports of mites (Onslaught® x PLT® 4 and Tempo® 9). One mite was observed in Credo® 17 on the twenty-first d. On the twenty-seventh d dried eggs were observed (Tempo® 15). The larval counts and hatch rate would have been impacted especially in the earlier observations. After the eggs were counted, the egg packets were segregated to their own Petri dishes and removed from the mating pairs. This physical control probably limited the population of mites and within a few days the number of round/misshapen eggs as well as mites became less numerous. However, it could have possibly reduced egg hatching up to ~40%. This may have affected the hatch rate and egg survivability of several mating pair dishes in this study.

Our study was able to demonstrate that environmental factors such as RH and pH can have a large impact on the efficacy of insecticides applied in conjunction with a litter acidifier. The field strain population tested in Oklahoma had high levels of resistance to all the compounds tested, especially Durashield® and Credo®. The combination with PLT® increased the LT_{50} for Tempo® and Onslaught® but decreased it for Credo®. Our study also revealed that sublethal exposure to these compounds may positively affect fecundity from mated pairs. Our research has demonstrated that the combination of insecticides and PLT® can positively affect the number of eggs oviposited but may also negatively impact the hatch rate. Further research is needed to focus on the fecundity and offspring survivability to determine if the lesser mealworm has a hormetic response from these compound combinations.

FIGURES

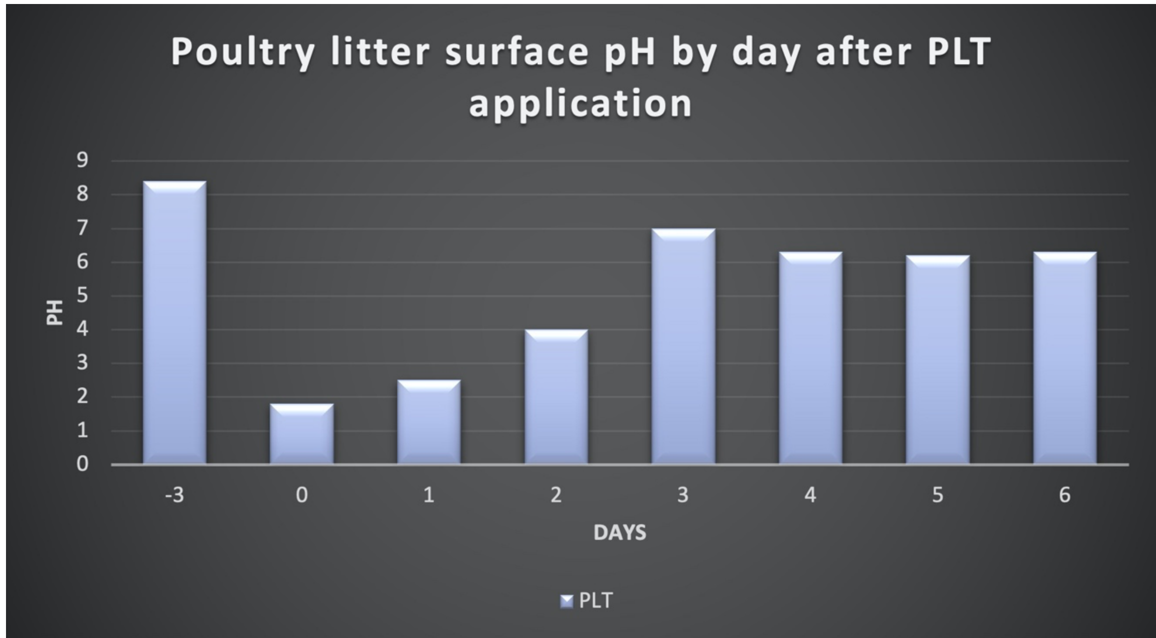


Fig. 5.1. Poultry litter surface pH measurements taken before and after litter amendment application in a poultry house. Litter amendment was applied on day -1

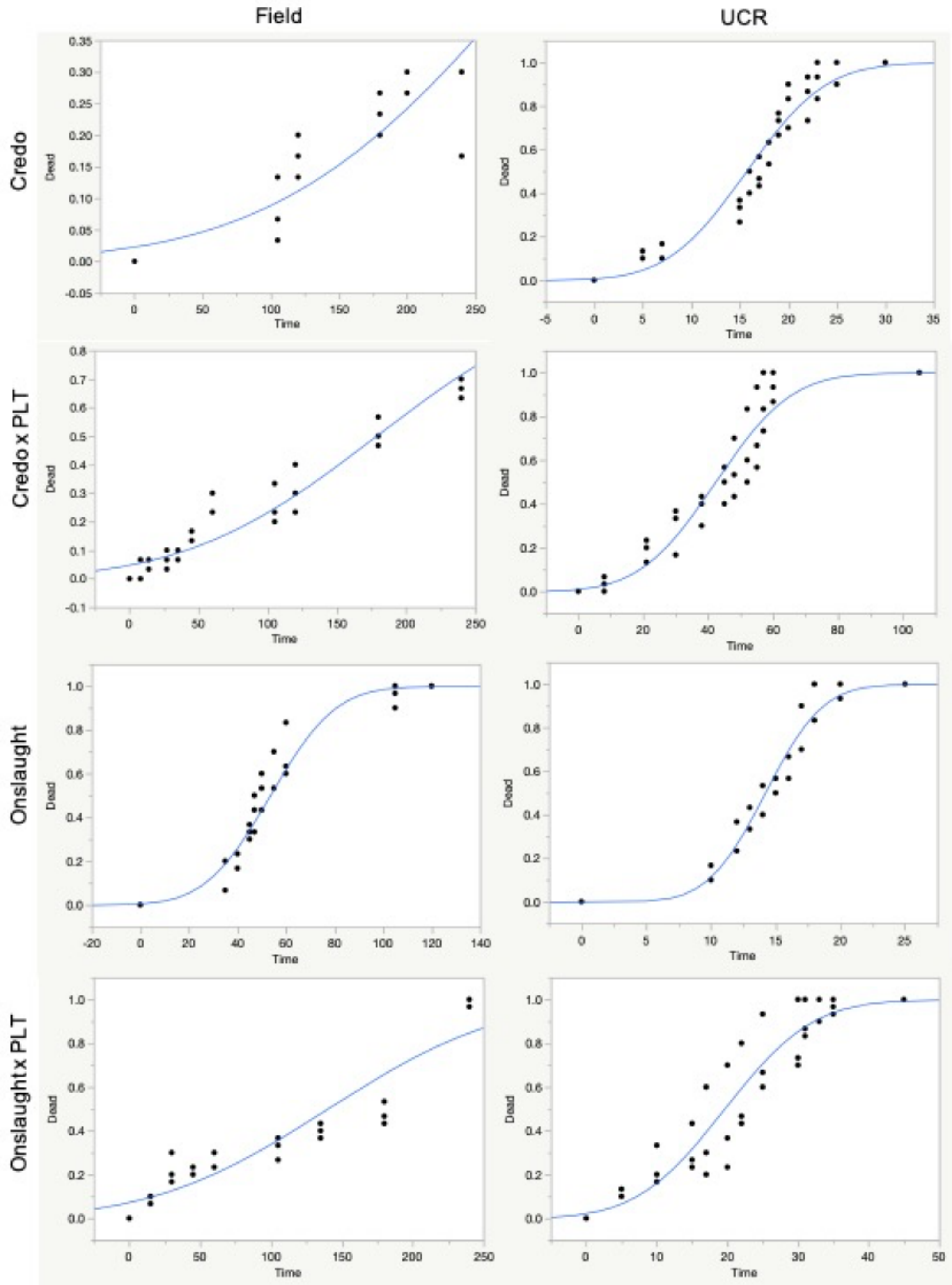


Fig. 5.2. Probits of Field and UCR (Susceptible) Strains of *Alphitobius diaperinus* adults treated with Distilled H_20 (Control), Poultry Litter Treatment (PLT®), Tempo SC Ultra®, Tempo SC Ultra® and PLT®, Onslaught®, Onslaught® and PLT®, Credo SC Ultra®, Credo SC Ultra® and PLT®, applied at label rate.

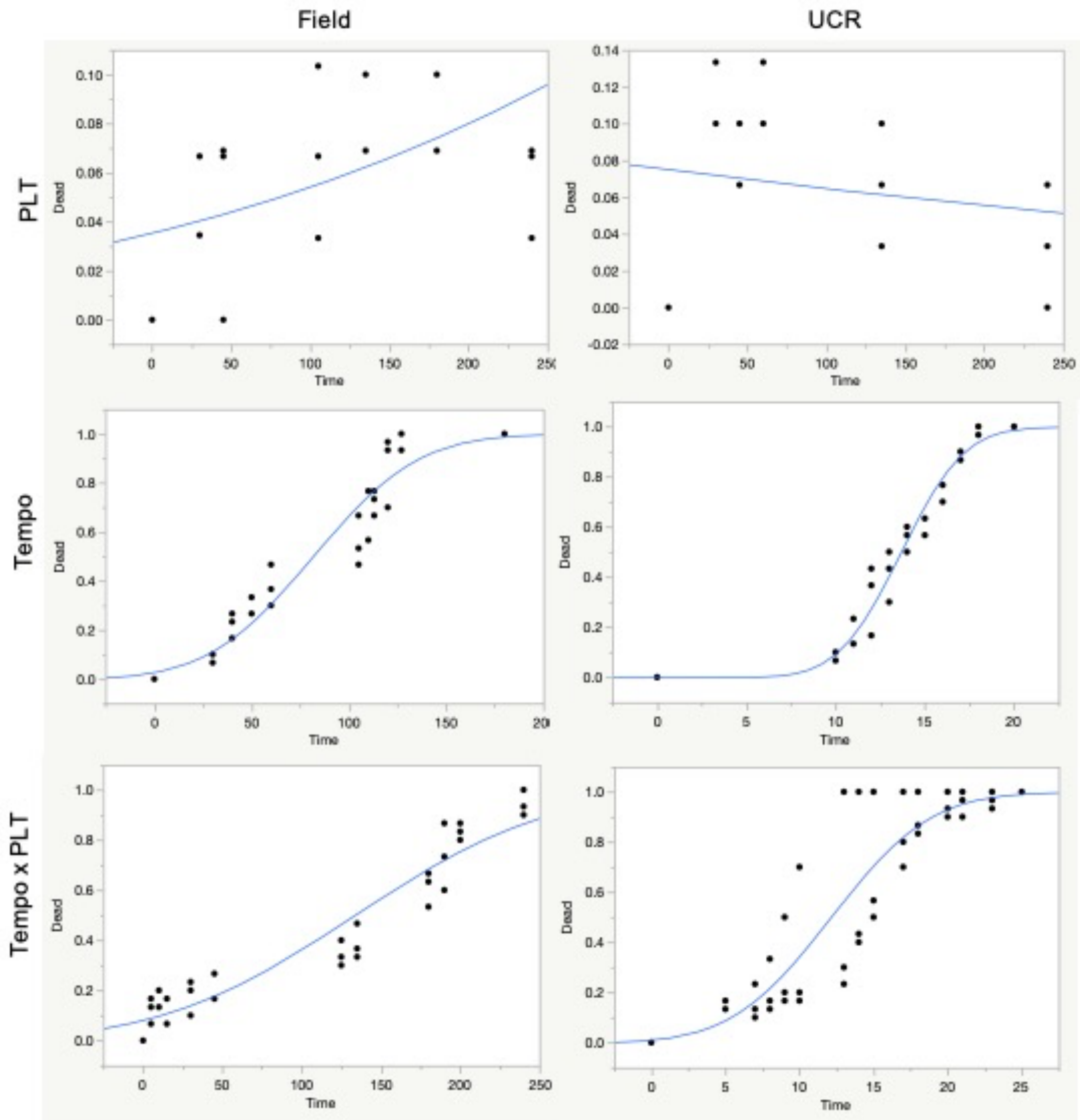


Fig. 5.2. Probits of Field and UCR (Susceptible) Strains of Alphitobius diaperinus adults treated with Distilled H_20 (Control), Poultry Litter Treatment (PLT®), Tempo SC Ultra®, Tempo SC Ultra® and PLT®, Onslaught®, Onslaught® and PLT®, Credo SC Ultra®, Credo SC Ultra® and PLT®, applied at label rate

TABLES

Table 5.1. Insecticide and litter amendments trade name, active ingredient, and insecticide class

Trade Name	Encapsulated	Active Ingredient	Insecticide Class
Insecticides			
Tempo SC Ultra®	No	*ß*-Cyfluthrin	Pyrethroids
Onslaught Microencapsulate®	Yes	Esfenvelerate	Pyrethroids
Durashield CS®	Yes	Chlorpyrifos	Organophosphates
Credo SC Ultra®	No	Imidacloprid	Neonicotinoids
Litter Amendments			
Poultry Litter Treatment (PLT)®	NA	Sodium Bisulfate	NA
Al^+ Clear A7®	NA	Aluminum Sulfate	NA

Table 5.2. Preliminary bioassay LT_{20} and LT_{50} , 95%CI, Slope (SE). RH 62-65%

Treatment	n	LT_{20} (95% CI)	LT_{50} (95% CI)	Slope (SE)
Control®	20	-	-	-
PLT®	20	6.09 (4.15-7.45)	10.06 (8.85-11.24)	0.2124 (0.0277)
Tempo®	20	36.78 (30.95-41.31)	56.39 (52.27-61.08)	0.0429 (0.0047)
Tempo x PLT®	20	3.49 (1.61-4.67)	6.27 (5.18-7.25)	0.3029 (0.0482)
Onslaught®	20	21.12 (17.42-23.92)	29.00 (26.41-31.51)	0.1068 (0.0121)
Onslaught® x PLT®	20	4.47 (1.77-6.24)	9.59 (8.09-10.98)	0.1644 (0.0229)
Credo®	20	40.11 (33.06-45.80)	67.26 (61.70-73.96)	0.0310 (0.0033)
Credo® x PLT®	20	6.80 (4.77-8.22)	11.12 (9.86-12.38)	0.1949 (0.0256)
Durashield®	20	55*	-	-
Durashied® x PLT®	20	5.98 (3.23-7.82)	11.77 (10.20-13.33)	0.1452 (0.0197)

* Durashield® only obtained 25% mortality. LT_{20} occurred at approximately 55 min.

Table 5.3. Used litter (pH=7.65) bioassay and wheat bran pH=6.74) substrate bioassays 24-hour mortality with PLT®, A7®, Tempo SC Ultra®, Durashield CS®, Onslaught®, and Credo SC® with adult beetles collected from Arkansas poultry houses.

	Used Poultry Litter (7.65 pH)				Wheat Bran (6.74 pH)			
Treatment	**n**	**Mean % Mortality (± SEM)**	**Upper 95 % CI**	**Lower 95% CI**	**n**	**Mean % Mortality (± SEM)**	**Upper 95 % CI**	**Lower 95 % CI**
Control	90	3.33 ± 2.28b	-8.49	15.16	93	0.00 ± 0.00d	-10.66	10.66
PLT®	181	4.96 ± 1.20b	-6.86	16.79	91	78.26 ± 5.13c	67.6	88.92
A7®	90	3.35 ± 1.21b	-8.47	15.18	94	100.00 ± 0.00a	89.34	110.66
Tempo®	91	39.44 ± 6.87a	27.62	51.27	90	82.89 ± 4.08b	72.22	93.55
Tempo® x A7®	90	27.22 ± 8.71a	15.4	39.05	89	100.00 ± 0.00a	89.89	111.22
Tempo® x PLT®	91	45.09 ± 2.12a	33.27	56.91	90	89.52 ± 7.85b	78.85	100.18
Onslaught®	90	9.44 ± 2.00b	-2.38	21.27	90	81.86 ± 4.06b	71.20	92.53
Onslaught® x A7®	90	25.54± 10.54a	13.72	37.36	91	100.00 ± 0.00a	89.34	110.66
Onslaught® x PLT®	90	25.00 ± 7.97a	13.18	36.82	98	80.94 ± 5.98c	70.28	91.6

* Values with different lower-case letters in each column indicate statistical significance ((LSD, $P \leq 0.05$).

Table 5.4. Used litter (pH=7.65) bioassay and wheat bran pH=6.74) substrate bioassays 168-hour mortality with PLT®, A7® Tempo SC Ultra®, Durashield CS®, Onslaught®, and Credo SC® with adult beetles collected from Arkansas poultry houses.

	Used Poultry Litter (7.65 pH)				Wheat Bran (6.74 pH)			
Treatment	**n**	**Mean % Mortality (± SEM)**	**Upper 95 % CI**	**Lower 95% CI**	**n**	**Mean % Mortality (± SEM)**	**Upper 95 % CI**	**Lower 95 % CI**
Control	90	4.44 ± 4.44cd	-1.05	15.16	93	0.00 ± 0.00c	-4.82	4.82
PLT®	181	7.18 ± 0.51cd	1.68	16.79	91	100.00 ± 2.22a	97.40	107.05
A7®	90	4.48 ± 1.09cd	-1.01	15.18	94	100.00 ± 2.30a	95.18	104.82
Tempo®	91	28.53 ± 4.28b	23.04	51.27	90	80.14 ± 5.19b	75.32	84.97
Tempo® x A7®	90	8.89 ± 3.31cd	3.39	39.05	89	100.00 ± 0.00a	95.18	104.82
Tempo® x PLT®	91	41.83 ± 2.62a	36.33	56.91	90	100.00 ± 0.00a	95.18	104.82
Onslaught®	90	10.00 ± 1.92c	4.51	21.27	90	82.44 ± 3.95b	77.61	87.26
Onslaught® x A7®	90	2.19 ± 1.09d	-3.31	37.36	91	100.00 ± 0.00a	95.18	104.82
Onslaught® x PLT®	90	7.78 ± 1.11cd	2.28	36.82	98	100.00 ± 0.00a	95.18	104.82

* Values with different lower-case letters in each column indicate statistical significance ((LSD, $P \leq 0.05$).

Table 5.5. Larval bioassay of field strain late stage larval *Alphitobius diaperinus* LT_{50} (minutes), 95%CI, Slope (SE).

	RH~60%			RH ~70%		
Treatment	**n**	**LT_{50} (95% CI)**	**Slope (SE)**	**n**	**LT_{50} (95% CI)**	**Slope (SE)**
Control	55	-	-	10	-	-
PLT®	58	280.80 (229.17-390.35)	0.0076 (0.0014)	10	72.13 (66.26-79.17)	0.0385 (0.0044)
Tempo®	60	104.01 (95.98-113.10)	0.0138 (0.0011)	10	113.11 (97.95-133.51)	0.0296 (0.0057)
Tempo® x PLT®	55	139.22 (127.36-154.58)	0.0120 (0.0011)	30	134.64 (119.62-153.08)	0.0120 (0.0013)
Onslaught®	36	92.95 (86.54-99.84)	0.0268 (0.0021)	20	61.37 (52.51-74.44)	0.0463 (0.0101)
Onslaught® x PLT®	58	210.68 (182.46-258.12)	0.0085 (0.0012)	30	119.28 (105.11-136.24)	0.0121 (0.0013)
Credo	58	211.11 (183.56-256.81)	0.0088 (0.0012)	10	265.27 (206.70-440.21)	0.0090 (0.0026)
Credo® x PLT®	59	194.50 (166.60-242.62)	0.0071 (0.0011)	30	132.72 (117.44-151.51)	0.0116 (0.0012)
Durashield®*	53	339.86 (244.73-675.49)	0.0042 (0.0012)	10	-	-
Durashield® x PLT®	58	256.93 (211.66-348.01)	0.0069 (0.0012)	30	216.81 (194.39-249.18)	0.0122 (0.0016)

*No mortality was reached during our experiments

Table 5.6. Resistance Ratios of Susceptible Colony (UCR) and Field Strain of *Alphitobius diaperinus* adults treated with Distilled H_20 (Control), Poultry Litter Treatment (PLT®), Tempo SC Ultra®, Tempo SC Ultra® and PLT®, Onslaught®, Onslaught® and PLT®, Credo SC Ultra®, Credo SC Ultra® and PLT®, applied at label rate. Mortality assessed within the first 24 hours. LT given in minutes

Treatment	Strain	n	LT_{50} (95% CI)	LT_{90} (95% CI)	RR_{50}	RR_{90}	Slope (SE)
PLT®	UCR	90	-1874.09 (<429.05)	-3543.53 (>868.75)	-	-	-0.0007 (0.0011)
	Field	89	900.18 (>505.90)	1539.43 (<826.57)			0.0020 (0.0010)
Tempo®	UCR	90	13.65 (13.37-13.923)	17.21 (16.78-17.74)	5.96*	7.90*	0.3596 (0.0214)
	Field	90	81.37 (77.24-85.44)	136.01 (129.62-143.62)			0.0235) (0.0013)
Tempo® x PLT®	UCR	90	12.10 (11.64-12.55)	18.88 (18.20-19.67)	11.01*	13.57*	0.1890 (0.0088)
	Field	90	133.20 (124.665-142.244)	256.16 (240.22-275.31)			0.0104 (0.0006)
Onslaught®	UCR	60	14.17 (13.72-14.59)	18.63 (17.95-19.53)	3.77*	4.20*	0.2872 (0.0239)
	Field	90	53.37 (51.22-55.79)	80.33 (75.37-87.16)			0.0475 (0.0041)
Onslaught® x PLT®	UCR	90	19.56 (18.65-20.438)	32.09 (30.77-33.65)	7.14*	7.85*	0.1022 (0.0052)
	Field	90	139.61 (129.091-151.69)	263.39 (241.59-291.45)			0.0104 (0.0007)
Credo®	UCR	90	15.66 (15.040-16.236)	23.86 (23.02-24.86)	19.62*	18.20*	0.1562 (0.0086)
	Field	90	307.13 (268.60-380.08)	504.47 (418.95-674.21)			0.0065 (0.0011)
Credo® x PLT®	UCR	90	42.13 (40.42-43.81)	65.59 (62.73-69.08)	4.23*	5.10*	0.0546 (0.0033)
	Field	90	178.17 (164.48-194.90)	316.17 (288.25-352.44)			0.0093 (0.0007)

* Significantly different from 1.0 based on non-overlap of 95% CI

Table 5.7. Nominal logistic fit model parameter estimates, SE, ChiSquare, Probability of ChiSquare and 95% confidence levels (CI)

Parameter	Estimate	SE	ChiSquare	Prob>ChiSq	Lower CL	Upper CL
Intercept	-0.97	0.08	147.21	<.0001*	-1.13	-0.81
Time (0-6 Days)	1.05	0.14	57.21	<.0001*	0.78	1.32
Time (6-12 Days)	0.46	0.14	10.95	0.0010*	0.19	0.74
Time (12-18 Days)	0.35	0.14	6.08	0.0148*	0.07	0.63
Time (18-24 Days)	-0.84	0.18	22.30	<0.0001*	-1.19	-0.49
Treatment (Control)	-0.43	0.22	3.78	0.05	-0.87	0.00
Treatment (Credo®)	-0.04	0.23	0.02	0.88	-0.48	0.41
Treatment (Credo® x PLT®)	-0.54	0.24	6.42	0.0196*	-1.00	-0.09
Treatment (Durashield®)	-0.74	0.25	8.42	0.0034*	-1.24	-0.25
Treatment (Durashield® x PLT®)	0.20	0.20	1.00	0.33	-0.20	0.59
Treatment (Onslaught®)	1.08	0.23	22.20	<0.0001*	0.63	1.53
Treatment (Onslaught® x PLT®)	0.02	0.22	0.01	0.92	-0.42	0.46
Treatment (PLT®)	0.11	0.22	0.26	0.63	-0.34	0.56
Treatment (Tempo®)	-0.15	0.22	0.39	0.51	-0.60	0.30

* Denotes significance $p<0.05$

Table 5.8. Odds ratios for treatment from nominal logistic fit model.

Treatment	vs Treatment	Odds Ratio	SE	Upper 95% CI	Lower 95% CI	Prob>ChiSq
Credo®	Control	1.49	0.53	0.77	2.86	0.2342
Credo® x PLT®	Control	0.90	0.33	0.46	1.74	0.7468
Credo® x PLT®	Credo®	0.60	0.22	0.31	1.18	0.1386
Durashield®	Control	0.73	0.28	0.36	1.48	0.3842
Durashield®	Credo®	0.49	0.19	0.24	1.00	0.0497*
Durashield®	Credo® x PLT®	0.82	0.32	0.40	1.67	0.5804
Durashield® x PLT®	Control	1.87	0.63	1.01	3.47	0.0455*
Durashield® x PLT®	Credo®	1.26	0.43	0.68	2.35	0.466
Durashield® x PLT®	Credo® x PLT®	2.09	0.72	1.11	3.93	0.0223*
Durashield® x PLT®	Durashield®	2.56	0.94	1.31	5.00	0.0061*
Onslaught®	Control	4.53	1.64	2.34	8.79	<0.0001*
Onslaught®	Credo®	3.05	1.12	1.56	5.94	0.0011*
Onslaught®	Credo® x PLT®	5.06	1.89	2.57	9.96	<0.0001*
Onslaught®	Durashield®	6.19	2.45	3.03	12.64	<0.0001*
Onslaught®	Durashield® x PLT®	2.42	0.83	1.29	4.53	0.0059*
Onslaught® x PLT®	Control	1.57	0.56	0.82	3.02	0.1726
Onslaught® x PLT®	Credo®	1.06	0.38	0.55	2.04	0.8668
Onslaught® x PLT®	Credo® x PLT®	1.75	0.64	0.90	3.42	0.0985
Onslaught® x PLT®	Durashield®	2.15	0.84	1.06	4.34	0.0335*
Onslaught® x PLT®	Durashield® x PLT®	0.84	0.28	0.45	1.56	0.5793
Onslaught® x PLT®	Onslaught	0.35	0.13	0.18	0.67	0.0018*
PLT®	Control	1.72	0.62	0.89	3.31	0.1055
PLT®	Credo®	1.16	0.42	0.60	2.24	0.6679
PLT®	Credo® x PLT®	1.92	0.71	0.98	3.75	0.0576
PLT®	Durashield®	2.35	0.92	1.15	4.76	0.0184*
PLT®	Durashield® x PLT®	0.92	0.31	0.49	1.71	0.7861
PLT®	Onslaught®	0.38	0.14	0.19	0.74	0.0045*
PLT®	Onslaught® x PLT®	1.09	0.39	0.57	2.11	0.792
Tempo®	Control	1.32	0.48	0.68	2.56	0.4038
Tempo®	Credo®	0.89	0.33	0.46	1.73	0.7339
Tempo®	Credo® x PLT®	1.48	0.55	0.75	2.90	0.2576
Tempo®	Durashield®	1.81	0.71	0.89	3.69	0.1034
Tempo®	Durashield® x PLT®	0.71	0.24	0.38	1.33	0.2796
Tempo®	Onslaught®	0.29	0.11	0.15	0.57	0.0003*
Tempo®	Onslaught® x PLT®	0.84	0.31	0.43	1.64	0.6119
Tempo®	PLT®	0.77	0.28	0.39	1.50	0.4451
Tempo® x PLT®	Control	2.53	0.88	1.34	4.77	0.0043*
Tempo® x PLT®	Credo®	1.70	0.60	0.89	3.23	0.1056
Tempo® x PLT®	Credo® x PLT®	2.82	1.01	1.47	5.41	0.0019*
Tempo® x PLT®	Durashield®	3.45	1.31	1.73	6.87	0.0004*
Tempo® x PLT®	Durashield® x PLT®	1.35	0.44	0.74	2.46	0.3311
Tempo® x PLT®	Onslaught®	0.56	0.20	0.29	1.06	0.0763
Tempo® x PLT®	Onslaught® x PLT®	1.61	0.56	0.85	3.04	0.1462
Tempo® x PLT®	PLT®	1.47	0.52	0.77	2.80	0.2411
Tempo® x PLT®	Tempo®	1.91	0.68	1.00	3.65	0.0511

* denotes significance $p<0.05$, Significance based on 1.0 not being in the 95% Confidence Interval

Table 5.8. Odds ratios for treatment from nominal logistic fit model.

Treatment	vs Treatment	Odds Ratio	SE	Upper 95% CI	Lower 95% CI	Prob>ChiSq
Control	Credo®	0.67	0.24	0.35	1.29	0.2342
Control	Credo® x PLT®	1.12	0.41	0.57	2.16	0.7468
Credo®	Credo® x PLT®	1.66	0.61	0.85	3.24	0.1386
Control	Durashield®	1.36	0.53	0.68	2.75	0.3842
Credo®	Durashield®	2.03	0.79	1.00	4.11	0.0497*
Credo® x PLT®	Durashield®	1.22	0.49	0.60	2.50	0.5804
Control	Durashield® x PLT®	0.53	0.18	0.29	0.99	0.0455*
Credo®	Durashield® x PLT®	0.79	0.27	0.43	1.48	0.466
Credo® x PLT®	Durashield® x PLT®	0.48	0.16	0.25	0.90	0.0223*
Durashield®	Durashield® x PLT®	0.39	0.14	0.20	0.77	0.0061*
Control	Onslaught®	0.22	0.08	0.11	0.43	<0.0001*
Credo®	Onslaught®	0.33	0.12	0.17	0.64	0.0011*
Credo® x PLT®	Onslaught®	0.20	0.07	0.10	0.39	<0.0001*
Durashield®	Onslaught®	0.16	0.06	0.08	0.33	<0.0001*
Durashield® x PLT®	Onslaught®	0.41	0.14	0.22	0.77	0.0059*
Control	Onslaught® x PLT®	0.64	0.23	0.33	1.22	0.1726
Credo®	Onslaught® x PLT®	0.95	0.34	0.49	1.82	0.8668
Credo® x PLT®	Onslaught® x PLT®	0.57	0.21	0.29	1.11	0.0985
Durashield®	Onslaught® x PLT®	0.47	0.18	0.23	0.94	0.0335*
Durashield® x PLT®	Onslaught® x PLT®	1.19	0.40	0.64	2.21	0.5793
Onslaught®	Onslaught® x PLT®	2.88	1.05	1.48	5.60	0.0018*
Control	PLT®	0.58	0.21	0.30	1.12	0.1055
Credo®	PLT®	0.87	0.31	0.45	1.68	0.6679
Credo® x PLT®	PLT®	0.52	0.19	0.27	1.02	0.0576
Durashield®	PLT®	0.43	0.17	0.21	0.87	0.0184*
Durashield® x PLT®	PLT®	1.09	0.37	0.58	2.03	0.7861
Onslaught®	PLT®	2.64	0.97	1.35	5.14	0.0045*
Onslaught® x PLT®	PLT®	0.92	0.33	0.47	1.77	0.792
Control	Tempo®	0.75	0.27	0.39	1.46	0.4038
Credo®	Tempo®	1.12	0.41	0.58	2.19	0.7339
Credo® x PLT®	Tempo®	0.68	0.25	0.34	1.33	0.2576
Durashield®	Tempo®	0.55	0.22	0.27	1.13	0.1034
Durashield® x PLT®	Tempo®	1.41	0.48	0.75	2.65	0.2796
Onslaught®	Tempo®	3.42	1.27	1.74	6.71	0.0003*
Onslaught® x PLT®	Tempo®	1.19	0.43	0.61	2.31	0.6119
PLT®	Tempo®	1.30	0.48	0.66	2.53	0.4451
Control	Tempo® x PLT®	0.40	0.14	0.21	0.75	0.0043*
Credo®	Tempo® x PLT®	0.59	0.21	0.31	1.12	0.1056
Credo® x PLT®	Tempo® x PLT®	0.35	0.13	0.18	0.68	0.0019*
Durashield®	Tempo® x PLT®	0.29	0.11	0.15	0.58	0.0004*
Durashield® x PLT®	Tempo® x PLT®	0.74	0.24	0.41	1.35	0.3311
Onslaught®	Tempo® x PLT®	1.79	0.63	0.94	3.43	0.0763
Onslaught® x PLT®	Tempo® x PLT®	0.62	0.22	0.33	1.18	0.1462
PLT®	Tempo® x PLT®	0.68	0.24	0.36	1.30	0.2411
Tempo®	Tempo® x PLT®	0.52	0.19	0.27	1.00	0.0511

* denotes significance $p<0.05$, Significance based on 1.0 not being in the 95% Confidence Interval

Table 5.9. Odds ratio for time

Time	Vs Time	Odds Ratio	SE	Upper 95% CI	Lower 95% CI	Prob>Chisq
6-12 Days	**0-6 Days**	0.56	0.12	0.37	0.84	0.0052*
12-18 Days	**0-6 Days**	0.49	0.11	0.33	0.75	0.0009*
12-18 Days	**6-12 Days**	0.89	0.20	0.58	1.36	0.591
18-24 Days	**0-6 Days**	0.15	0.04	0.09	0.25	<0.0001*
18-24 Days	**6-12 Days**	0.27	0.07	0.16	0.45	<0.0001*
18-24 Days	**12-18 Days**	0.30	0.08	0.18	0.50	<0.0001*
24-30 Days	**0-6 Days**	0.13	0.03	0.08	0.21	<0.0001*
24-30 Days	**6-12 Days**	0.23	0.06	0.13	0.38	<0.0001*
24-30 Days	**12-18 Days**	0.25	0.07	0.15	0.43	<0.0001*
24-30 Days	**18-24 Days**	0.84	0.26	0.47	1.50	0.5517
0-6 Days	**6-12 Days**	1.80	0.39	1.19	2.72	0.0052*
0-6 Days	**12-18 Days**	2.02	0.44	1.33	3.06	0.0009*
6-12 Days	**12-18 Days**	1.12	0.25	0.74	1.71	0.591
0-6 Days	**18-24 Days**	6.64	1.75	4.05	10.90	<0.0001*
6-12 Days	**18-24 Days**	3.69	0.98	2.24	6.08	<0.0001*
12-18 Days	**18-24 Days**	3.29	0.88	1.99	5.43	<0.0001*
0-6 Days	**24-30 Days**	7.93	2.19	4.73	13.30	<0.0001*
6-12 Days	**24-30 Days**	4.41	1.22	2.62	7.42	<0.0001*
12-18 Days	**24-30 Days**	3.93	1.10	2.33	6.62	<0.0001*
18-24 Days	**24-30 Days**	1.19	0.38	0.67	2.14	0.5517

* denotes significance $p<0.05$, Significance based on 1.0 not being in the 95% Confidence Interval

Table 5.10. Mean (± SE) number of eggs collected from *Alphitobius diaperinus* adults that were treated with Distilled H_20 (Control), Poultry Litter Treatment (PLT®), Tempo SC Ultra®, Tempo SC Ultra® and PLT®, Onslaught®, Onslaught® and PLT®, Credo SC Ultra®, Credo SC Ultra® and PLT®, Durashield CS®, or Durashield CS® and PLT® applied at label rate and exposed for LT_{20}[a]

	Time (Days)				
Treatment	**0-6**	**6-12**	**12-18**	**18-24**	**24-30**
Control	6.94 (± 3.89) C	4.63 (± 3.17) CD	4.06 (± 2.57) A	2.12 (± 1.15) A	0.25 (± 1.22) B
PLT®	21.00 (± 4.49) AB	5.79 (± 3.39) BCD	7.29 (± 2.75) A	1.79 (± 1.22) A	0.00 (± 1.30) B
Tempo®	8.75 (± 3.10) C	12.42 (± 3.66) ABCD	6.33 (± 2.97) A	2.58 (± 1.32) A	0.33 (± 1.41) B
Tempo® x PLT®	12.93 (± 4.02) BC	9.20 (± 3.27) ABCD	8.13 (± 2.65) A	1.40 (± 1.18) A	1.13 (± 1.26) B
Onslaught®	31.75 (± 4.92) A	14.58 (± 3.59) AB	8.33 (± 2.97) A	1.42 (± 1.32) A	3.08 (± 1.41) B
Onslaught® x PLT®	8.70 (± 3.37) BC	3.80 (± 3.66) CD	11.10 (± 3.25) A	1.30 (± 1.45) A	1.00 (± 1.54) B
Credo®	13.50 (± 4.49) BC	13.50 (± 3.66) ABC	5.00 (± 2.97) A	1.17 (± 1.32) A	0.82 (± 1.41) B
Credo® x PLT®	10.23 (± 4.31) BC	5.85 (± 3.51) BCD	3.54 (± 2.85) A	0.38 (± 1.27) A	0.25 (± 1.35) B
Durashield®	15.40 (± 4.92) BC	2.00 (± 4.01) D	3.70 (± 3.25) A	0.50 (± 1.45) A	2.40 (± 1.54) B
Durashield® x PLT®	15.31 (± 4.31) BC	16.85 (± 3.51) A	7.62 (± 2.85) A	3.85 (± 1.27) A	7.23 (± 1.35) A
***p*-value**	0.0051*	0.0444*	0.7403	0.7808	0.0077*
DF	9	9	9	9	9
F-Ratio	2.81	2.01	0.66	0.78	2.66

[a] Values with different upper-case letters indicate statistical significance within a time between treatments (LSD, $P \leq 0.05$).
* denotes significance with one way analysis of variance between treatments within a time frame.

Table 5.11. Mean (± SE) number of eggs collected from *Alphitobius diaperinus* adults treated with Distilled H_20 (Control), Poultry Litter Treatment (PLT®), Tempo SC Ultra®, Tempo SC Ultra® and PLT®, Onslaught®, Onslaught® and PLT®, Credo SC Ultra® and PLT®, Durashield CS®, or Durashield CS® and PLT® applied at label rate and exposed for LT_{20}. [a]

	Time (Days)							
Treatment	**0-6**	**6-12**	**12-18**	**18-24**	**24-30**	***p*-value**	**DF**	**F-Ratio**
Control	6.94 (± 1.64) A	4.63 (± 1.64) A	4.06 (± 1.64) A	2.12 (± 1.64) A	0.25 (± 1.64) A	0.0555	4	2.45
PLT®	21.00 (± 3.20) A	5.79 (± 3.20) B	7.29 (± 3.20) B	1.79 (± 3.20) B	0.00 (± 3.20) B	0.0001*	4	7.11
Tempo®	8.75 (± 2.90) AB	12.42 (± 2.90) A	6.33 (± 2.90) ABC	2.58 (± 2.90) BC	0.33 (± 2.90) C	0.0205*	4	3.24
Tempo® x PLT®	12.93 (± 2.75) A	9.20 (± 2.75) A	8.13 (± 2.75) AB	1.40 (± 2.75) B	1.13 (± 2.75) B	0.0064*	4	3.99
Onslaught®	31.75 (± 3.59) A	14.58 (± 3.59) B	8.33 (± 3.59) BC	1.42 (± 3.59) C	3.08 (± 3.59) C	<0.0001*	4	13.25
Onslaught® x PLT®	8.70 (± 2.77) AB	3.80 (± 2.77) BC	11.10 (± 2.77) A	1.30 (± 2.77) C	1.10 (± 2.77) C	0.0074*	4	4.14
Credo®	13.50 (± 2.74) A	13.50 (±2.74) A	5.00 (± 2.74) B	1.17 (± 2.74) B	0.75 (± 2.74) B	0.0009*	4	5.66
Credo® x PLT®	10.23 (± 2.16) A	5.85 (±2.16) AB	3.54 (± 2.16) B	0.38 (± 2.16) B	0.23 (± 2.16) B	0.0038*	4	4.45
Durashield®	15.40 (± 3.03) A	2.00 (± 3.03) B	3.70 (± 3.03) B	0.50 (± 3.03) B	2.40 (± 3.03) B	0.0100*	4	3.89
Durashield® x PLT®	15.31 (± 4.23) AB	16.85 (± 4.23) A	7.62 (± 4.23) BC	3.85 (± 4.23) BC	7.23 (± 4.23) C	0.0245*	4	3.08

[a] Values with different upper-case letters indicated statistical significance within a treatment between time (LSD, $P \leq 0.05$)

[*] Denotes significance with one-way analysis of variance between time within a treatment

Table 5.12. Mean (± SE) number of emerged larvae collected from *Alphitobius diaperinus* mated pairs that were treated with Distilled H_20 (Control), Poultry Litter Treatment (PLT®), Tempo SC Ultra®, Tempo SC Ultra® and PLT®, Onslaught®, Onslaught® and PLT®, Credo SC Ultra®, Credo SC Ultra® and PLT®, Durashield CS®, or Durashield CS® and PLT® applied at label rate and exposed for LT_{20}. [a]

Treatment	Time (Days) 0-6	6-12	12-18	18-24	24-30
Control	6.44 (± 2.68) B	3.75 (± 1.81) BCD	1.56 (± 1.85) A	0.75 (± 0.77) A	0.69 (± 0.81) BC
PLT®	12.36 (± 2.87) B	3.50 (± 1.94) BCD	6.43 (± 1.97) A	1.29 (± 0.82) A	0.43 (± 0.88) BC
Tempo®	7.31 (± 2.97) B	7.62 (± 2.01) ABC	6.00 (± 2.05) A	1.85 (± 0.85) A	2.00 (± 0.90) ABC
Tempo® x PLT®	10.63 (± 2.68) B	5.13 (± 1.81) ABCD	4.75 (± 1.85) A	1.13 (± 0.77) A	2.00 (± 0.81) BC
Onslaught®	21.09 (± 3.23) A	10.55 (± 2.18) A	7.09 (± 2.23) A	3.27 (± 0.93) A	2.91 (± 0.98) AB
Onslaught® x PLT®	7.60 (± 3.39) B	3.40 (± 2.29) BCD	8.40 (± 2.33) A	1.50 (± 0.97) A	1.30 (± 1.03) BC
Credo®	6.64 (± 2.87) B	6.14 (± 1.94) ABCD	2.71 (± 1.97) A	1.43 (± 0.82) A	1.29 (± 0.87) BC
Credo® x PLT®	6.50 (± 2.53) B	2.61 (± 1.71) CD	2.06 (± 1.74) A	1.00 (± 0.73) A	0.39 (± 0.77) C
Durashield®	8.14 (± 2.87) B	1.29 (± 1.94) D	1.71 (± 1.97) A	0.36 (± 0.82) A	1.14 (± 0.87) BC
Durashield® x PLT®	12.38 (± 2.97) B	8.46 (± 2.01) A	6.38 (± 2.05) A	3.38 (± 0.85) A	4.46 (± 0.90) A
***p*-value**	0.0265*	0.0363*	0.1428	0.2068	0.0406*
DF	9	9	9	9	9
F-Ratio	2.19	2.07	1.53	1.37	2.03

[a] Values with different upper-case letters indicate statistical significance within a time between treatments (LSD, $P \leq 0.05$).
* denotes significance with one way analysis of variance between treatments within a time frame.

Table 5.13. Mean (± SE) number of emerged larvae collected from *Alphitobius diaperinus* mated pairs that were treated with Distilled H_20 (Control), Poultry Litter Treatment (PLT®), Tempo SC Ultra®, Tempo SC Ultra® and PLT®, Onslaught®, Onslaught® and PLT®, Credo SC Ultra®, Credo SC Ultra® and PLT®, Durashield CS®, or Durashield CS® and PLT® applied at label rate and exposed for LT_{20}. [a]

Treatment	Time (Days) 0-6	6-12	12-18	18-24	24-30	*p*-value	DF	F-Ratio
Control	6.44 (± 1.19) A	3.75 (± 1.19) AB	1.56 (± 1.19) B	0.75 (± 1.19) B	0.69 (± 1.19) B	0.0021*	4	4.78
PLT®	12.36 (± 2.04) A	3.50 (± 2.04) BC	6.43 (± 2.04) B	1.29 (± 2.04) BC	0.43 (± 2.04) C	0.0007*	4	5.72
Tempo®	7.31 (± 2.20) A	7.62 (± 2.20) A	6.00 (± 2.20) A	1.85 (± 2.20) A	2.00 (± 2.20) A	0.1289	4	1.88
Tempo® x PLT®	10.63 (± 1.71) A	5.13 (± 1.71) B	4.75 (± 1.71) B	1.13 (± 1.71) B	2.00 (± 1.71) B	0.0009*	4	5.42
Onslaught®	21.09 (± 3.37) A	10.55 (± 3.37) B	7.09 (± 3.37) BC	3.27 (± 3.37) C	2.91 (± 3.37) C	<0.0001*	4	9.94
Onslaught® x PLT®	7.60 (± 2.67) A	3.40 (± 2.67) A	8.40 (± 2.67) A	1.50 (± 2.67) A	1.30 (± 2.67) A	0.0953	4	2.15
Credo®	6.64 (± 1.47) A	6.14 (± 1.47) ABCD	2.71 (± 1.47) A	1.43 (± 1.47) A	1.29 (± 1.47) BC	0.0052*	4	4.18
Credo® x PLT®	6.50 (± 1.27) A	2.61 (± 1.27) B	2.06 (± 1.27) B	1.00 (± 1.27) B	0.39 (± 1.27) B	0.0043*	4	4.18
Durashield®	8.14 (± 1.53) A	1.29 (± 1.53) B	1.71 (± 1.53) B	0.36 (± 1.53) B	1.14 (± 1.53) B	0.0048*	4	4.25
Durashield® x PLT®	12.38 (± 2.68) A	8.46 (± 2.68) AB	6.38 (± 2.68) B	3.38 (± 2.68) B	4.46 (± 2.68) B	0.0184*	4	3.29

[a] Values with different upper-case letters indicate statistical significance within a treatment between time (LSD, $P \leq 0.05$).
* denotes significance with one way analysis of variance between time within a treatment.

Table 5.14. Multiplier based on Percent Reproductive Control (PRC) displaying proportion of number of eggs between treatment and control by days.

	Days				
PRC (Treatment)	**0-6**	**6-12**	**12-18**	**18-24**	**24-30**
PLT®	2.65	1.09	1.57	0.74	0.00
Tempo®	0.95	2.01	1.17	1.09	1.00
Tempo® x PLT®	1.75	1.86	1.88	0.62	4.50
Onslaught®	3.43	2.36	1.54	0.50	9.25
Onslaught® x PLT®	0.78	0.51	1.71	0.38	2.75
Credo®	1.46	2.19	0.92	0.41	2.25
Credo® x PLT®	1.20	1.03	0.71	0.15	0.75
Durashield®	1.39	0.27	0.57	0.15	6.00
Durashield® x PLT®	1.79	2.96	1.52	1.47	23.50

* Value > 1.0 indicates stimulatory effect, < 1.0 suppressive

Table 5.15. Multiplier based on Percent Ovicidal Activity (POA) displaying proportion of number of hatched larvae between treatment and control by days.

	Days				
POA (Treatment)	**0-6 days**	**6-12 days**	**12-18 days**	**18-24 days**	**24-30 days**
PLT®	1.78	0.82	4.50	1.60	0.00
Tempo®	0.96	1.63	3.40	2.30	0.00
Tempo® x PLT®	1.74	1.37	3.50	0.90	2.50
Onslaught	2.36	1.93	3.80	0.60	13.00
Onslaught® x PLT®	0.78	0.57	4.05	1.30	2.50
Credo®	0.95	1.42	1.85	0.70	3.00
Credo® x PLT®	1.21	0.77	1.35	0.40	0.00
Durashield®	1.18	0.30	0.90	0.30	5.00
Durashield® x PLT®	1.66	1.83	4.15	2.90	26.00

* Value > 1.0 indicates stimulatory effect, < 1.0 suppressive

CHAPTER VI

SUMMARY

The poultry industry has pushed production of birds used for meat consumption such that since 1925 the industry has shortened the grow-out times of broilers from 112 d to 47 d while more than doubling the slaughter weight up to about ~2.7 kg (Mishler 2020, NCC 2021). As the industry shifted from extensive systems to increasing levels of confinement starting with open sided houses to fully confined housing units, the microhabitat has become optimal for growth and development of the lesser mealworm. Ideal humidity is 50-60% RH, and above 70% RH at 29℃ will impact weight gain of the birds (Aviagen 2018). Rearing temperatures are kept at 30℃ for 1 d old chicks and slowly decreased to 25℃ by d 12 and then 21℃ by d 24. Development time for the lesser mealworm is the lowest around 30℃ (Rueda and Axtell 1996). Also, a generation of the lesser mealworm can be completed within 5 to 8 wk (Axtell and Arends 1990). This timeline falls in line with the amount of time it takes for a broiler chick to get to market weight. The health of the birds can be a concern as the beetles have been revealed to be reservoirs for

several pathogens including bacteria and fungi (Axtell and Arends 1990, Despins and Axtell 1994, Goodwin and Waltman 1996, Bates et al. 2004, Crippen et al. 2009). An emerging pathogen in the broiler industry, *Enterococcus cecorum*, has been implicated in the outbreaks of Enterococcus spondylitis (Aziz and Barnes 2007, Armour et al. 2011). Pathogenic strains of *E. cecorum* can survive on a litter substrate for 168 h (37°C: 78% RH) to an estimated 4200 hours (15°C: 32% RH) (Grund et al. 2021). Environmental sources were tested in houses exposed to the bacteria but had no success (Robbins et al. 2012). Infection of successive flocks suggests that there is a reservoir or environmental source of the pathogen (Borst et al. 2012, Jung et al. 2018). Transmission of bacteria from the lesser mealworm to birds has been demonstrated for *Salmonella* and *Camplobacter* (Hazeleger et al. 2008). In this study we have demonstrated that the lesser mealworm can mechanically transfer *E. cecorum* on its cuticle. If a beetle is exposed to an infected surface, the lesser mealworm can pick up the pathogen and introduce it to a "clean" surface such as feed. A bird may then consume inoculated feed which may cause gastric enteritis.

Further research is needed to measure the bacteria persistence in the frass and gastrointestinal track (GIT) of the beetle so we can better assess its reservoir competency for *E. cecorum*. As the beetle can number into the millions in each broiler house and can escape sanitation efforts, the lesser mealworm may be the source of this pathogen and help the pathogen persist between flocks. To help identify the presence of *E. cecorum* a set of primers was developed in this study that uses a commercially available PCR mastermix. Future researchers can utilize this primer and PCR protocol to test environmental samples from poultry broiler houses and also verify the bacteria is in the GIT or frass of lesser mealworms.

A Knowledge, Attitudes and Practices survey was given to growers in northeast Oklahoma. In our survey, most growers understood that the lesser mealworm could spread pathogens and affect the growth performance of their birds. The growers surveyed in our study already have a high rate of pest monitoring and sufficient general knowledge about the lesser

mealworm pest. The responses demonstrate that insecticides are the most common, and usually only, method for pest management in broiler houses. Most growers that were surveyed properly windrowed their litter. Windrowing also helps to dry the litter which along with ammonia control, can improve the performance of birds, their welfare, and improve their foot pad quality (De Jong et al. 2014). Chemicals classes available for use can be limited and overuse has led to resistance issues (Hamm et al. 2006, Tomberlin et al. 2008). Until more techniques are made available for lesser mealworm control in the field, we must improve the efficacy of our chemical treatments.

Unfortunately, the survey had a low response rate due to the impact of Covid-19. Because of Covid-19 outbreaks the extension program halted in person meetings and soon thereafter the university was closed. It is possible with resumption of in person meetings for PWME courses surveys can be redistributed to broiler growers in several counties to increase the response rate and allow statistical analysis.

The litter acidifier PLT®, reduced the pH of poultry litter, down from 8 to 2. This can possibly impact the efficacy of insecticides and litter amendments. The 24 h mortality of insecticides and litter amendments tested was greatest on the wheat bran substrate possibly due to having a lower pH. After 48 h, the recovery of compounds on wheat bran was less than on the used litter substrate. Our study has revealed a synergistic relationship in mortality between PLT® and Credo® in our field strain. Without PLT® there was a near 20 fold difference in resistance between the susceptible and field strains, but with PLT® the difference fell to 4-5 fold, which was similar to Onslaught®. An antogonistic response to PLT® was observed for the two pyrethroids in our study, greater for Tempo® than Onslaught®. Most pesticides are stable around a pH of 5 to 6 (Schilder 2008). The low pH caused by PLT® may be affecting the chemistry of the active ingredient.

One objective of this study was to measure how much resistance was present from consistent chemical use for lesser mealworm control in eastern Oklahoma broiler houses. We compared LT_{50}, LT_{90} and their resistance ratios (RR) between a susceptible strain not subjected to insecticides to our field strain from eastern Oklahoma. The field strain was at least 3.77-fold more resistant than the susceptible colony (Onslaught®). The highest measurable resistance in this study was Credo SC Ultra® which had an RR_{50} (19.62) and an RR_{90} (18.20). Tempo® x PLT® had the next highest RR's at both RR_{50} (11.01) and RR_{90} (13.57). Durashield CS® was also tested but the levels of resistance present in our field strain prevented the calculation of RR. The levels of resistance were greatest for Durashield® in our larval and adult bioassays.

This study measured an increase in fecundity resulting from sublethal exposure at LT_{20}. No mated pairs were less likely to produce eggs than Control and several treatments including Onslaught® (OR=0.22) and Tempo® x PLT® (OR=0.40) had a stimulatory effect. Mating pairs produced more eggs in the first 2 wk and decreased throughout the 30 days, which was broken up into five (6 d) intervals. The number of eclosed eggs that resulted in viable larvae did not coincide with increased number of eggs. For example, Onslaught® had 31.75 mean number of eggs but only 21.09 mean number of those eclosed during the first 6-day interval. In that same period, all other treatments had statistically similar mean number of eclosed eggs with viable larvae as control. The increases in fecundity translated to greater number of eggs and larvae for several treatments. Durashield® x PLT® and Onslaught® had over twice as many emerged larvae as Control. Each female can lay as many as 2,000 eggs; however, it is expected to be closer to 200-400 per female (Dunford and Kaufman 2006).

Our study demonstrates that the performance of PLT® as an insecticidal compound was shown to be impacted by the RH of the environment. As sodium bisulfate, which is the principal component of PLT, is hygroscopic and will use the moisture in the air and dissociate. Our study shows PLT® had no mortality at RH ~60%. Through various bioassays conducted in our lab, we

have observed the PLT® granules will dissociate fully at RH 65%-70%. Prior experiments revealed that when PLT is allowed to fully dissociate any dish treated with PLT® had complete mortality within 15-20 minutes. At a lower RH, PLT® decreased the efficacy of the two pyrethroids used in this study, Onslaught® and Tempo® as it is seen to increase the LT_{50}. As the humidity is expected to vary from 50% to 70% inside a broiler house (Vučemilo et al. 2008, Reece and Lott 1982), the RH at time of application is important for improving the efficacy of insecticides applied. If there is less compound in spray droplets on lower surfaces, then less of it is available to diffuse through the cuticle of the insect. With less compound diffusing across the cuticle, the insect can metabolize more effectively with enzymes to detoxify compounds. In the field PLT® has been demonstrated to be an effective means to control the lesser mealworm. A field study did find the sodium bisulfate, the a.i. of PLT®, did reduce the lesser mealworm population inside a broiler house out to 34 d (McWard and Taylor 2000). The environmental conditions for this study were not mentioned. As most of the broiler industry is in the southeastern United States, the summer months would be hot and humid. Our study has shown that using PLT® in a higher humidity setting can increase the efficacy of some insecticides and improves the insecticidal properties of PLT® if used alone.

A microencapsulated pyrethroid, Onslaught®, was compared to a non-controlled release pyrethroid, Tempo®. The field strain of lesser mealworm treated with Tempo® had higher levels of resistance than those treated with Onslaught®. When PLT® was used with both products Tempo® still had greater resistance. The opposite trend in resistance was observed with Credo®. When the litter amendment was used in conjunction the mortality was significantly less.

Onslaught® had a greater stimulatory effect on egg production than Tempo®. Tempo® treated mated pairs produced a similar mean number of eggs compared to Control but Onslaught® was significantly greater from d 0-18. The mean number of eggs was greater than Control for Onslaught® from d 0-12. Our study has shown that PLT® can suppress the mean number of eggs

negating some increased fecundity response from some insecticide treatments. This was especially apparent in the first six days, which is the most critical interval observed in our study. PLT® suppressed the egg production of mated pairs exposed to Onslaught®, Tempo® and Credo® but had a stimulatory effect when used with Durashield® on some intervals throughout the experiment. Overall, Onslaught® and Durashield® x PLT® treated mated pairs produced the greatest number of eggs and emerged larvae in our study.

In a poultry house the contact time of the lesser mealworm to compounds sprayed on the various surfaces is likely limited to a few minutes. As a result, not enough compound may have been in contact with the exocuticle to deliver a lethal dose to the individual. Understanding how these sublethal doses impact the fecundity and net population rates on this beetle would be an important consideration in a pest management program. Our research suggests that using the field rate of insecticides at approximately 20% lethal time may cause a hormetic response. Pest resurgence is typically associated with reduced natural enemies but as insecticide induced hormesis studies are becoming more prevalent more instances of increased fecundity are observed (Morse 1998). The mechanisms are still poorly understood and need to be further persued but there has been some progress. We believe that this is the first study that has addressed the increase in fecundity in the lesser mealworm and suggest insecticide induced hormesis may be impacting the population possibly leading to pest resurgence post treatment.

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APPENDICES

KAP Survey given to Poultry Waste Management Education course

Poultry Operation

1. How many birds do you manage on a yearly basis?

   ____

2. How many grow-outs (how many flocks) do you have annually?

   ____

Pests of Poultry Industry (General)

1. Have you observed these pests in your operation?

   House Flies
   Mites
   Litter Beetles/Darkling Beetles/Lesser Mealworm
   Ticks
   Other; Please list________________________

2. Have you seen any insect related damage to your facility?

   Yes; Please explain______________________
   No
   Don't Know

Litter Beetle Knowledge

1. Are litter beetles a major pest in the poultry industry?
   Yes
   No
   Don't Know
2. Are litter beetles capable of spreading pathogens?
   Yes
   No
   Don't Know
3. Can the consumption of litter beetles by birds lead to problems?
   Yes
   No
   Don't Know

4. What do litter beetles consume inside a poultry house? (Check all that apply)
   Bird feed
   Bird feces
   Live healthy birds
   Live sick birds
   Other; Please list________________________________
   Feathers
   Grain
   Wood shavings
   Insulation
5. Would a high population of litter beetles impact energy costs?
   Yes
   No
   Don't Know

Insecticide Program

1. How often do you monitor pest populations?
   Each flock
   Rarely
   Don't Know
   Other___________
   I do not actively monitor the beetle population
2. Do you use methods other than insecticides to control pests?
   Yes, explain__________________________
   No, insecticides are only used

3. What insecticides have you used in the past 2 years? (List as many as possible)

    ____
    ____
    ____
    ____
    ____
    ____

4. What was your target pest(s) for your insecticide applications?

    ____
    ____
    ____
    ____
    ____
    ____
    ____
    ____

5. Do you rotate pesticides? If yes, please list product names:

    Yes; ____
    ____
    No
    Don't Know

6. How do you apply your insecticides? (Check all that apply)

    Targeted (only in certain areas)
    Broadcast sprayer application (whole sprayer including concrete footings)

7. If you apply in a targeted fashion where do you apply?

    Along the walls
    Cracks
    Under feeders
    Under water lines
    Along concrete footing
    Other_____________

Litter Management

1. Do you windrow your litter?

    Yes
    No (If no, skip to question 6)

2. If you windrow, How many days after you first construct the windrow do you turn the windrows?

    0 days
    1 days
    2 days
    3 days
    4 days

Other; ________________

3. How often do you turn the windrows?
   Once
   Every 3-4 days
   Every 5 days
   Other; ______________
4. Do you check the windrow for moisture content?
   Yes
   No
5. What is the layout time for windrowing?
   Less than 12 days
   12 days
   More than 12 days
   Other; __________________
6. How often do you decake the house?
   Once a year
   Once every two years
   Other; __________________
7. Do you apply insecticides on top or on the sides of your windrow piles?
   Yes
   No
   Don't Know
8. What material(s) do you use for bedding/litter
   Pine shavings
   Rice hulls
   Other wood shaving types
   Other_____________
9. Do you fully replace the litter in the house or do a partial cleanout?
   Fully replace; how often _________________
   Partial cleanout; how often ________________
10. What is the average depth of your litter? ______________
11. Do you use any acidifying agents?
    PLT
    A7 Liquid
    A7 dry
    Other___________
    i. Application rate for litter amendment if applicable________________
    ii. Application method for litter amendment if applicable______________

Waste Management

1. How do you address bird mortalities on the farm?

Compost
Incinerator
Offsite rendering
Other; ____________________________

Dear Brandon Lyons,

The Oklahoma State University Institutional Review Board (IRB) has reviewed the following application:

Number: IRB-20-87
PI: Brandon Lyons
Title: Poultry litter management knowledge and practices survey
Review Level: Not Human Subjects

The IRB has determined that your study does not qualify as human subjects research. You will find a copy of your Determination Letter in the generated documents section on IRBManager.
Click IRB - Initial Submission to go directly to the event page. Please click attachments in the upper left of the screen to access the letter.

If you make modifications that could change the determination, please contact the IRB prior to implementation.

If you have questions about the IRB procedures or need any assistance from the Board, please contact the IRB office at 405-744-3377 or irb@okstate.edu.

Best of luck with your research,

Sincerely,

Dawnett Watkins, CIP
Whitney McAllister, MS

Oklahoma State University
Institutional Review Board
Office of University Research Compliance
223 Scott Hall, Stillwater, OK 74078
Website: https://irb.okstate.edu/
Ph: 405-744-3377 l Fax: 405-744-4335l irb@okstate.edu

VITA

Brandon Nicholas Lyons

Candidate for the Degree of

Doctor of Philosophy

Dissertation: LESSER MEALWORM (*ALPHITOBIUS DIAPERINUS*) ASSOCIATION WITH *ENTEROCOCCUS CECORUM* AND PEST MANAGEMENT CONSIDERATIONS FOR THIS PEST IN BROILER HOUSES

Major Field: Entomology

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Entomology at Oklahoma State University, Stillwater, Oklahoma in July, 2021.

Completed the requirements for the Master of Science in Entomology at Texas A&M University, College Station, Texas in 2014.

Completed the requirements for the Bachelor of Science in Animal Science at Texas A&M University, College Station, Texas in 2010.

Experience:

Teaching assistant, Oklahoma State University
Courses: ENTO 2993 Introduction to Entomology: 2018, 2019, 2020, 2021
ENTO 3003 Livestock Entomology: 2015, 2016, 2019

Research Assistant: 2014, 2015, 2016

Professional Memberships:

Entomological Society of America