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Chemical Ecology, Population Dynamics and Insecticide Susceptibility of lesser Mealworm Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae)

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CHEMICAL ECOLOGY, POPULATION DYNAMICS AND INSECTICIDE SUSCEPTIBILITY OF LESSER MEALWORM *ALPHITOBIUS DIAPERINUS* (PANZER) (COLEOPTERA: TENEBRIONIDAE)

CHEMICAL ECOLOGY, POPULATION DYNAMICS AND INSECTICIDE SUSCEPTIBILITY OF LESSER MEALWORM *ALPHITOBIUS DIAPERINUS* (PANZER) (COLEOPTERA: TENEBRIONIDAE)

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of the requirements for the degree of
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ABSTRACT

The lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), is a serious, cosmopolitan pest present in poultry production facilities, where it slows down weight gain in chicks, carries disease-causing organisms, and causes damage to poultry insulation. The first study estimated that the lesser mealworm densities in poultry litter applied to pastures were 3.5, 2.2 and 0.4 million larvae, adults and pupae, respectively, per hectare in Savoy, Arkansas. The repercussions of these adult beetles are that they often disperse to and reinfest poultry houses with the potential to vector disease to poultry, and disperse to invade human dwellings resulting in litigation. The second experiment noted an initial increase in beetle trap catches after heat was turned on in the poultry house in preparation for placement of chicks. There was a reduction in sampled densities of beetles shortly after chick placement. These observations will help decision making regarding lesser mealworm sampling and timing pesticide use.

The third experiment consisted of two behavioral bioassays. In field experiments, significantly higher numbers of beetles were found in pitfall traps treated with the lesser mealworm aggregation pheromone lure as compared to untreated checks. The laboratory two-choice pitfall trap bioassay found a combination of the aggregation pheromone and chicken droppings (CD) to be attractive to lesser mealworm larvae and adults than either odor source alone. Results indicate the potential for combining the synthetic pheromone lure with the potential attractive CD compounds to enhance trap efficacy.

The fourth objective determined respective percent mortalities and baseline dose-responses of beetles to three different classes of insect growth regulators (IGR's) including fenoxycarb, diflubenzuron, and 20-hydroxyecdysone, through topical, residual

contact and feeding bioassays. In feeding bioassays, fenoxycarb and diflubenzuron were more toxic to 1st instars than 7th instars or adults, whereas ecdysone was equally more toxic to 1st instars and 7th instars than to adults. The feeding bioassay was more suitable for the 1st and 7th instar stages than adults. The residual contact bioassay exhibited slightly less percent mortality of 7th instars and adults but this bioassay most closely simulated field application of insecticides. All three bioassay methods produced usable dose-response curves for 7th instar and adults and may be used for surveying temporal changes in the IGR susceptibility to lesser mealworm.

The fifth objective determined the comparative susceptibility and cross-resistance to selected insecticides from different classes in beetles collected from two broiler farms having different insecticide application histories. No pre-existing resistance and cross-resistance to imidacloprid, spinosad and chlorfenapyr were found in cyfluthrin and tetrachlorvinphos resistant M population. The sixth objective examined the baseline susceptibility of lesser mealworm to imidacloprid, metaflumizone and diflubenzuron in residual bioassays. Solutions of 0.25% imidaclorprid + 0.25% metaflumizone, and 0.25% imidaclorprid + 0.50% metaflumizone were more effective against adult beetles. Diflubenzuron was more effective against larvae as compared to adults at 5, 7 or 10 d post exposure. An integrated resistance management approach is discussed for lesser mealworm in poultry houses.

Key Words: poultry, litter, lesser mealworm, pheromone, susceptibility, resistance

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

The United States is the world leader in the poultry industry producing 33% of the world's broiler meat, 53.7% of the turkey meat, and 11% of egg production (Watts 2007). Poultry products in the U.S. have the highest per capita consumption, and are used in a variety of ways including: eggs, buffalo wings, chicken nuggets, chicken-patties, fried, turkey ham, and hot dogs (US-EPA 2001). Eggs are also used in the production of therapeutic vaccines, antibodies and pharmacological proteins (Mine and Kovacs-Nolan 2002). Chicken meat has the highest per capita consumption of 87 pounds in U.S.

Arkansas is the nation's second leading chicken producer, third in turkey production and eighth in table egg production. It produces 1.2 billion chickens, 29 million turkeys and 3.4 billion eggs per year that value $2.6 billion, $284 million and $363 million, respectively. Arkansas is the only U.S. state ranked in the top ten of all three categories of broiler, turkey, and egg production in 2009 (The Poultry Federation 2009).

Many stored grains pest species have been introduced from tropical or subtropical regions to temperate regions due to increased international trade of stored grains and cereals (Peters 1977). The mechanization of farming in the twentieth century resulted in increased and excess production of grains and cereals requiring large scale environmentally controlled storage facilities suitable for the propagation of stored insect-pests (Calibeo 2002). In addition, the increased demand for a growing list of poultry products worldwide caused a rapid transition from a low density, free range poultry rearing system to large scale, and environmentally controlled poultry production houses. This large scale system increased poultry production and decreased the potential for disease outbreaks and pollution from poultry house litter of chicken manure, feed and

bedding (Asaniyan et al. 2007). However, the stable environment in poultry houses of warm temperatures, high humidity and accumulated poultry manure has lead to increased problems with insect-pests (Axtell and Arends 1990).

A typical broiler growout house in Arkansas has an earthen floor that is covered with approximately 8 cm thick bedding material. Depending upon availability and cost, typical bedding materials covering the floor include peanut hulls, sawdust, straw, corncobs, chopped newspaper, and wood shavings (Asaniyan et al. 2007). In Arkansas, pine wood shavings, rice hulls and other wood by-products are the most commonly used bedding materials in broiler production (Davis et al. 2002). The combinations of bedding materials, feed and chicken manure yield litter. The litter may be reused at the end of growout season or refreshed by adding a layer of wood shavings or rice hulls to the old litter. At least yearly, the litter accumulated on the floor is removed and spread on pasture or fields as an organic fertilizer (Steelman 2009).

Bacteria degrade the accumulated moisture rich chicken droppings in the litter to produced ammonia and other noxious gases in the environment (Ritz et al. 2005), which are hazardous to both bird and human health. The litter also supports many insect-pests, such as the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), the house fly, *Musca domestica* (L.) (Diptera: Muscidae), the larder beetle, *Dermestes lardarius* L. and the hide beetle, *Dermestes maculatus* De Geer (Coleoptera: Dermestidae) (Kaufman 2005).

The lesser mealworm (Coleoptera: Tenebrionidae), is a serious, cosmopolitan pest present in poultry production facilities, where it consumes poultry feed and litter, carries disease-causing organisms, poultry lack chitinases to properly digest beetles consumed in

place of more nutritious feed leading to delay of weight gain, and beetles causes damage to building insulation (Eidson et al. 1966). Lesser mealworm is a member of the tenebrionid tribe Alphitobiini (Doyen 1989), which comprises four genera and eleven known *Alphitobius* spp. worldwide (Green 1980; Aalbu et al. 2002). Adult lesser mealworm beetles are also known by other common names: litter beetle, darkling beetle, shining black wheat beetle, black fungus beetle, black poultry bugs, Schmittle beetle, and the shiny black moldy grain beetle (Nolan 1982; Guatam 1989).

Distribution

The lesser mealworm is thought to have originated in sub-Saharan Africa as an inhabitant of bird's nests (Hopkins 1990), and was first introduced in North America from Europe before 1910 (Lambkin 2001; Majka et al. 2008). The beetle has been found in Florida (Peck and Thomas 1998), North Carolina, West Virginia (Castrillo and Brooks 1998), Virginia (Geden and Steinkraus 2003), Georgia, Indiana, Michigan, New York, Ohio, and Wisconsin (Dunford and Young 2004), Maine (Kaufman et al. 2005), and Arkansas (Steelman 1996). The lesser mealworm has been reported as an important pest of poultry houses in England, Italy, Ireland, Belgium, Finland, Poland, Budapest, Russia, South Africa, Nigeria, Chile, Brazil, India, and and New Zealand. It has also been reported as the most abundant coleopteran in caged-layer houses in the United States and South Africa (Rueda and Axtell 1997; Banjo and Soyoye 1999).

Host Range

Lesser mealworm is a stored-product pest infesting stored wheat, barley, rice, oatmeal, soybeans, cowpeas, peanuts, linseed, cottonseed, oilseed products, tobacco, and skims already damaged by other biological agents (Hosen et al. 2004). They are found in

areas with spilled grains around feed bins, especially in poorly maintained grain processing plants (Spilman 1991). Increased survival rates were reported with larvae feeding on broken and spoiled grains (Wilson and Miner 1969). Francisco and Prado (2001) reported larvae and adults feeding on chicken droppings, spilled feed and dead birds in poultry house. They were also reported to feed on bat guano, mold, dead bats and pigeons in caves inhabitated by bats and pigeons in Texas and Kenya (Falomo 1986). Adult lesser mealworm beetles require a protein rich diet (Sarin 1978). They have also been observed feeding on live animals such as chicks, pigeon squabs, snakes and rats (Vaughan 1982; Kumar 1983), manure, house fly eggs and small house fly larvae (Hulley and Pfleiderer 1988).

Biology and Reproduction

Wilson and Miner (1969) reported the lesser mealworm female laid 3.5 eggs/d and that fecundity was positively correlated with an increase in temperature. Rueda and Axtell (1996) reported that females laid 4-7 eggs/d at 25 °C, no egg hatch or larval development occurred at or below 17 °C, and development times from oviposition to adult decreased from 164 d at 20 °C to the shortest of 29 d at 35 °C which is close to the temperature maintained in poultry houses for chicken production. The optimum temperature for larval development is 30° to 33°C with 80 to 90% relative humidity (RH) (Dunford and Kaufman 2006). Eggs are laid in cracks and crevices of poultry house floor, within walls, and in poultry litter (Wilson and Miner 1969). Larvae undergo six to eleven instars depending upon nutrition before pupating. Larvae feed on poultry litter, spilled feed, cracked eggs and dead birds inside the poultry house.

Many last instar lesser mealworms climb the walls and the supporting structures of the poultry houses in search of a pupation site, and often make tunnels in the thermal insulation. Beetle tunneling gradually degrades the insulation and its thermal resistance or R-value (Vaughan et al. 1984). Pupation can also occur in poultry litter, in the earthen floor under the litter or in cracks and crevices in walls. The pupa ecloses to adult in 4 to 17 d (Rueda and Axtell 1996), and the adults usually have a life span of 1 year. Adults have been reported to live more than two years under experimental conditions (Falomo 1986). After emergence from the pupa, it requires 7 d for males to reach sexual maturity and females to become receptive to mating (Hopkins et al. 1992).

Larval and adult stages are primarily nocturnal, with greatest activity occurring at dusk. The adult males and females are identical in appearance and size, and usually found in a 1:1 male to female sex ratio (Wilson and Miner 1969; Pospischil 1996). The sexes may be separated by observing the meso- or metathoracic tibial spines (Hewlett 1958) or posterior edge of the eighth sternite. Sexes can be easily separated in the pupal stage based on an externally visible genital appendage or the second valvifer in females which is absent in males (Barke and Davis 1967).

Survival Under Adverse Environmental Conditions

The 1^{st} to 5^{th} instars can live without food and water for an average of 10.6 d (Rahman et al. 1991), and late instars pupate in situations of prolonged starvation. In a study by Preiss (1971), newly emerged adults survived an average of 19.5 d without food or water whereas the older adults survived a slightly longer period of time. The highest temperature at which insect movement has been observed (thermostupor point) was 47.4 °C (Vannier 1987). Chill-coma, the low temperature at which insect activity ceases, was

6 °C, and the supercooling temperature of the adult beetle was -14.5° to -10.3 °C (Renault et al. 1999). The thicker cuticle of rectal intima aids adults in the conservation of water (McAllister et al. 1995), and this cuticular adaptation allows them to survive extreme dry environment conditions especially during removal of poultry litter in the poultry house (Salin et al. 1999; Salin et al. 2000a).

Habitat and Population Structure

Adults and larvae tend to congregate in higher numbers under caked manure, areas beneath the waterers (Salin et al. 2000b), feeding trays, dead birds and any object lying on the surface of the litter in a broiler house (Lancaster and Simco 1967). Cogan et al. (1996) reported 90% of beetles under the support pillars and feeders in a heavily infested turkey growout house in England. Beetles were found initially on the perimeter of houses, and started moving toward the center as the density of beetles increased over the time (Strother and Steelman 2001). Beetle populations have been known to increase with the addition of successive flocks in a broiler growout house (Strother and Steelman 2001).

Densities of lesser mealworm in a poultry house can reach 10,000 to 20,000 individuals per m^2 (Arends 1987). In Arkansas, a 1,386 m^2 poultry broiler growout house where Arends tube traps and soil/litter samples from the 1,268 m^2 area of open litter estimated an average weekly number of 2.2×10^6 adult lesser mealwom beetles (Strother 1998). Larvae were most numerous in May, whereas adult numbers peaked in late August in simulated laboratory population studies (Sarin 1978, 1980). In South Africa, numbers of lesser mealworm were greatest from March until June, and reduced during the winter, where the population overwinters as an adult in poultry manure in a caged-layer house

(Pfeiffer and Axtell 1980). Schmitz and Wohlgemuth (1988) examined the behavioral activity of larvae in response to poultry flock rearing schedules. The larvae migrated to the walls of the house about 13 d prior to clean out and remained hidden until the house temperature was again increased, and a new flock was introduced in poultry houses in Sweden.

Pest Status

Inhibit Chick Weight Gain: Adults and larvae feed and hide in large numbers in the litter of poultry houses. Broiler chicks eat these beetles and may consume as much as 33 g live weight of larvae/d (Despins and Axtell 1995). The chick's crop becomes distended and the beetle larvae may be visible through the stretched skin. Weight gains by chicks consuming larvae were significantly less than those that fed on chicken feed, and the difference continued even after chicks were provided normal feed (Despins and Axtell 1995). Moisture starved beetles chew the skin at the base of feathers of the birds to get moisture during dry conditions (Savage 1992). Feeding by the beetles causes annoyance to the birds and keeps them moving at night rather than eating which can result in decreased weight gain. Lesser mealworm adults and larva are difficult to digest due to lack of chitinases in the bird's digestive tract. This can cause intestinal blockage and microscopic lesions to develop along the bird's intestinal wall (Elowni and Elbihari 1979).

Structural Damage: Mature beetle larvae seeking pupation sites will climb poultry house walls, support structures, and chew holes in styrofoam, fiberglass, and polystyrene insulation panels in the walls of poultry houses (Vaughan et al. 1984; Despins et al. 1987). Resulting damage can cause gradual decrease in R-value resulting in

increased heating bills, and additional building repair costs. Energy costs in beetle-damaged broiler houses are reported to be 67% higher than in houses without beetle damage (Geden and Hogsette 2001). It was estimated that in 1996 Georgia spent over $1,000,000 in control, and $8,476,000 for damage repair costs (Riley et al. 1997)**.**

Invasions: Beetles get spread with the litter on nearby pastures and fly back to lighted, poultry houses (being prepared for new chicks and those producing chickens) or fly to and infest nearby residences or granaries (Gall 1980). This practice can be a potential source of beetles that can invade other nearby lighted buildings leading to poor community relations and litigation (Axtell 1999). Lesser mealworm beetles can fly over a mile, and have been captured in suction traps placed several miles from the nearest granary or farm (White et al. 1995).

Effect on Other Insects: Lesser mealworm may have a negative impact on the establishment of other insects. Lesser mealworms in caged-layer poultry houses made the substrate less favorable for house fly reproduction by their tunneling activity that dries out the manure (Geden 1990; Geden and Hogsette 2001). Similarly, they were facultative predators of larvae of the house fly predator, *Carcinops pumilio* Erichson (Coleoptera: Histeridae), which reduced its efficiency to suppress populations of house fly (Watson et al. 2001; Kaufman et al. 2002). Lesser mealworm larvae and adults were also observed to feed on eggs of the Angoumois grain moth, *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) (Guatam 1989), and eggs and larvae of the rice moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) (Dass et al. 1984).

Vector Disease: Lesser mealworm beetles feed on dead diseased poultry carcasses, and vector disease causing organisms. Healthy birds that eat infected beetles

can become infected. Lesser mealworm beetles serve as mechanical vectors, and reservoirs of several avian viral pathogens and diseases such as the Marek's disease, Newcastle disease, avian influenza (Hosen et al. 2004), infectious bursal disease (Gumboro) (Falomo 1986), turkey coronavirus (Calibeo 2002; Watson et al. 2000), Poult Enteritis Mortality Syndrome, avian leukosis virus (Calibeo 2002); bacterial pathogens, *Salmonella typhimurium* Loeffler and *Escherichia coli* Migula (McAllister et al. 1994); fungal pathogens, *Aspergillus* and *Candida* (Des Las Casas 1970; 1972); protozoans such as *Eimeria* spp. that cause coccidiosis (Goodwin and Waltman 1996; Hosen et al. 2004); helminths such as *Subulura brumpti* Lopes-Neira (Karunamoorthy et al. 1994), and *Hymenolepsis minutissima*, a causal agent of fowl cestodes (Gogoi and Chaudhuri 1982).

Lesser mealworm is an omnivore that scavenges, continuously feeds and lacks a crop (McAllister et al. 1995), which allows large numbers of pathogens to increase in the gut (Calibeo 2002). Larvae exposed to *S. typhimurium* were able to transmit pathogens even after they were surface sterilized. Transstadial transmission of *S. typhimurium* appears to occur from the larvae to the adults. This may be the reason why certain diseases remain endemic in a particular poultry house despite extensive cleaning and disinfecting efforts (Calibeo 2002).

Toxins and Allergens: Adult beetles produce defensive compounds such as quinones and benzoquinones, for their protection against predation (Tschinkel 1975). These compounds are hazardous to human health, and long term exposure can cause health ailments such as asthma, headaches, dermatitis, allergic angiodema, rhinitis, erythema (reddening), conjunctivitis and corneal ulceration, and formation of papules (Tseng et al. 1971; Falomo 1986). The mycotoxin F-2 (Eugenio et al. 1970) produced by

the fungus *Fusarium roseum* Gibbosum, responsible for deformation of sex organs and abortions in swine persist in the lesser mealworm through metamorphosis.

Management

Accurate monitoring of lesser mealworm in the poultry house is the first step towards successful management. Sampling methods available include: counting holes on "sentinel" pieces of insulation placed on walls and posts; counting larvae on walls and posts during early evening (Geden and Hogsette 2001); Berlese funnels to drive beetles out of soil/litter samples (Safrit and Axtell 1984); and Arends tube trap (Arends 1987). This Arends tube trap consists of rolled corrugated cardboard inside a PVC pipe (20 cm long and 3.8 cm diameter), and is a very effective trap for monitoring lesser mealworm in poultry houses (Strother and Steelman 2001).

Cultural and Mechanical Control: Checking waterer's for leaks, and keeping manure dry through adequate ventilation reduced numbers of house flies and lesser mealworm beetles inside poultry houses (Turner 1986). Cleaning up spilled feed in feed storage areas or outside poultry houses can help in preventing new infestations. A more expensive approach to maintain a low beetle population is frequent removal of litter and replacement with fresh litter bedding in broiler breeder and broiler growout systems (Geden and Hogsette 2001; Hinton and Moon 2003).

Mechanical barriers have been used to prevent larval dispersal from the manure to walls in caged-layer poultry house in order to prevent tunneling into thermal insulation. Application of toxic bands of a strain of the fungal pathogen *Beauveria bassiana* (Balsamo) Vuillemin (Geden et al. 1998), and attachment of bands of polyethylene terepthalate thermoplastic polymer to wooden posts were both 100% effective at

preventing lesser mealworm beetle larval dispersal in a poultry house (Geden and Carlson 2001). A plastic collar wrapped around building support posts proved highly effective in preventing movement of adult and larval lesser mealworm, the larder beetle, and the hide beetle in caged-layer poultry facilities in New York and Maine (Kauffman et al. 2005).

Biological Control: The fungal pathogen *B. bassiana* isolate provided 60 to 90% suppression in young lesser mealworm larvae (Geden and Steinkraus 2003). The warm and humid conditions inside the poultry house during the growout period favor the fungal propagation that makes this fungus a potential candidate for biological control (Steinkraus et al. 1991). A total of 12 strains of nematodes, *Steinernema carpocapsae*, *S. feltiae*, and *S. scapterisci*, were tested for their infectivity and virulence against adults of the lesser mealworm beetle in laboratory bioassays. The infectivity of *S. carpocapsae* and *S. feltiae* against lesser mealworm adults varied from 16.8 to 98.3%, and 0 to 100%, respectively (Szalanski et al. 2004). Nematodes applied at the time of clean out became ineffective because the beetle population increased very slowly for the first three weeks (Geden et al. 1987). Nematodes did not survive more than three weeks at a temperature above 24 ºC and were not present when the beetle's population increased inside the house (Geden and Axtell 1988).

The predaceous female mite, *Acarophenax mahunkai* (Steinkraus & Cross), parasitized 51% of the lesser mealworm egg masses in the laboratory, but its potential in the field has not been tested (Steinkraus and Cross 1993). Many isolates of *Bacillus thuringiensis* (BT) were tested but all of them were ineffective against the lesser mealworm adults and larvae in laboratory assays, with *B. thuringiensis* subsp. *finitimus* achieving the highest mortality of only 9.9 % (Lonc et al. 2001). The virulent isolate (K)

of *Metarhizium anisopliae* (Metchnikoff) Sorokin reduced the number of early instars by 8 to 15 fold, and produced 80-90% mortality in mature larvae for up to 14 d after containers with a concrete bottom covered with wood shavings were treated (Gindin et al. 2008). Two protozoans, *Gregarina alphitobii* (an undescribed spp.) (Eugregarinorida, Gregarinidae) and *Farinocystis tribolii* Weiser (Neogregarinorida, Lipotrophidae), failed to produce epizootic infections in poultry houses (Steinkraus et al. 1992), but reduced fecundity and longevity of beetles in Africa, Asia, Europe and the United States (Bala et al. 1990).

Chemical Control: Majority of broiler and turkey growout houses in AR are treated with chemical insecticides between flocks and during clean out, a time between removal of poultry litter and introduction of new flock. Many insecticides have been reported as effective and are registered against the lesser mealworm, including carbaryl, mevinphos, fenitrothion, pyrethrum, azamethiphos, chlorpyrifos and lindane (Saxena and Sarin 1972), naled, metrifonate, fenitrothion with permethrin, fenitrothion with resmethrin and pipernyl butoxide (Toyoshima et al. 1996), cyfluthrin, tetrachlorvinphos (Kaufman et al. 2008), fenitrothion (Lambkin 2005), and fenpropathrin (Tabassum et al. 1998).

Boric acid and lime hydrate (calcium hydroxide) are effective against adult and larval lesser mealworm in the laboratory but their potential has not been tested in the field (Watson et al. 2003). Insect juvenile hormone analogs, methoprene and fenoxycarb (Edwards and Abraham 1985), provided 100% control, and the chitin synthesis inhibitors, hexafluron and triflumuron caused 95 to 100% mortality in laboratory assays against larval beetles (Weaver and Kondo 1987; Weaver 1996). The neem extract RB-a caused

70% mortality in adults (Tabassum et al. 1998). An insect juvenile hormone analog, ivermectin was added to the poultry feed at a rate of 2 ppm, resulting in reduced beetle numbers in field trials, but also reduced the bird's weight gain and feed conversion efficiency (Miller 1990). Impregnating insulation with insecticide (tetrachlovinphos and pirimiphosmethyl) or painting insulations with insecticide (chlorpyrifos) impregnated paint provided good control against mature larvae (Despins et al. 1991).

Due to resistance, none of the currently available insecticides provide satisfactory control when lesser mealworms are at outbreak levels (Lambkin 2005; Ronda et al. 2006). Steelman (2009) confirmed resistance to carbaryl, methoxychlor, DDT, cyfluthrin, cypermethrin, tetrachlorvinphos, and chlorpyrifos in adult and larval lesser mealworm beetles. These bioassayed beetles were collected from broiler chicken production farms with different insecticide application history to organochlorine, organophosphate, carbamate and pyrethroid insecticides for over 40 y. However, spinosad was found to be very effective against cyfluthrin (pyrethroid) resistant lesser mealworm populations in Australian broiler houses (Lambkin and Rice 2007).

RATIONALE FOR THE RESEARCH

Restrictions imposed on many insecticides by Federal and State agencies, restricted use of insecticides in presence of birds, and the development of insecticide resistance in lesser mealworm populations limit the chemical control options for management of lesser mealworm. Dust accumulated on treated surfaces and a build up of high manure levels in poultry houses adsorb applied insecticide and protects the larvae from exposure (Despins et al. 1991). Moreover, these insecticides are unable to maintain their potency for longer periods due to the deleterious effect of high pH (8-9.5) caused by

chicken droppings. Most of the pyrethroids and carbamates breakdown rapidly in high pH conditions (Tomlin 1997). Larvae that pupate in the soil floor may not be affected by topical insecticide applications during cleanout. Adults and larvae may also hide in cracks and crevices or climb the walls to the roof of the poultry house to avoid contact with the insecticides (Wohlgemuth 1989). Beetles also disperse to cleaned houses from land where litter was applied or the removed litter pile after insecticide residues have dropped below toxic levels.

There are many natural enemies and promising biological control agents documented, but none provided satisfactory biological control of lesser mealworm beetle populations in poultry houses. Research was conducted to find better alternative tactics for managing the lesser mealworm populations.

The objectives of my dissertation were:

1. Estimate lesser mealworm, *Alphitobius diaperinus* (Panzer), densities in poultry litter applied to pasture field in Arkansas.
2. Determine movement of lesser mealworm adults and larvae inside poultry house.
3. Laboratory and field evaluations of an aggregation pheromone lure and fresh chicken dropping volatile compounds in their attractiveness to the adults and larvae of lesser mealworm.
4. Measure baseline dose-responses of lesser mealworm to insect growth regulators.
5. Establish baseline susceptibility and cross-resistance in adult and larval lesser mealworms collected from poultry houses in Arkansas.
6. Measure susceptibility of lesser mealworm larvae and adults to imidacloprid, metaflumizone and diflubenzuron.

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CHAPTER II

AN ESTIMATION OF THE DENSITIES OF LESSER MEALWORM *ALPHITOBIUS DIAPERINUS* (PANZER) IN POULTRY LITTER APPLIED TO PASTURE FIELD IN ARKANSAS

ABSTRACT

The lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) infested litter applied to the pasture fields serve as a potential source of disease transmission, reinfestation of poultry houses and invasion of human dwellings. A study was conducted to estimate densities of larvae, pupae and adult lesser mealworms from poultry house litter that was applied to a pasture in Savoy, Arkansas. Plastic trays were distributed along the four strips of pasture to catch the litter from the manure spreader truck that passed directly overhead. Greater numbers of larvae were found than adults or pupae. This study determined that there were 3.5, 2.2 and 0.4 million larvae, adults and pupae, respectively, per hectare of pasture land spread with litter. This study showed the potential of pasture applied litter as a source of reinfestation in the poultry houses. Some alternative solutions to control the beetles such as removing and spreading the litter in the pasture fields during coldest part of winter or covering and heat composting the litter are discussed.

Key Words: poultry, litter, pasture, density, larvae, reinfestation

INTRODUCTION

Poultry litter is a mixture of chicken manure and wood-shavings or rice hull material. Application of poultry litter to pasture land in Arkansas is an effective way of litter management and recycling the nutrients back to the soil. However, the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), inhabits the poultry litter (Turner 1986; Axtell and Arends 1990) since it feeds on the manure, spilled feed and dead birds inside poultry houses. When lesser mealworm infested litter is applied to pastures, the beetles become a potential source of disease transmission as well as a noxious pest by immigrating away from litter and invading human dwellings. Adult beetles can fly as far as 1.6 km overnight toward residential artificial lights from fields treated with beetle-infested litter as described by Savage (1992); however methods to measure flight distances were not described. Local residents in La Rue, Ohio, were awarded a $25 million lawsuit against a poultry farm for invasion of beetles inside their houses due to application of litter on land in close proximity to their dwellings (Miller 1997).

In an experiment simulating land application of turkey litter in fields, adult beetles survived and emerged even at 28 d after they were buried in central North Carolina Cecil red clay at different depths. Effectiveness of mechanical incorporation of turkey litter on reducing adult lesser mealworm emergence from North Carolina field clay and sandy soils was documented (Calibeo-Hayes et al. 2005). Little is known about the densities of beetles in poultry litter that has been applied to the pastures in Arkansas. A study was conducted to estimate the densities of immature and adult lesser mealworms in poultry house litter applied to pasture.

MATERIAL AND METHODS

A pasture field was selected 0.5 miles east of a broiler chicken house at Washington County, Savoy, AR on 30 April 2009. Four long strips of pasture of approximately equal size and similar vegetation were selected for making estimates of the numbers of lesser mealworms in a pasture treated with beetle-infested litter. Sixty plastic trays (0.2 m^2) were distributed along these four strips (15 trays each) of pasture to catch the litter from a manure spreader truck as it passed directly overhead. Litter from each tray was transferred immediately into garbage bags, transported to the laboratory and stored in a -20 °C freezer to kill the beetles. Weight of the litter in each bag was recorded. Beetles from each bag were sifted from the litter and counted. The number of beetles was used to estimate the number of beetles per g of litter or per tray (0.2 m^2). Mean number of adults, larvae and pupae spread per hectare or present in about 75 tons of poultry litter from one poultry house were calculated. Differences in mean counts among beetle growth stages were analyzed with ANOVA and the means found to be significantly different ($P = 0.05$) were separated using a Tukey's HSD test (JMP 8.0 2009).

RESULTS AND DISCUSSION

Average weight (g) of litter spread on the pasture and collected per tray was 231 ± 15.7. Lesser mealworm adults, larvae and pupae were estimated at 2.2, 3.5, and 0.4 million per hectare, respectively (Table 2.1). For each poultry house, the lesser mealworm numbers that were thrown on the pasture were estimated to be 34.7 million. Beetle numbers derived from Arends tube traps set in the litter at several locations inside a broiler house were estimated as high as 25 million per broiler house in Arkansas (Strother 1998). Surprisingly, pupae were found in large numbers in the litter spread on

the field. Pupae are normally restricted to soil beneath the litter, insulation and in cracks or crevices of poultry house structures. Larvae were found in higher numbers than adults or pupae (Table 2.1). These larvae might pupate in pastures, and then emerge as adults. This study showed the potential of pasture applied litter as a source of reinfestation in the poultry houses. Savage (1992) speculated that if litter applied is 0.4 km from the poultry house, and the beetle dispersal is random, approximately 60,000 beetles for every one million applied to the field would return to the poultry house, although the methods used to determine numbers were not described.

This litter is normally removed from the poultry houses in summer time every year when the outside temperatures are suitable for larval development, adult emergence and adult flight. One effective way of controlling lesser mealworm populations might be to harvest the birds during the coldest part of the winter, remove and spread the poultry litter outside and open the house to expose beetles to sub-freezing temperatures for as long as possible. A more likely alternative would be in house litter composting through windrowing and covering piles to restrict beetle dispersal. Biocidal temperatures are achieved quickly when composting is done properly. This would quickly expose all stages of the beetles to 50 to 60 °C for three days, turn the pile and repeat for three more composting cycles (Brake 1992). There is a need to estimate the numbers of adult beetles leaving the litter applied fields and find the relationship between their density and dispersal. Number of beetles that return back into the poultry house from the litter applied pasture should be determined with mark recapture studies. This estimation of lesser mealworm infestation in litter supports the need for further investigation of the survivability of all life stages in litter applied to pastures during warm and cold seasons.

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Table 2.1 Number of lesser mealworm beetles by growth stage collected from a pasture where litter was applied from a broiler chicken house in Savoy, AR on April 30 2009.

Stage	Mean per tray ($0.2\ m^2$)	In 1 Hectare (millions) ($10{,}117 m^2$)	In 1 house (millions) (75 tons)
Adults	42.2 ± 5.9b[a]	2.2	12.4
Larvae	68.4 ± 6.9a	3.5	20.1
Pupae	7.2 ± 0.5c	0.4	2.1

[a]Means followed by the same letter in each column are not significantly different (Tukey's HSD test, $P > 0.05$).

CHAPTER III

MOVEMENT PATTERNS OF LESSER MEALWORM, *ALPHITOBIUS DIAPERINUS* (PANZER), ADULTS AND LARVAE INSIDE BROILER PRODUCTION FACILITY

ABSTRACT

Knowledge of lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) movement patterns inside a poultry houses is important for the development and implementation of effective control strategies. Field experiments using pitfall traps were conducted in a poultry house to determine the response of beetles to temperature and presence/absence of chicks. There was a reduction in beetle movement and numbers when the chicks were first introduced into the house but the beetle counts increased within a few days. More adults and larvae were captured when the temperatures were raised from 28 to 34 ºC inside the house because of higher numbers of beetles present on the surface of litter. Numbers of adults captured were significantly greater in the southwest part of the house irregardless of absence or presence of chicks. Importance of the findings for sampling and management decisions for lesser mealworm is discussed.

Key Words: poultry, litter, lesser mealworm, movement, temperature, chicks, sampling

INTRODUCTION

The lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) requires approximately 50 d to complete its development from egg to adult under controlled temperatures of 30-33 °C inside the poultry house (Wilson and Miner 1969). This time span also closely correlates with number of days necessary to rear a flock of broilers to market weight (nearly 60 d) (Erichsen and Jespersen 1997; Salin et al. 2000). Rueda and Axtell (1996) reported that females laid 4-7 eggs/d at 25 °C, no egg hatch or larval development occurred at or below 17 °C, and development times from oviposition to adult decreased from 164 d at 20 °C to the shortest of 29 d at 35 °C. The optimum temperature for larval development is 30° to 33°C with 80 to 90% relative humidity (RH) (Dunford and Kaufman 2006). Controlled temperature, moisture and a thick layer of litter or manure provide favorable conditions for the development of beetles throughout the year (Stafford and Collison 1987). Californian turkey brooder houses had larger lesser mealworm populations at higher temperatures during the summer and lower populations during the winter (Voris et al. 1994).

Knowledge of insect population levels and dynamics is an important tool for effective integrated pest management. Sampling of lesser mealworm beetles to determine their population cycles during chicken growout is very important for optimizing effectiveness of pesticide applications (Safrit and Axtell 1984). Strother (2002) did an extensive study to find the correlation between environmental variables (temperature, litter pH and percent moisture content) and beetles distribution inside the litter in open areas and under feeder pans inside the poultry house. In open areas, adult preffered low temperatures (12-15 °C) and high moisture content (25-33%). Highest numbers of large

larvae were associated with high moisture content (29-37%), medium larvae with higher temperatures (33-36 °C) and small larvae with pH < 8.5. Underneath the feeder pans, highest numbers of large, medium and small larvae were associated with temperatures of 33-39 °C, 36 °C and 30-33 °C, respectively. Beetle density was greater under feeder pans but the total population was higher in open areas due to larger size of open areas. Understanding the importance of temperature and chick activity on the movement of beetles on the surface of litter is crucial for beetle sampling and pest management decisions. Beetle counts from pit fall trap samples from different house locations were compared. The objective was to determine the in-house movement patterns and changes in beetle densities in response first to no heat and no chickens during cleanout, followed by a short period of heating the house and then placement of chickens inside the poultry house.

MATERIAL AND METHODS

To evaluate seasonal effect on lesser mealworm populations, two field trials were conducted with the first field test begining 10 April 2008 during the cool season and the second test beginning on 29 July 2008 during the hot season. Both trials were conducted in a same broiler chicken house at Applied Broiler Research Unit, Savoy, AR. The building was 14 m wide and 99 m long with the long axis orientated east to west and was used to produce 6 or 8 week old broiler chickens. The barn did not contain birds at the start of the study. Two automatic feeder lines ran the length of the house, 2 m out from the wall. The floor of the house was covered with pine wood-shavings 8 cm deep. Plastic trays (56 × 34 cm) containing chick feed were present and were used to supplement automatic feeders so that newly hatched chicks have optimum feed access during the first

10 d of a production cycle. The trays were located in three parallel lines. One row of trays was located under feed spouts on either side of the permanent feeder pans. The remaining two rows were on either side of feed line beneath each automatic feeder line and the tray spacing was 2 m. The beetle pitfall traps (Photograph 4.1) were placed parallel to the feeder lines or feeder pans. Trap spacing was approximately 1 m. There were four blocks and each block contained 12 traps. The locations of blocks inside the poultry house were in the: northwest side; northeast side; southeast side; and southwest side (Fig. 3.1).

Effects of house locations (space), sampling dates (time) and factor interactions on lesser mealworm catches in pitfall traps in this house were determined. On 10 April (test 1) and again on 29 July (test 2), the house just had litter replaced and the temperature was maintained at 28 °C when pitfall traps were initially placed. The beetles were collected from the traps after 24 h and 48 h. Then, propane heaters raised the house temperature to 34 °C on day 3 (the rearing temperature of the young birds). The beetles were collected again after 24 h. The chicks were released into the facility on day 4, and once again, beetles were collected from the traps at 24 h interval for the next 5 d. Thus, the beetle numbers were monitored for 1, 2 and 3 d prior to chick introduction, and for 5 d post chick placement.

The analysis of variance (ANOVA) indicated no interaction between lesser mealworm population and season so the seasonal data was combined for analysis. A multifactorial ANOVA of space and time was performed on transformed log (X + 1) trap catch data subjected to the SAS regression and general linear models procedures (SAS Institute 2004).

RESULTS AND DISCUSSION

The significant interaction between trap location and time suggested that the adult (F = 3.2; df = 21, 735; *P* = 0.0001) and larval (F = 2.18; df = 21, 735; *P* = 0.0001) population densities and distribution changed over space and time. The number of adults (F = 0.56; df = 1, 735; *P* = 0.45) and larvae (F = 0.14; df =1, 735; *P* = 0.30) present in the poultry house in two experiments for different dates in April and July were not significantly different (Table 3.1). The greatest numbers of adults (334) and larvae (336) were found on day 3 when the heat was turned on but before chicks were present inside the poultry house. After the introduction of chicks on day 4, adult (43) and larval (156) catch decreased, becoming the lowest on day 5, and then increased from day 6 through day 8. More adults and larvae were captured when the heat was turned on (34 ºC) even if chicks were present as compared to when heat was turned off (24-28 ºC) (Table 3.2). This might be due to higher number of beetles present on the litter surface**.**

Beetle counts were affected by poultry house location and absence or presence of chickens. The numbers of adults captured were significantly higher in the southwest part of the house regardless of absence or presence of chicks when heat was turned on. The solar-radiant heating of the southwest wall and interior substrate of the house or thick litter layer during most of the day might be the reason for higher beetle numbers. The number of adults captured was not significantly different among northwest, northeast and southeast house locations in absence of chicks. With chickens present, the northeast house location had significantly lower captures than northwest and southwest but significantly similar captures as southeast. Prior to introduction of chicks, the number of larvae trapped was not significantly different among northwest, northeast and southwest although

southwest was significantly greater than southeast. With introduction of chicks, larvae trapped in the southwest became significantly higher than the other three blocks that were similar (Table 3.3).

Larvae were apparently less affected by the presence of chicks than by house temperature changes. There was a reduction in beetle movement and counts per trap when the chicks were first introduced into the house but the beetle counts did rise after a couple of days of propane heating that probably warmed up the soil and the litter. Higher temperatures led to increased insect activities and significantly greater trap catch than when the heat was turned off. These results differ from the results reported by Chernaki-Leffer et al. (2007) who recorded no temperature effect (range from 20.5 to 28.3 °C) on weekly beetle counts from Arends tube traps over six growout periods in Brazilian poultry houses. In AR, higher numbers of larvae and adults present in the southwest side of poultry house than other quadrants indicated possibly warmer, more suitable conditions for lesser mealworm. Sampling should be done during the flock free period with heat on to maximize beetle activity on the litter surface and exposure to applied insecticide. If sampling warrants beetle management, insecticides should be applied to litter after the heat is turned on and prior to flock placement.

Future research should include evaluating the effects of varying factors such as temperature, moisture and litter depth at different locations in the poultry house and their relationship with beetle distribution. By establishing correlations between factors that promote beetle populations in particular locations, the poultry industry could use beetle sampling of houses to make decisions about the need and timing of control strategies.

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Table 3.1 Field experiment conducted to determine the effect of season, pitfall trap location and sampling date (day) on numbers and movement of lesser mealworm adults and larvae in response to temperature and chicks.

ANOVA					
Source	DF	SS	MS	F- Ratio	*P*-value
Adults					
Day	7	595.03	85.00	131.60	0.0001
Location	3	123.51	41.33	63.73	0.0001
Field test	1	0.36	0.36	0.56	0.45
Day X Location	21	43.40	2.06	3.20	0.0001
Error	735	475.00	0.64		
C. Total	767	1237.00			
Larvae					
Day	7	1248.34	178.33	268.71	0.0001
Location	3	129.44	43.14	65.01	0.0001
Field test	1	0.14	0.14	0.21	0.64
Day X Location	21	45.95	2.18	3.29	0.0001
Error	735	488.44	0.66		
C. Total	767	1912.20			

Experiment had a complete block design with four locations (Blocks) and twelve traps per location. Analysis was on log (X+1) transformed data.

Table 3.2 Mean number (± SE) of lesser mealworm adults and larvae captured in pitfall traps in response to temperature and absence or presence of chicks in the poultry house.

	Mean (± SE)	
Day	Adults	Larvae
1	21.9 ± 2.1g[a]	5.3 ± 0.4g
2	28.7 ± 2.2f	16.0 ± 1.9f
3	334.0 ± 21.0a	335.6 ± 26.2a
4	43.6 ± 4.6e	156.2 ± 16.3c
5	43.6 ± 6.1f	37.2 ± 5.2e
6	82.9 ± 7.8d	108.4 ± 13.1d
7	119.7 ± 10.2c	203.5 ± 20.7bc
8	144.0 ± 11.1b	245.2 ± 22.0b

[a]Means followed by the same letter in each column are not significantly different (LSD, α=0.05). Analysis was on log (X+1) transformed data, but actual mean values used in this table.
Day 1-3: Chicks absent; day 4-8: Chicks present
Day1-2: Heat off; day 3-8: Heat on

Table 3.3 Mean number (± SE) of lesser mealworm adults and larvae captured in pitfall traps from four blocks in absence or presence of chicks.

		Mean (± SE)			
Chicks	Stage	Northwest	Northeast	Southeast	Southwest
Absent	Adults	75.0 ± 8.8b[a]	73.6 ± 11.6b	80.2 ± 12.6b	325.5 ± 43.4a
Present	Adults	96.8 ± 9.9b	58.5 ± 6.8c	62.3 ± 5.4bc	136.2 ± 9.9a
Absent	Larvae	73.1 ± 9.1ab	97.5 ± 16.9ab	69.6 ± 12.6b	250.6 ± 28.1a
Present	Larvae	137.4 ± 16.9b	119.6 ± 15.7b	105.4 ± 12.6b	241.8 ± 18.9a

[a]Means followed by the same letter in each row are not significantly different (LSD, α=0.05). Analysis was on log (X+1) transformed data, but actual mean values used in this table.

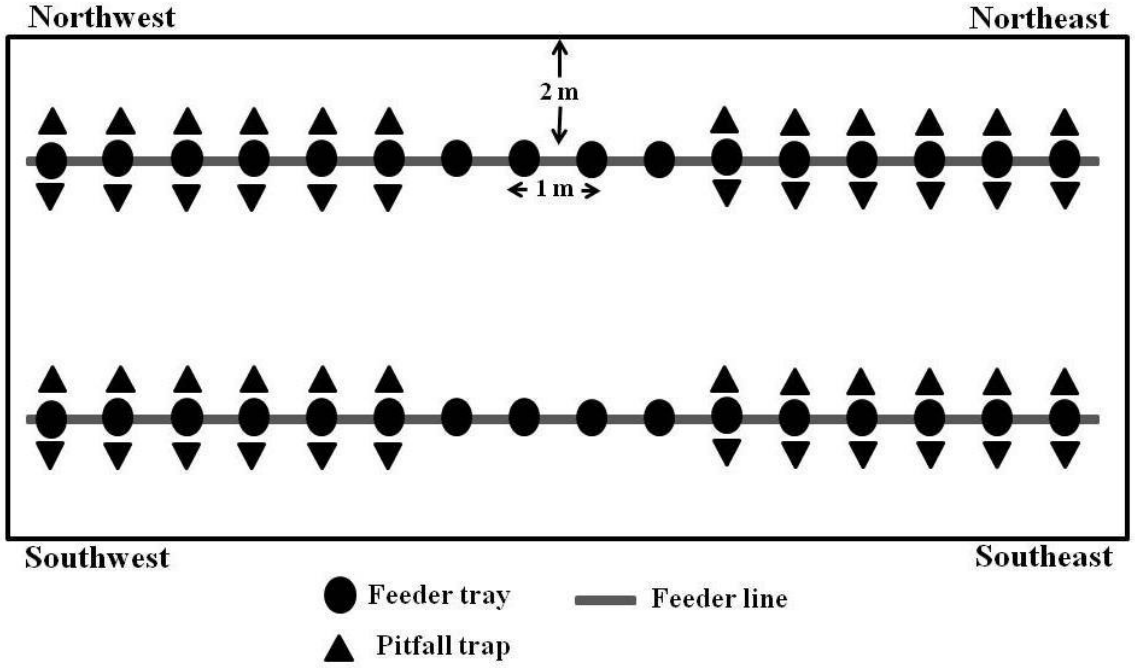

Fig 3.1 Schematic diagram of pitfall trap locations inside a broiler production house.

CHAPTER IV

LABORATORY AND FIELD EVALUATION OF AGGREGATION PHEROMONE LURE AND CHICKEN DROPPING IN THEIR ATTRACTIVENESS TO THE LARVAE AND ADULTS OF LESSER MEALWORM, *ALPHITOBIUS DIAPERINUS* (PANZER)

ABSTRACT

The chemical cues by which lesser mealworm, A*lphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) find each other is among the least understood aspects of the beetle's chemical ecology. The attractiveness of the lesser mealworm synthetic aggregation pheromone lure and its individual components to the larvae and adults were examined in laboratory two-choice pitfall bioassays. The compounds identified from a gas chromatographic analysis of chicken dropping volatiles (CDV) included: 2-methyl-propanoic acid; 2, 3-butanediol; butanoic acid; 2-pentanone; 1-octen-3-ol; 2-chlorocyclohexanol; pentanoic acid; and dodecanal. Laboratory two-choice pitfall bioassay was found to be a useful and convenient tool for evaluating the potential attractants for lesser mealworm adults and larvae before testing them in the poultry house. Adults and larvae were more attracted to a dose of 20-30 µg aggregation pheromone lure than other doses evaluated in laboratory two-choice pitfall bioassays. A combination of 2 g fresh chicken droppings (CD) + 20 µg lesser mealworm aggregation pheromone lure was very attractive to adults and larvae in laboratory two-choice pitfall bioassays. The attractiveness of CDV compounds were 3-fold less attractive than the fresh CD. The low attraction of limonene and linalool in the laboratory two-choice pitfall bioassays suggest that either they are non attractive or attractive only at a different range of concentrations yet to be tested. Baited pitfall trapping experiments were also done in poultry houses with low, moderate and high lesser mealworm populations to evaluate the attraction of aggregation pheromone lures. Significantly higher numbers of beetles were found in traps treated with pheromone lure as compared to untreated checks in all field experiments. Results generally indicate that there is a potential for combining the

synthetic lesser mealworm pheromone lure with whatever compound(s) is attractive in fresh CD to enhance trap efficacy. The best combination that attracts beetles can be used to monitor lesser mealworm populations and may prove effective when combined with insecticide baits(s) to attract and kill lesser mealworm in poultry houses.

INTRODUCTION

The lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), is a serious, cosmopolitan pest present in poultry production facilities, where it consumes poultry feed and litter, and causes decreased weight gains in broiler chicks that eat these beetles (Despins and Axtell 1995). The beetles transmit several disease agents such as avian influenza, Marek's disease, Coronavirus, the Newcastle disease virus (De las Casas et al. 1973, 1976), *Salmonella typhimurium* (McAllister et al. 1994), *Campylobacter jejuni* (Strother et al. 2005), infectious bursal disease (McAllister et al. 1995), and cause damage to poultry insulation by tunneling (Despins et al. 1987). Control of lesser mealworm adults and larvae is mainly through use of contact insecticides, which has resulted in insecticide-resistant strains (Lambkin 2005). Due to environmental and resistance concerns related to the use of conventional insecticides, research needs to be focused toward alternative tactics such as attractants (pheromones or kairomones) and other behavior-modifying chemicals. Pheromones can be used to detect early infestations and to attract and kill beetles in spot treatments rather than spraying the whole poultry house. Bray et al. (2010) sprayed experimental chicken sheds with insecticide and used lures of the synthetic pheromone (±)-9-methylgermacrene-B to attract and kill greater numbers of male and female sand fly, *Lutzomyia longipalpis* (Diptera: Psychodidae) than attracted and killed at pheromone-free sheds (check).

Lesser mealworm adults and larvae are unevenly distributed in poultry houses in clumps or in aggregations. Adults and larvae placed in a Petri dish with wood shavings form tight aggregations indicating some sort of assembling scent present that leads to their aggregation behavior (Folomo 1986). These aggregations also suggest the existence

of some sort of pheromonal communication among adults and larvae. Attraction of the adults to pheromones is well documented in many tenebrionids including the red flour beetle, *Tribolium castaneum* Herbst (Rangaswamy and Sasikala 1991), the yellow mealworm, *Tenebrio molitor* L. (Tanaka et al. 1986), the broad-horned flour beetle, *Gnatocerus cornutus* (F.) (Tebayashi et al. 1998; Tashiro et al. 2004), and the desert tenebrionid beetle, *Parastizopus transgariepinus* Koch (Gieselhardt et al. 2008).

Falomo (1986) reported the first evidence for the presence of pheromones in adult males and females of lesser mealworm, but no chemical identifications were made. Falomo (1986) reported that during the calling period, male and female beetles rubbed the seventh and eighth abdominal tergites with the hind tarsi in a wiping motion as if to disperse pheromone into the air. Solvent extracts of secretions from setiferous patches attracted both sexes of lesser mealworm adults in olfactometer bioassays suggesting a sex and/or aggregation pheromone present. The secretions obtained from a pair of eversible posterior abdominal glands caused sexual excitement in both sexes (Wilson and Miner 1969). Four male-specific compounds were found, including: (R)-(+)-limonene, (E)-β-ocimene, (S)-(+)-linalool and (R)-(+)-daucene. A fifth compound, 2-nonanone, was identified from male lesser mealworms and in small amounts from female lesser mealworms (Bartelt et al. 2009). Lesser mealworm synthetic aggregation pheromone lure composed of these five compounds was found to be attractive to male and female adult beetles in laboratory pitfall bioassays (Bartelt et al. 2009).

Responses of larvae to these adult produced pheromones in tenebrionids have received relatively less attention. Larval *T. castaneum* were attracted to contact and vapor of synthetic adult aggregation pheromone 4, 8-dimethyldecanal at concentrations of 1, 5

and 10 µg/g medium (Mondal and Port 1984). The predatory larvae of genera *Elatophilus*, *Hemerobius* and *Sympherobius* were attracted to the sex pheromone of their respective host prey, *Matsucoccus josephi* Bodenheimer & Harpaz, *M. feytaudi* Ducasse and *M. matsumurae* Kuwana (Homoptera: Margarodidae), in a similar fashion as exhibited by conspecific predator adults in the field and laboratory choice tests (Branco et al. 2006). The codlemone pheromone mediated aggregation of last instar codling moth, *Cydia pomonella* L. seeking pupation sites confirming the communication within and among development stages (Duthie et al. 2003). A synthetic blend of the larval aggregation pheromone comprised of 3-carene, octanal, nonanal, decanal, (*E*)-2-octenal, (*E*)-2-nonenal, sulcatone, and geranylacetone, enhanced captures of fifth-instar codling moths (Jumean et al. 2007).

Successful mass trapping using male-produced synthetic aggregation pheromones and plant co-attractants has been reported for many coleopteran insect-pests including sap beetles, *Carpophilus* spp. (Hossain et al. 2007), the bean weevil, *Acanthoscelides obtectus* (Say), pea leaf weevil, *Sitona lineatus* (L.) (Blight et al. 1984), smaller European elm bark beetle, *Scolytus multistriatus* (Marsham) (Dickens et al. 1990), the coconut Rhinoceros beetle, *Oryctes rhinoceros* L. (Hallet et al. 1995), and the green June beetles, *Cotinus nitida* (L.) (Johnson et al. 2009). Adults and larvae of lesser mealworm were observed to be attracted to fresh chicken droppings (CD) in a poultry house. One or more components of chicken droppings volatiles (CDV) may act as an attractant to these beetles in the poultry house.

The objectives of this study include: 1) Identification and characterization of head space volatiles emitted from CDV; 2) To evaluate the attractiveness of the lesser

mealworm aggregation pheromone lure, individual components of lesser mealworm aggregation pheromone lure, and CDV compounds, and to fresh CD to the larvae and adults in laboratory pitfall bioassays; and 3) To determine the attractiveness of the aggregation pheromone lure to adults and larvae in field trials.

MATERIAL AND METHODS

Collection of headspace volatiles

A 100 g fresh sample of CD was placed inside a glass headspace volatile collection chamber. Volatiles were collected for 4 h from chamber containing chicken droppings (CD) and the blank collection chamber (check). Carbon-filtered air flowing at the rate of 0.5 l/min entered the glass chambers. Any CD volatiles (CDV) exiting the cylinders were adsorbed in a trap containing 30 mg SuperQ (Alltech Associates, Deerfield, IL). Traps were eluted with 300 µl dichloromethane + 10 µl ethyl caprate (100 ng/µl, internal standard) and stored at -80 °C.

Gas Chromatography/Mass Spectrometer (GC/MS)

A 1 µl aliquot of each CDV sample was first analyzed on Varian Chrompack CP-3800 GC equipped with a thick film EC-5 Capillary column of 1mm film thickness, 0.25 x 0.25 mm ID x 30 m length (Alltech. Associates, Deerfield, IL) and a flame ionization detector (FID). Injector and detector temperatures were 250 °C and 270 °C, respectively. Injections were made in splitless mode at 250 °C using helium as a carrier gas. The oven was programmed to initially hold for 3 min at 50 °C and then ramp at rate of 5 °C/min to 120 °C, then ramp at rate of 10 °C/min to 270 °C with a final hold time of 5 min. These runs were made to confirm the presence of compounds in the samples. To prepare

samples for injection into the GC/MS, each sample was diluted or concentrated depending upon the peak intensity obtained in the chromatograms.

Each volatile sample was then analyzed on Varian 450 GC coupled with Varian 320 MS at Statewide Mass Spectrometry Facility, University of Arkansas. The GC is equipped with a factory four Capillary column of 0.25 x .0.25 mm ID x 30 m length (Varian Inc., Palo Alto, CA). Injector temperature was set at 270 °C in split mode (1:50) using helium as a carrier gas with a constant column flow of 1ml/min. The oven was programmed to initially hold at 50 °C for 3 min, and then ramp at 5 °C/min to 120 °C and ramp at 10 °C/min to 270 °C with a final hold time of 5 min. Each GC peak produced an ion spectrum after entering the MS. To identify each peak, its MS ion pattern was compared to known ion patterns of compounds in the National Institute of Standards and Technology (NIST) mass spectral database.

Quantifying Volatiles

Four serial dilutions of ethyl caprate (99% pure) (Sigma-Aldrich Co., Milwaukee, WI) ranged from 1 nanogram (ng) to 1 micrograms (µg) were made in dichloromethane. A 1 µl aliquot of each dilution was injected in GC/MS following the procedure described above. The area was calculated for each chromatogram peak for each dilution and was plotted against four concentrations to generate the calibration curve. Area under each GC peak for each volatile peak from an injected CDV sample was related to the area for ethyl caprate peak in the sample. The area under the ethyl caprate peak in each sample was adjusted with calibration curve to quantify the compounds in each sample. Eight CDV compounds were tentatively identified as: 2-methyl-propanoic acid; 2, 3-butanediol, butanoic acid, 2-pentanone, 1-octen-3-ol, 2-chlorocyclohexanol, pentanoic acid; and

dodecanal (Table 4.1). Based on total amount of all compounds present per microliter, the amount of CDV sample needed to get 10 µg of total compounds were calculated and used in laboratory pitfall bioassay (Table 4.1).

Rearing

Lesser mealworm adults and larvae for all bioassays were collected from the Applied Broiler Research Unit, Savoy, AR. Beetles were extracted from litter or manure by sieving samples through 2.8 and 2.0 mm screens. They were transported to the laboratory and reared in covered plastic chambers maintained at 28 °C ± 1 °C and 14:10 h L:D photophase. Water and chick starter feed were provided, and rolled cardboard was added to serve as shelter and pupation sites.

Laboratory Two-Choice Pitfall Bioassay

A two-choice pitfall bioassay had a group of 6-8 instar lesser mealworm larvae in an observation arena with two holes in the arena floor. Aluminum roasting pans (38 cm long x 25 cm wide x 3.8 cm deep) were used as test arenas. Two 3.8 cm dia. circular holes were drilled in a straight line in the floor of the pans 15 cm apart and 2.5 cm from the pan lip. The pans rested on two open 120 ml glass specimen cups directly below the holes and these cups had either an odor stimulus or no odor (check). Two additional 120 ml specimen cups were placed at the other end of the pan to keep it level. The test arena tops were 43 cm long x 30 cm wide glass sheets. The glass sheets had two outlet holes 15 cm apart parallel to holes on pan floor but on the opposite end. Air containing each odor or check stimulus led separately to either of the inlet ports of the 120 ml specimen cups at 0.5 l/min through the air delivery system (ARS, Gainsville, FL). The air then entered into test arenas and was exhausted from the outlet holes at a rate of 1 l/min with a vacuum.

Lesser mealworm aggregation pheromone lure consisted of five compounds: (*R*)-(+)-limonene, (E)-β-ocimene, (S)-(+)-linalool, and (R)-(+)-daucene and 2-nonaone. The two commercial components, 2-nonanone and (R)-(+)-limonene (Aldrich Chemical Co., Milwaukee, Wisconsin) were used as received. (E)-β-ocimene, (S)-(+)-linalool, and (R)-(+)-daucene were prepared in the USDA-ARS laboratory at Peoria, IL, by the procedures described in Bartelt et al. (2009). Bioassays were conducted to determine responses of lesser mealworm larvae to doses ranging from 1 µg to 100 µg of lesser mealworm aggregation pheromone lure. Since 20 µg of aggregation pheromone lure was found to be more attractive in preliminary bioassays, this amount of lure or individual components of aggregation lure were used in subsequent bioassays.

Fresh chicken droppings volatiles (CDV) eluted in methylene chloride were tested alone or in combination with 20 µg aggregation pheromone lure. Fresh CD was also tested singularly or in combination with 20 µg aggregation pheromone lure. Red rubber septa (11 mm, Wheaton, Millville, New Jersey) were cleaned in advance by soaking them in methylene chloride overnight. Emission characteristics of the aggregation pheromone compounds placed on septa were measured in the laboratory by Bartelt et al. (2009) (Table 4.2). The synthetic aggregation pheromone lure, individual components and CDV compounds were applied to the rubber septa followed by 300 µl methylene chloride, allowed to soak in and methylene chloride allowed to evaporate.

Lesser mealworm beetles are nocturnal and inhabit a darkened or subdued light environment. Thus, all bioassays were conducted in a darkened room. An 8 h period was shown to allow optimal insect dispersal in preliminary bioassay tests. Bioassays were conducted for 8 h in complete darkness at 28°C and 50 ± 10% relative humidity. One

hundred and fifty test beetles were used in each replicate and four replicates were used for each treatment. Beetles were starved for 12 h before the bioassays. Beetles were placed under an inverted glass funnel (5 cm diam. at widest point) at the center of arena for 3 h prior to release to allow for acclimation to the experimental conditions. These acclimated beetles were released at the down-wind end of the arena, and their distribution recorded after 8 h. The treatment cup positions were reversed after each replicate to eliminate any positional cues. Two testing arenas 2.5 cm apart were used simultaneously. The beetles trapped in the odor or check cups and that made no choice were collected and counted. Each arena was washed with acetone and air-dried before any subsequent use.

Pitfall Trap Design for Field Test

The trap type used for the field study was a modified pitfall trap to exploit the lesser mealworm larvae and adults thigmotactic behavior (Photograph 4.1). The trap base was a 5 mm thick, 20 cm square piece of plywood with a 5 cm circular hole in the center. A threaded metal canning ring, 7 cm in dia., from a 250 ml canning jar, (Ball Inc., Muncie, Indiana) was attached, concentric to the hole, with 3 flat-head, machine screws, size 6-32 by 1.25 cm, and hex nuts. Holes were predrilled, spaced equally around the canning ring. The trap top was a 10 cm^2, 5 mm thick piece of plywood. Three holes drilled through the top aligned with the machine screws protruding through the trap base. The ends of these screws served as "pins" to keep the trap top centered above the 5 cm hole, and the hex nuts provided a suitable separation between the trap top and base so that beetles could enter into the trap. Trap assembly was completed by screwing the jar into the canning ring (Photograph 4.1)

A pheromone blend solution consisting of the five synthetic components mentioned in laboratory pitfall bioassays (proportions as in Table 4.2) was applied to each rubber septum (100 μg total, in 10 μl hexane), followed by 300 μl methylene chloride. The septa were loaded 1 h before use, stored in a tightly closed bottle and transported in an ice chest to the poultry house. The treated septa with check or pheromone were then dropped into the glass jar. A hole was made in the loose poultry house litter, and then the assembled trap was set in place, with the trap top slightly below the surface of the litter layer. Traps were cleaned with Alconox detergent, and dried at the end of the experiment.

Field Test to Determine Pheromone Lure Attractiveness to Adults and Larvae

Trapping experiments were done in poultry houses located in Savoy and Tontitown, AR to evaluate the attraction of the pheromone lure blend to lesser mealworm larvae and adults. The first field test was conducted in a broiler production house with moderate lesser mealworm infestation (1000-2000 lesser mealworm adults per Arends trap), at the Applied Broiler Research Unit in Savoy, AR. The building was 14 m wide and 99 m long with the long axis orientated east to west and was managed to produce 6 or 8 week-old broiler chickens, but it did not contain birds at the start of this study. Two automatic feeder lines ran the length of the house, 2 m out from the wall. The floor of the house was covered with pine wood-shaving litter approximately 8 cm deep. Plastic trays (56 × 34 cm) containing chick feed were present and were used to supplement automatic feeders so that newly hatched chicks have optimum feed access during the first 10 d of a production cycle. The trays were located in three parallel lines. One row of trays was located under feed spouts on either side of the permanent feeder pans. The remaining two

rows were on either side of feed line beneath each automatic feeder line and the tray spacing was 2 m. Beetles became abundant under these trays within a day of placement. The beetle traps were placed parallel to the feeder lines or feeder pans and spaced about 1 m apart. The experiment was a randomized complete block (RCB) design with four blocks: 1 and 2 on the north side of the house and 3 and 4 on the south side. Each block consisted of two treatments randomly assigned within each block: six pitfall traps with pheromone-treated septa; and six traps with untreated (check) septa. Trapping was conducted over three consecutive days from 20 to 23 August 2008. The house temperature was 28 °C when the traps were initially placed and baited in the afternoon. The beetles were collected from the traps after 24 h and trap baits were replaced. By then, propane heaters raised the house temperature to 34 °C (the rearing temperature of the young birds). After the second 24 h period, trapped beetles were again removed and baits replaced. At this time, the chicks were released into the facility, and once again, beetles were collected from the traps after 24 h. Thus, the beetle response to the treated septa was monitored for 1 and 2 d prior to chick introduction and for 1 d afterward, and the time period of the study included a defined temperature change.

The second field test was conducted from 15 to 19 September 2008 in a broiler production house with low lesser mealworm infestation (<1000 lesser mealworm adults per trap), in Tontitown, AR. The building had the same dimensions, and the internal feeders and waterers set up as described in the first field test experiment. Forty eight pitfall traps were used in the paired design. Treatments were randomly assigned within the pairs with one trap receiving the pheromone and the other trap being the untreated check. The house temperature was 28 °C when the traps were initially placed and baited

the afternoon of 15 September 2008. The beetles were collected from the traps after 24 h and 48 h, and the trap baits were replaced each time. Then, the house temperature was raised to 34 °C with propane heaters. At this time, the chicks were released into the facility, and once again, beetles were collected from the traps after 24 h and 48 h. Thus, the beetle response to the treated septa was monitored for 1 and 2 d prior to chick introduction and for 1 and 2 d afterward.

The third field test was conducted from 8 to 12 November 2008 in a broiler production house with high lesser mealworm infestation (>2000 lesser mealworm adults per trap), at the Applied Broiler Research Unit in Savoy, AR. The experimental set up, temperature change, introduction of chicks, collection schedule of beetles and replacement of baits procedure was the same as in the second field test. Trapped beetles were transported back to the laboratory and counted.

Statistical Analyses

Volatiles: Differences in amounts of identified compounds were analyzed by completely randomized one-way analysis of variance (ANOVA) (JMP 2009) followed by Tukey-HSD test.

Laboratory Two-Choice Pitfall Bioassay: Attractiveness or repellency of treatments was expressed as a response index (RI) or percent attraction shown by Suzuki and Sugawara (1979). It was calculated as RI = (T - C/Tot)*100, where T is the number responding to the treatment, C is the number responding to check, and Tot is the number of adults/larvae released per replicate into the test arena. Positive RIs indicate attraction to the treatment and negative RIs indicate repellency; and the values range from -100 (complete repellency) to + 100 (complete attraction). The mean RIs among the treatments

were compared using the non-parametric Kruskal-Wallis test followed by Tukey type multiple comparisons ($P = 0.05$) (JMP 8.0 2009).

Field Test: Data for adults and larvae captured in traps were $\log_{10}(X + 1)$ transformed to meet assumptions of normality and homogeneity of variance (Zar 1999). Data for the RCB design experiments were analyzed by ANOVA (SAS Institute 2004). For the paired design experiments, means were analyzed by Wilcoxon's paired sample test for two choice experiments ($P = 0.05$).

RESULTS

Laboratory Two-Choice Pitfall Bioassay for Adults

The treatments are abbreviated as follows: lesser mealworm = lesser mealworm synthetic aggregation pheromone lure; CDV = fresh chicken dropping volatiles collected on SuperQ adsorption powder trap; and CD = freshly collected chicken dropping. "Beetle " in this section refers to both lesser mealworm adult and larvae.

Significant differences in the mean RI values revealed the variability among fifteen different treatments tested for attractiveness to lesser mealworm adults ($\chi^2 = 58.32$, df = 14, $P < 0.0001$, Kruskal-Wallis Test). The bait combination of 2 g CD + 20 µg lesser mealworm attracted significantly more beetles than all other treatments. More adults responded to 2 g CD as compared to the other treatments except 2 g CD + 20 µg lesser mealworm or 30 µg lesser mealworm. The mean number of adults trapped in 30 µg lesser mealworm (59) was significantly greater than 20 µg 2-nonanone (42), 10 µg lesser mealworm (34.5), 100 µg lesser mealworm (30), 10 µg CDV + 20 µg lesser mealworm (29), and 50 µg lesser mealworm (27). Also, 30 µg lesser mealworm (59), 20 µg lesser mealworm (44), 20 µg (R)-(+)-daucene (43.17), or 20 µg 2-nonanone (42) were more

effective in arrestment of adults than 20 µg (E)-β-ocimene (22.13), 10 µg CDV (21.2), 1 µg lesser mealworm (18.67), 20 µg (S)-(+)-linalool (5.5), and 20 µg (R)-(+)-limonene (2.2). A 10 µg of CDV were 3-fold less attractive than the fresh CD, whereas the fresh 5 g CD + 20 µg lesser mealworm was 3.6-fold more attractive than a combination of 10 µg CDV + 20 µg lesser mealworm (Fig. 4.1).

Field Test to Determine Pheromone Lure Attractiveness to Adults

Low Lesser Mealworm Population: The trap catches of adults decreased 6-fold with the introduction and presence of birds (days 1 and 2 vs. day 3 and 4) which may cause lesser mealworm to elicit predator avoidance behavior. Significant differences in adult numbers were found in traps treated with pheromone lure as compared to untreated checks for all the four days (Table 4.3). The treatment-by-day interaction was not significant suggesting the stable effect of pheromone over time ($F = 2.51$; df = 3, 69; $P = 0.07$). The pheromone lure baited traps caught 3.5 times more adults than their corresponding checks for the combined four days. The significant interaction between blocks and days suggested that the population distribution did change over space and time ($F = 2.14$; df = 69, 69; $P = 0.0009$). The inconsistent effect of pheromone over space in the poultry house was revealed by significant interactions between treatment and blocks ($F = 2.8$; df = 23, 69; $P = 0.0006$) (Table 4.3).

Moderate Lesser Mealworm Population: The synthetic pheromone lure was significantly attractive in the moderate lesser mealworm population house. The statistical analysis revealed the significant effect of pheromone on total trap catch ($F = 83.40$; df = 1, 126; $P = 0.0001$). Overall, the pheromone-baited traps caught 3.3 times more adults than checks. Captures decreased about 10-fold after the chicks were introduced (days 1

and 2 vs. day 3). Nevertheless, the factor difference between the pheromone and checks was stable among the days (treatment-by-day interaction was not significant) (F = 0.84; df = 2, 126; *P* = 0.43). The significant interaction between blocks and days suggested that the population distribution changed over time (F = 5.25; df = 6, 126; *P* = 0.0001). Importantly, though, no interactions involving blocks and treatments were significant, indicating a consistent pheromone effect over space (F = 1.25; df = 3, 126; *P* = 0.30) (Table 4.4)

High lesser mealworm population: Significant differences were found in day-by-treatment interaction suggesting the variable effect of pheromone over time (F = 2.9; df = 3, 69; *P* = 0.04). The significant interaction among days and blocks revealed that population distribution inside the poultry house changed over space and time (F = 1.85; df = 69, 69; *P* = 0.005). Interactions involving blocks and treatments were non-significant, indicating a consistent pheromone effect over space (F = 1.35; df = 23, 69; *P* = 0.17). A 30-fold reduction in adults trapped was observed after the introduction of chicks (days 1 and 2 vs. day 3 and 4). The pheromone-baited traps caught 2.6 times more adults than checks (Table 4.5).

Laboratory Two-Choice Pitfall Bioassay for Larvae

Significant differences in the mean RI values were found among fifteen different treatments exposed to lesser mealworm larvae ($\chi^2 = 52.56$, df = 14, *P* = 0.0001, Kruskal Wallis Test). The lure of 2 g CD alone (78.0%) or combined with 20 µg lesser mealworm (76.0%) was statistically more attractive to larvae than all other treatments. A significantly higher percentage of larvae were trapped in traps baited with 10 µg lesser mealworm (46.0%) than 1 µg lesser mealworm (8.5%), 50 µg lesser mealworm (12.0%),

100 µg lesser mealworm (17.0%), 10 µg CDV, 10 µg CDV + 20 µg lesser mealworm or 20 µg (E)-β-ocimene. However, larval captures in traps baited with lures charged with 10 µg lesser mealworm (46.0%), 20 µg lesser mealworm (39.0%) and 30 µg lesser mealworm (32.0%) were not significantly different from each other. Percent larval captures in traps baited with 20 µg (R)-(+)-limonene (0.17%) or (S)-(+)-linalool (1.3%) were not significantly attractive to larvae as compared to 10 µg lesser mealworm (46.0%), 30 µg lesser mealworm (32.0%), 20 µg 2-nonanone (33.0%), or 20 µg (R)-(+)-daucene (30.0%). Larvae were most attractive at 10 µg of lesser mealworm pheromone lure but attractiveness decreased with increased doses. A 10 µg of CDV was 5-fold less attractive than the fresh CD, whereas the fresh 5 g CD+ 20 µg lesser mealworm was 4-fold more attractive than a combination of 10 µg CDV + 20 µg lesser mealworm. A 10 µg CDV might not be a sufficient amount to elicit attraction of lesser mealworm (Fig. 4.2).

Adults were more attracted to traps baited with 30 µg lesser mealworm (59.0), 50 µg lesser mealworm (27.0) and 100 µg lesser mealworm (30.0) than were larvae at same treatments (31.5, 12.2 and 17.4, respectively). Larvae (77.5) were more responsive to fresh CD than were adults (61.2) (Fig.1 and 2).

Field Test to Determine Pheromone Lure Attractiveness to Larvae

Low Lesser Mealworm Population: Significant differences in larval numbers were found in traps treated with lesser mealworm pheromone lure as compared to untreated checks for all the four days (Table 4.6). Overall, the baited traps caught 3 times more larvae than checks. Trap catches only decreased 2-fold with the introduction of chicks (days 1 and 2 vs. day 3 and 4). The treatment-by-day interaction was not

significant suggesting the stable effect of pheromone over time (F = 0.80; df = 3, 69; *P* = 0.50). The significant interactions among treatment and block (F = 3.6; df = 23, 69; *P* = 0.0001) suggested that the effect of pheromone was not consistent over the space. The larval population distribution did not change significantly inside the poultry house over the space and time as evident from non-significant interactions among block and day (F = 0.95; df = 69, 69; *P* = 0.60). The statistical differences in total capture among the day's suggest the unchanged larval distribution inside the poultry house (F = 49; df = 3, 69; *P* = 0.0001) (Table 4.6).

Moderate Lesser Mealworm Population: The pheromone-baited traps caught two times more larvae than checks. The introduction of chicks decreased the captures by 9-fold (days 1 and 2 vs. day 3) (Table 4.7). The pheromone baited traps significantly caught more larvae than the checks over the time (treatment-by-day interaction was not significant) (F = 0.46; df = 2, 126; *P* = 0.62). The distribution of the adult population changed somewhat over space and time as revealed from the significant interaction between blocks and days (F = 6.30; df = 6, 126; *P* = 0.0001). Interactions involving blocks and treatments were significant, indicating an inconsistent pheromone effect over space (F = 3.9; df = 3, 126; *P* = 0.011) (Table 4.7).

High Lesser Mealworm Population: Captures decreased about 25-fold after the chicks were introduced (days 1 and 2 vs. day 3 and 4). Non-significant differences were found in day-by-treatment interaction suggesting the consistent effect of pheromone over time (F = 0.90; df = 3, 69; *P* = 0.44). The significant interaction between days and blocks revealed the population distribution change over space and time (F = 1.66; df = 69, 69; *P* = 0.02). The pheromone effect was consistent over space (treatment-by- block interaction

was not significant) (F = 1.32; df = 23, 69; *P* = 0.19). Overall, the pheromone-baited traps caught 2.16 times more larvae than did the check (Table 4.8).

DISCUSSION

The laboratory two-choice pitfall bioassay was found to be a useful and convenient tool for evaluating the potential attractants for lesser mealworm adults and larvae before testing them in poultry houses. Similar studies were already done to assess the pheromone performance in laboratory pitfall bioassays for the rice weevil, *Sitophilus oryzae* (Phillips and Burkholder 1981), the sawtoothed grain beetle, *Oryzaephilus surinamensis* (Mikolajczak et al. 1984), the maize weevil, *Sitophilus zeamais* Motschulsky and the red flour beetle, *Tribolium castaneum* Herbst (Phillips et al. 1993).

Adults and larvae were strongly attracted to a dose of 20-30 µg synthetic lesser mealworm aggregation lure in these laboratory pitfall bioassays. This dose range can be combined with other potential attractants from fresh CD to determine the synergistic/additive effect of various combinations in their attractiveness to adults and larvae.

Chicken dropping volatiles (CDV) were 3-fold less attractive than the fresh chicken dropping (CD), whereas the fresh CD + 20 µg lesser mealworm was 3.6-fold more attractive than a combination of 10 µg CDV + 20 µg lesser mealworm. Some compounds are highly attractive at lower concentrations but elicit repellent behavior at higher concentrations (Szausman-Szumski et al. 1998). The higher concentrations of some of the compounds in the CDV samples may act as repellents, and account for the low attractiveness of CDV to adults and larvae in the laboratory pitfall bioassays. The fresh CD might have released the volatile profiles in a fashion similar to that released in

the poultry house, and also the larvae and adults perceived the volatiles as they do in their natural habitat. The fresh CD compounds that are attractive to lesser mealworm adults and larvae should be identified and tested in different combinations with the aggregation pheromone lure.

The very low attraction of limonene and linalool suggest that either they are non-attractive or attractive presented in too low or too high a range of concentrations to be attractive. Limonene is a monoterpene that is found to be repellent against certain pest insects (Ibrahim et al. 2001). Limonene sprayed at the concentration of 1% was found to be repellent to mealybugs and whiteflies (Hollingsworth et al. 2005). Linalool repelled significantly 93% more mosquitoes than the unprotected control (Muller et al. 2009). The attractiveness of limonene or linalool in combination with lesser mealworm aggregation pheromone lure needs to be assessed, and these compounds should be excluded if found non-attractant or repellent.

A combination of fresh CD and lesser mealworm aggregation pheromone lure was observed to be very attractive to adults and larvae in laboratory two-choice pitfall bioassays. Adults were more attracted to combination of pheromone lure and fresh CD than the larvae, whereas larvae were more attracted to fresh CD alone than adults. These results were in agreement with many other insect-pests that had increased attraction to host volatiles. Human urine and chicken feces increased lure attraction to fruit flies (*Anostrepha* spp.) (Diptera: Tephritidae) in a commercial mango orchard in Veracruz, Mexico (Pinero et al. 2003). Scarab dung beetles were most attracted to swine and opossum feces in laboratory bioassays (Fincher et al. 1979). Odors from fresh chicken

feces in water elicited upwind flight of host-seeking female *Culex quinquefasciatus* Say mosquitoes in a two-choice olfactometer (Cooperbanda et al. 2008).

The numbers of adults caught in pitfall traps baited with daucene or nonanone were higher than the other three components of lesser mealworm aggregation pheromone lure: 20 µg (E)-β-ocimene (22.13), 20 µg (S)-(+)-linalool (5.5), or 20 µg (R)-(+)-limonene (2.17). The foreign grain beetle, *Ahasverus advena* (Waltl) responds to 2-nonanone and 2-undecanone derived from pods of the carob tree, *Ceratonia siliqua* (L.) in behavioral pitfall bioassays (Wakefield et al. 2005). The behavioral activity of 2-nonanone and 2-undecanone as identified from the literature appears to range from highly attractive (Szausman-Szumski et al. 1998) to highly repellent (Lwande et al. 1992). Adults can perceive pheromones at lower doses, and were also more attracted to pheromones at higher doses (30 µg, 50 µg, and 100 µg) than the larvae at corresponding doses.

The laboratory and field results confirm the larval lesser mealworm attraction to adult aggregation pheromones. Males of the spined soldier bug, *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) attracted mates with a pheromone, but the nymphs were also found to be attracted to the pheromone in a wind tunnel and in field studies (Sant'Ana et al. 1997). It is not known whether larvae of lesser mealworm produce compounds similar to adult emitted volatile profiles or not. Both larva and adults are attracted to and feed on poultry manure. The larvae may have adapted and evolved the behavioral responses to male-produced aggregation pheromones for exploiting food resources and shelter occupied by adults.

There was a reduction in lesser mealworm beetle movement and numbers in the presence of the chicks as compared to when chicks were not present in the poultry houses. The consistent effect of pheromone over time was found for larvae in low, moderate and high lesser mealworm infestation poultry houses, and for adults in low and moderate lesser mealworm infestation poultry houses. A 30-fold reduction in adults trapped after the chicks were introduced might be responsible for inconsistent pheromone trap catch in the high lesser mealworm infestation house over time. The population distribution of lesser mealworm adults and larvae in low, moderate and high infestation houses changed over time. The presence of chicks affected distribution of beetles and affected trap catch. The low larval population or clumped aggregations in the house (Strother and Steelman 2001) might be the reason for the inconsistent attractiveness of pheromone over space for adults in a low infestation house, and for larvae in low and moderate infestation houses.

Our results generally indicate that there is a potential for combining the synthetic lesser mealworm pheromone lure with the attractive components of fresh CD to enhance trap efficacy. Future research should involve confirming all the identified compounds by injecting the commercially available compounds into the GC/MS. The biological activity of lesser mealworm to these identified compounds needs to be evaluated in electroantennogram and in laboratory two-choice pitfall bioassays. The CD volatile compounds that are attractive to lesser mealworm adults and larvae should be identified and tested in different combinations with the aggregation pheromone lure. Successful candidate compounds should be tested in poultry house to demonstrate their attractiveness to lesser mealworm larvae and adults. The combinations that attract more

beetles can be used to monitor the lesser mealworm populations and also combined with insecticide baits(s) to manage lesser mealworm populations in poultry houses.

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Table 4.1 Gas chromatograph/mass spectrometer analysis (N=3) of fresh chicken droppings volatiles (CDV) collected by SuperQ trap for 4 h.

Compound	Retention time (min)	Mean amount (ng/µl) ± SE
2-methyl-propanoic acid	3.72	21.0 ± 3.6d[a]
2,3-butanediol	3.83	179.3 ±12.9a
Butanoic acid	4.74	69.4 ± 7.5c
2-pentanone	5.75	34.9 ± 4.5d
1-octen-3-ol	10.34	77.4 ± 6.1c
2-chlorocyclohexanol	11.18	111.3 ± 12.2b
Pentanoic acid	12.72	112.8 ± 8.0b
Dodecanal	15.43	35.8 ± 4.8 d

[a]Means in same column with different letters are significantly different ($P < 0.05$, Tukey Kramer HSD-test).

Table 4.2 Preparation of lures and release characteristics of five components of the aggregation pheromone of lesser mealworm, *A. diaperinus* (Bartelt et al. 2009).

Compound	Relative abundance			Emission rate (ng/hr)[b]		Change emission rate/hr
	Applied to septum[a]	Emitted from septum				
		Initial	After 24 hr	Initial	After 24 hr	
(*R*)-(+)-limonene	0.16	0.16	0.16	502	110	-6.5%
(*E*)-β-ocimene	1.00	1.00	1.00	3228	665	-6.8%
2-nonanone	0.058	0.054	0.067	174	45	-5.8%
(*S*)-(+)-linalool	0.16	0.092	0.17	298	115	-4.0%
(*R*)-(+)-daucene	0.29	0.053	0.18	173	122	-1.4%

[a] Total amount applied = 100 μg/septum.
[b] Based on regression analysis of volatiles collected in laboratory

Table 4.3 Field experiment and results of analysis of variance (ANOVA) conducted to determine attractiveness of an aggregation pheromone lure to lesser mealworm adults at a broiler production facility with low lesser mealworm infestation, Tontitown, AR, from 15 to 19 September 2008.

Treatment	Log (X+1) mean ± SE	F-ratio	*P*-value	Untransformed mean ± SE
Single day means (N = 24)				
Day 1				
Pheromone	2.51 ± 0.07a	28.9	0.0001	485.4 ± 96.0
Check	1.98 ± 0.05b			123.3 ± 22.5
Day 2				
Pheromone	2.44 ± 0.08a	16.5	0.0002	439.9 ± 95.7
Check	2.01 ± 0.06b			141.7 ± 30.3
Day 3				
Pheromone	1.68 ± 0.06a	13.6	0.0006	69.2 ± 11.5
Check	1.33 ± 0.05b			24.9 ± 3.1
Day 4				
Pheromone	1.83 ± 0.07a	39.9	0.0001	85.3 ± 11.1
Check	1.28 ± 0.05b			21.3 ± 2.6
Overall means (N= 96)				
Pheromone	2.11 ± 0.05a	40.4	0.0001	265.7 ± 39.0
Check	1.65 ± 0.04b			77.6 ± 11.0

ANOVA

Source	DF	SS	MS	F- ratio	*P*-value
Treatment	1	9.5	9.50	256.4	0.0001
Day	3	25.3	8.40	226.4	0.0001
Block	23	8.8	0.38	10.3	0.0001
Treatment X Day	3	0.3	0.10	2.5	0.065
Day X Block	69	5.5	0.08	2.1	0.0009
Treatment X Block	23	2.3	0.10	2.8	0.0006
Error	69	2.5	0.04		
C. Total	191	53.9			

Experiment had a paired design with twenty four traps for each treatment. Each pair had either a check or pheromone treatment, replicated on 4 days. Analysis was on log (X+1) transformed data. Within a day, or for overall means, values followed by the same letter are not significantly different (LSD, α=0.05).

Table 4.4 Field experiment and results of analysis of variance (ANOVA) conducted to determine attractiveness of an aggregation pheromone lure to lesser mealworm adults at a broiler production facility with moderate lesser mealworm infestation, Savoy, AR, from 20 to 23 August 2008.

Treatment	Log (X+1) mean ± SE	F-ratio	*P*-value	Untransformed mean ± SE
Single day means (N = 24)				
Day 1				
Pheromone	3.10 ± 0.07a	6.9	0.0001	1555.5 ± 162.0
Check	2.54 ± 0.10b			535.1 ± 72.1
Day 2				
Pheromone	3.30 ± 0.03a	26.3	0.0001	2110.1 ± 170.9
Check	2.58 ± 0.09b			568.1 ± 84.2
Day 3				
Pheromone	1.97 ± 0.12a	5.6	0.0002	207.6 ± 56.4
Check	1.17 ± 0.16b			54.8 ± 17.7
Overall means (N= 72)				
Pheromone	2.80 ± 0.08a	26.2	0.0001	1290.1 ± 123.6
Check	2.10 ± 0.10b			386.3 ± 46.2

ANOVA

Source	DF	SS	MS	F-ratio	*P*-value
Treatment	1	17.0	17.0	83.4	0.0001
Day	2	55.2	27.6	134.9	0.0001
Block	3	3.9	1.3	6.4	0.0005
Treatment X Day	2	0.3	0.1	0.8	0.43
Day X Block	6	6.4	1.0	5.2	0.0001
Treatment X Block	3	0.8	0.3	1.2	0.30
Error	126	25.8	0.2		
C. Total	143	109.5			

Experiment had a randomized complete block design, with six traps for each treatment within each of four blocks, replicated on 3 days. Analysis was on log (X+1) transformed data. Within a day, or for overall means, values followed by the same letter are not significantly different (LSD, α=0.05).

Table 4.5 Field experiment and results of analysis of variance (ANOVA) conducted to determine attractiveness of an aggregation pheromone lure to lesser mealworm adults at a broiler production facility with high lesser mealworm infestation, Savoy, AR, from 8 to 12 November 2008.

Treatment	Log (X+1) mean ± SE	F-ratio	*P*-value	Untransformed mean ± SE
Single day means (N = 24)				
Day 1				
Pheromone	3.65 ± 0.03a	88.1	0.0001	4799.3 ± 288.2
Check	3.22 ± 0.03b			1782.7 ± 113.0
Day 2				
Pheromone	3.45 ± 0.05a	22.7	0.0001	3351.5 ± 321.9
Check	3.07 ± 0.04b			1398.9 ± 192.2
Day 3				
Pheromone	2.06 ± 0.08a	15.2	0.0003	176.0 ± 38.3
Check	1.53 ± 0.10b			59.6 ± 12.5
Day 4				
Pheromone	2.21 ± 0.06a	30.4	0.0001	202.6 ± 27.9
Check	1.51 ± 0.11b			58.0 ± 11.8
Overall means (N= 96)				
Pheromone	2.84 ± 0.08a	17.4	0.0001	2132.4 ± 232.2
Check	2.33 ± 0.09b			824.0 ± 96.9

ANOVA

Source	DF	SS	MS	F- ratio	*P*-value
Treatment	1	12.5	12.5	156.1	0.0001
Day	3	111.9	37.3	467.3	0.0001
Block	23	5.5	0.2	3.0	0.0002
Treatment X Day	3	0.7	0.2	2.9	0.04
Day X Block	69	10.2	0.2	1.9	0.005
Treatment X Block	23	2.5	0.1	1.4	0.17
Error	69	5.6			
C. Total	191	159.3			

Experiment had a paired design with twenty four traps for each treatment. Each pair had either a check or pheromone treatment, replicated on 4 days. Analysis was on log (X+1) transformed data. Within a day, or for overall means, values followed by the same letter are not significantly different (LSD, α=0.05).

Table 4.6 Field experiment and results of analysis of variance (ANOVA) conducted to determine attractiveness of an aggregation pheromone lure to lesser mealworm larvae at a broiler production facility with low lesser mealworm infestation, Tontitown, AR, from 15 to 19 September 2008.

Treatment	Log (X+1) mean ± SE	F-ratio	*P*-value	Untransformed mean ± SE
Single day means (N = 24)				
Day 1				
Pheromone	2.51 ± 0.06a	22.6	0.0001	441.0 ± 78.0
Check	2.08 ± 0.06b			157.0 ± 26.3
Day 2				
Pheromone	2.42 ± 0.08a	13.2	0.0007	401.2 ± 79.0
Check	2.07 ± 0.05b			136.6 ± 16.9
Day 3				
Pheromone	2.17 ± 0.07a	26.3	0.0001	206.7 ± 38.1
Check	1.69 ± 0.06b			59.8 ± 8.5
Day 4				
Pheromone	2.17 ± 0.07a	19.9	0.0001	207.6 ± 38.4
Check	1.75 ± 0.06 b			70.8 ± 11.4
Overall means (N= 96)				
Pheromone	2.32 ± 0.07a	65.1	0.0001	314.1 ± 32.3
Check	1.90 ± 0.06b			106.0 ± 9.4

ANOVA

Source	DF	SS	MS	F- ratio	*P*-value
Treatment	1	8.50	8.50	225.2	0.0001
Day	3	5.20	1.73	49.0	0.0001
Block	23	11.40	0.50	13.1	0.0001
Treatment X Day	3	0.09	0.03	0.8	0.50
Day X Block	69	2.45	0.04	0.9	0.60
Treatment X Block	23	3.07	0.13	3.6	0.0001
Error	69	2.60	0.04		
C. Total	191	33.30	0.17		

Experiment had a paired design with twenty four traps for each treatment. Each pair had either a check or pheromone treatment, replicated on 4 days. Analysis was on log (X+1) transformed data. Within a day, or for overall means, values followed by the same letter are not significantly different (LSD, α=0.05).

Table 4.7 Field experiment and results of analysis of variance (ANOVA) conducted to determine attractiveness of an aggregation pheromone lure to lesser mealworm larvae at a broiler production facility with moderate lesser mealworm infestation, Savoy, AR, from 20 to 23 August 2008.

Treatment	Log (X+1) mean ± SE	F-ratio	*P*-value	Untransformed mean ± SE
Single day means (N = 24)				
Day 1				
Pheromone	3.10 ± 0.04a	11.1	0.001	1465.8 ± 180.9
Check	2.80 ± 0.07b			812.0 ± 95.15
Day 2				
Pheromone	3.10 ± 0.08a	8.5	0.005	1746.8 ± 254.6
Check	2.71 ± 0.10b			751.4 ± 93.0
Day 3				
Pheromone	2.07 ± 0.12a	7.3	0.009	256.6 ± 57.4
Check	1.62 ± 0.10b			90.2 ± 25.7
Overall means (N= 72)				
Pheromone	2.75 ± 0.07a	10.8	0.0001	1156.4 ± 129.5
Check	2.38 ± 0.08b			551.2 ± 59.0

ANOVA

Source	DF	SS	MS	F-ratio	*P*-value
Treatment	1	5.08	5.08	35.8	0.0001
Day	2	37.33	18.67	131.5	0.0001
Block	3	4.06	1.35	9.5	0.0001
Treatment X Day	2	0.13	0.07	0.5	0.62
Day X Block	6	5.36	0.89	6.3	0.0001
Treatment X Block	3	1.64	0.55	3.9	0.011
Error	126	17.90	0.14		
C. Total	143	71.51	0.50		

Experiment had a randomized complete block design, with six traps for each treatment within each of four blocks, replicated on 3 days. Analysis was on log (X+1) transformed data. Within a day, or for overall means, values followed by the same letter are not significantly different (LSD, α=0.05).

Table 4.8 Field experiment and results of analysis of variance (ANOVA) conducted to determine attractiveness of an aggregation pheromone lure to lesser mealworm larvae at a broiler production facility with high lesser mealworm infestation, Savoy, AR, from 8 to 12 November 2008.

Treatment	Log (X+1) mean ± SE	F-ratio	*P*-value	Untransformed mean ± SE
Single day means (N = 24)				
Day 1				
Pheromone	3.14 ± 0.04a	39.0	0.0001	1522.6 ± 136.1
Check	2.84 ± 0.03b			730.9 ± 54.3
Day 2				
Pheromone	3.09 ± 0.05 a	24.9	0.0001	1430.5 ± 172.2
Check	2.72 ± 0.04b			632.0 ± 75.4
Day 3				
Pheromone	1.70 ± 0.09a	8.0	0.007	81.9 ± 22.4
Check	1.32 ± 0.06b			38.0 ± 4.3
Day 4				
Pheromone	1.50 ± 0.08a	3.0	0.09	51.0 ± 13.2
Check	1.30 ± 0.09b			29.7 ± 5.6
Overall means (N= 96)				
Pheromone	2.36 ± 0.08a	7.0	0.008	771.5 ± 90.0
Check	2.04 ± 0.08b			357.7 ± 40.6

ANOVA

Source	DF	SS	MS	F- ratio	*P*-value
Treatment	1	4.8	4.80	61.9	0.0001
Day	3	107.2	35.73	459.2	0.0001
Block	23	6.2	0.27	3.5	0.0001
Treatment X Day	3	0.2	0.07	0.9	0.44
Day X Block	69	8.9	0.13	1.7	0.019
Treatment X Block	23	2.4	0.10	1.3	0.18
Error	69	5.4	0.08		
C. Total	191	135.1			

Experiment had a paired design with twenty four traps for each treatment. Each pair had either a check or pheromone treatment, replicated on 4 days. Analysis was on log (X+1) transformed data. Within a day, or for overall means, values followed by the same letter are not significantly different (LSD, α=0.05).

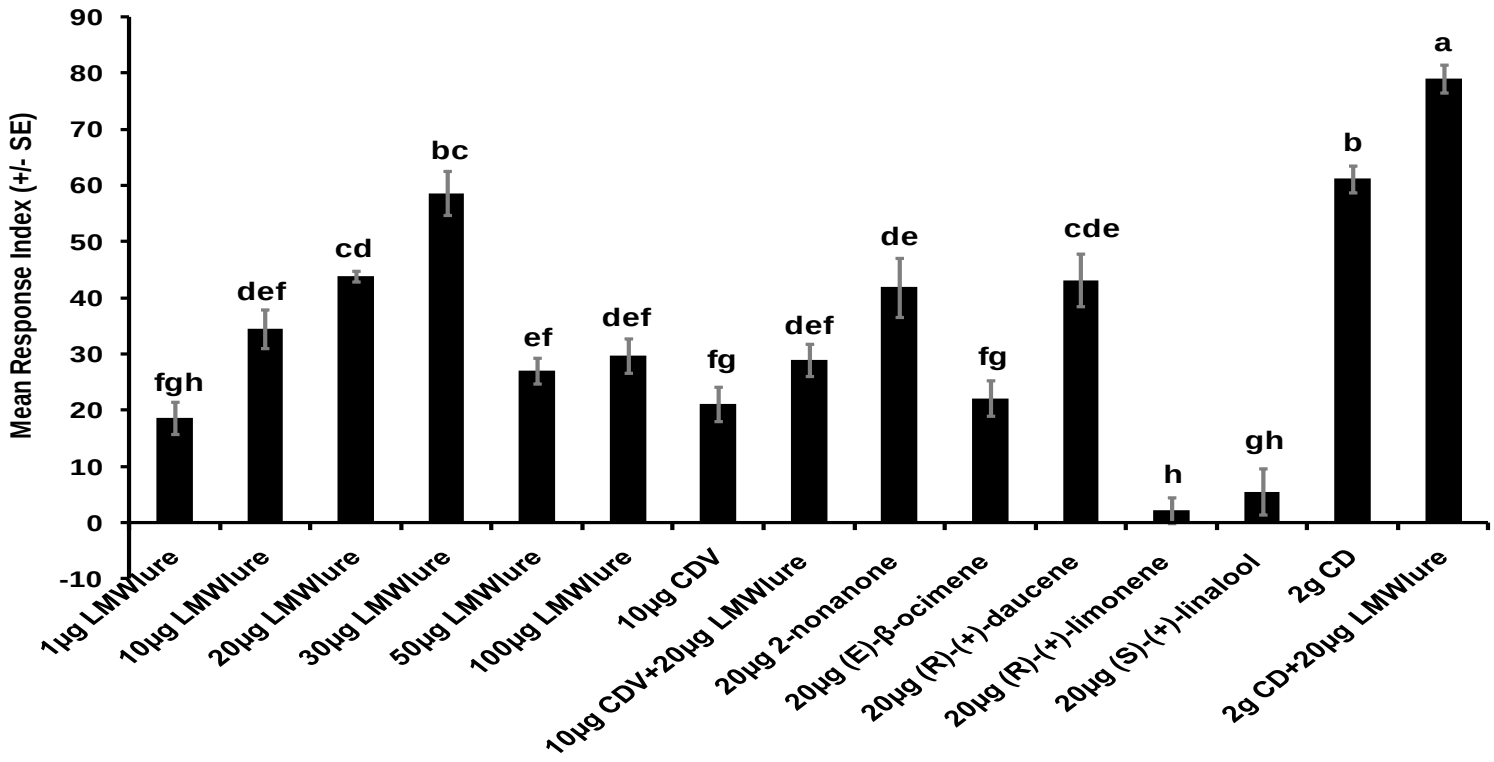

Fig. 4.1 Response of lesser mealworm adults to a range of aggregation pheromone lure doses, 20 µg each component of aggregation pheromone lure, 10 µg chicken dropping volatiles (CDV) and fresh chicken dropping (CD) in laboratory two-choice pitfall bioassays at air flow rate of 0.5 l/min. Response Index = (T - C/Tot)*100, for which T is the number responding to the treatment, C is the number responding to the check, and Tot is the total number released. Data are expressed as means ± SE (N = 4). Bars with different letters are significantly different ($P < 0.05$, Tukey Kramer HSD-test).

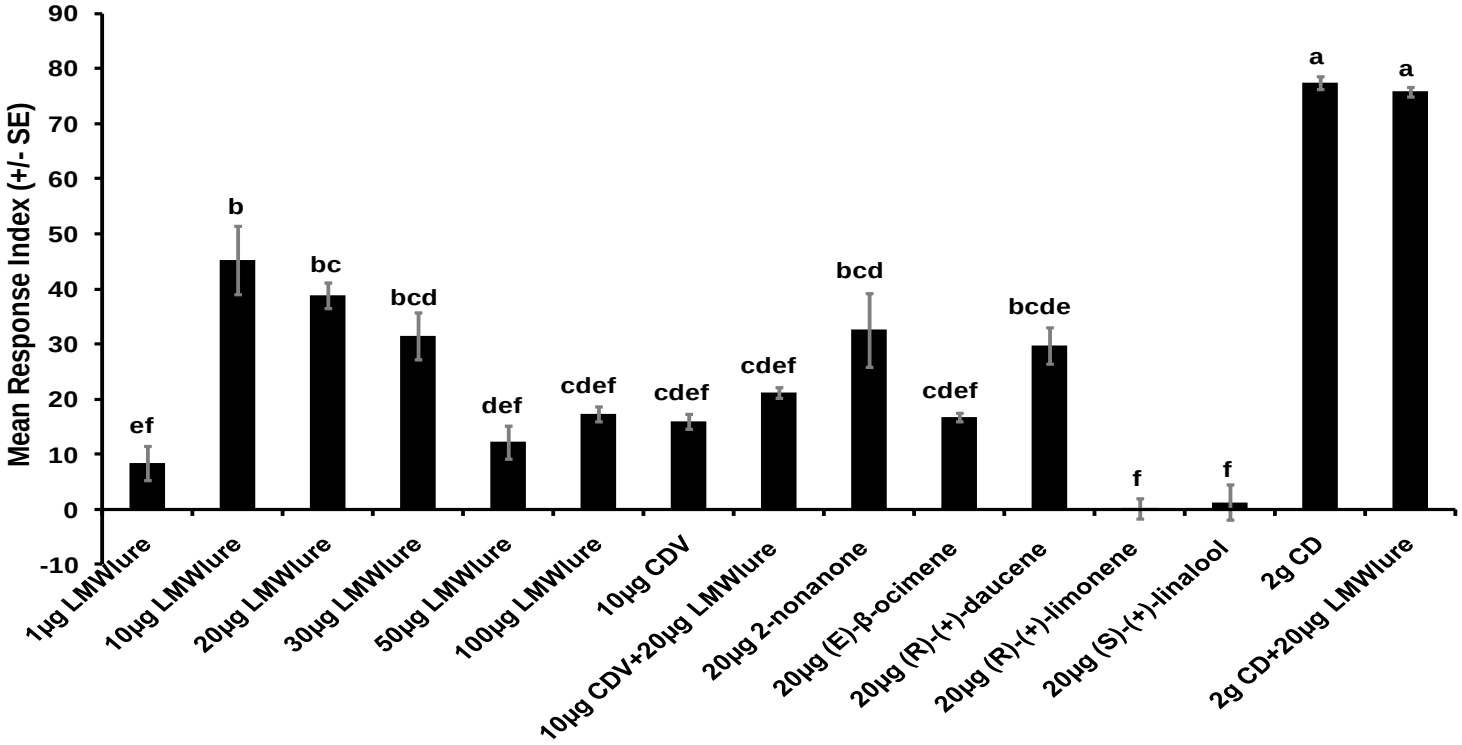

Fig. 4.2 Response of lesser mealworm larvae to a range of aggregation pheromone lure doses, 20 µg each component of aggregation pheromone lure, 10 µg chicken dropping volatiles (CDV) and fresh chicken dropping (CD) in laboratory two-choice pitfall bioassays at air flow rate of 0.5 l/min. Response index = (T - C/Tot)*100, for which T is the number responding to the treatment, C is the number responding to the check, and Tot is the total number released. Data are expressed as means ± SE (N = 4). Bars with different letters are significantly different ($P < 0.05$, Tukey Kramer HSD-test).

Photograph 4.1 Different parts of newly designed pitfall trap that was used in the field experiments to collect lesser mealworms.

a) This cross-section shows one of the three base screws that attach the canning ring to the trap base

b) The trap top attached to the trap base

c) The full trap including 250 ml jar fitted to canning ring that is attached to plywood trap base and top

CHAPTER V

BASELINE RESPONSES OF THE LESSER MEALWORM, *ALPHITOBIUS DIAPERINUS* (PANZER) TO INSECT GROWTH REGULATORS

ABSTRACT

The cancellation of most organophosphate and carbamate insecticides and the emerging resistance to currently available insecticides in the lesser mealworm, *Alphitobius diaperinus* (Panzer), necessitate efficacy studies of other classes of insecticidal compounds. Baseline dose-response bioassays were conducted with three different types of insect growth regulators (IGR's) including juvenile hormone analog (fenoxycarb), chitin synthesis inhibitor (diflubenzuron), and the ecdysone agonist (20-hydroxyecdysone) on 1st and 7th instars, 2 d old pupae, and 1 wk old adult beetles. Beetles were exposed to a range of concentrations through topical application, residual contact with treated wood shavings, and feeding on treated chicken feed that simulated the ways insecticides could possibly be acquired by beetles in poultry facilities. Beetles were observed at 10 d intervals to estimate percentage mortality, pupation and abnormal growth for each treatment. All three insecticides applied to 1st or 7th instars by feeding caused a significant reduction in mean percent pupation and significantly increased the mean numbers of days to pupation compared to untreated checks. The 7th instars exposed to fenoxycarb by feeding, residual and topical bioassays continued to molt and gain weight, and died as deformed larvae, abnormal pupae or intermediate larval-pupal and pupal-adult forms. Fenoxycarb, ecdysone, and diflubenzuron caused less than 48, 35 and 68% mortality, respectively, to adult lesser mealworms exposed by feeding, residual and topical bioassays with high LC_{50} and LC_{90} concentrations. Adults died from significantly lower concentrations of all three IGR's in a topical bioassay than in feeding or residual contact bioassays. In comparison, fenoxycarb, ecdysone, and diflubenzuron at 100 ppm caused significantly greater mortality in: feeding bioassays of 1st instars (100, 92.5 and

90%, respectively) and 7th instars (94, 92.5 and 77%, respectively); residual bioassays of 7th instars (87.5, 55 and 66%, respectively); and topical bioassays of 7th instars (93.9, 80 and 100%, respectively) and pupae (100, 88.3 and 91.6%, respectively) than the < 20% mortality in the untreated checks. In feeding bioassays, fenoxycarb and diflubenzuron were more toxic to 1st instars than 7th instars and adults, whereas ecdysone was equally more toxic to 1st instars and 7th instars than to adults. The feeding bioassay caused the most percent mortality of 1st and 7th instars. The residual contact bioassay exhibited slightly less percent mortality of 7th instars and adults but this bioassay most closely simulated field application of insecticides. All three bioassay methods produced usable dose-response curves and may be used for surveying temporal changes in IGR susceptibility in lesser mealworm populations.

Key Words: feeding bioassay, weight gain, toxicity, susceptibility, pupation, LC_{50}

INTRODUCTION

There has been some concern about using insect growth regulators (IGRs) against lesser mealworm in poultry facilities. It was reported that an IGR-treated diet prolonged the larval feeding period of the Indian mealmoth, *Plodia interpunctella* (Hübner) (Firstenberg and Silhacek 1976), and increased larval size by completing extra larval molts in the Khapra beetle, *Trogoderma granarium* Everts (Metwally and Sehnal 1973). If IGR treatments cause lesser mealworm larvae to prolong feeding and/or increase size, these larvae could eat more chicken feed, fill the crop of chickens faster, carry and transmit disease for a longer time and further delay weight gain and cause larger tunnels in insulation causing more heat loss. However, some IGR's may have a role in resistance management program.

The IGR subgroups of juvenile hormones (JH's) and ecdysones regulate the physiological and behavioral processes in insects during metamorphosis (Willis 1974). Molting is a complex process that involves up and down regulation of genes, and is initiated by binding of 20-hydroxyecdysone (20E) to ecdysone receptors (Retnakaran et al. 2003). Ecdysone analogs bind strongly with ecdysone receptors and are not easily detached (Dhadialla et al. 1998). These analogs activate all upregulated genes but repress down-regulated genes that activate in absence of 20E. Events required for ecdysis do not happen and there is a developmental freeze resulting in incomplete precocious molt (Park et al. 2002; Zitnan et al. 2002). Ecdysone agonists caused premature cuticle synthesis around the head region where occlusion of the functional mouth parts resulted in feeding inhibition within 24 h regardless of the age or instar treated (Wing and Aller 1990). Treatment of two ecdysone agonists, methoxyfenozide (RH-2485) and halofenozide (RH-

0345) at concentrations of 25, 50 and 100 mg/liter on multicolored Asian lady beetle, *Harmonia axyridis* Pallas, caused premature induction of larval molting, cessation of feeding and incomplete pupation in the last instar (Carton et al. 2003). The newly ecdysed 6th instar of spruce budworm, *Choristoneura fumiferana* (Clemens), fed with 100 ng of ecdysone agonist, Tebufenozide (RH-5992), produced incomplete precocious larval molt. Apolysis happened but larvae failed to pass through ecdysis 48 h post treatment (Retnakaran et al. 1997).

Metamorphosis (larval-pupal and pupal-adult) is induced by 20E but needs to happen in the absence of JH (Goodman and Granger 2005). JH is secreted and maintained in all instars to retain the larval-larval molt except in the last instar where it is absent. Juvenile hormone analogs (JHAs) are toxic during the embryonic, last-larval, and reproductive stages of insects. JH treatment at any immature stage reduced adult emergence in stored-product insects (Pallos et al. 1971). There was 45.5% adult mortality and 95% reduction in offspring produced by maize weevil, *Sitophilus zeamais* Motschulsky, when wheat grains treated with fenoxycarb at 10 mg/kg were exposed to adults for 3 wk (Letellier et al. 1995). Fenoxycarb caused 93% reduction of several stored product pests, e.g., rice weevil, *Sitophilus oryzae* (L.), confused flour beetle, *Tribolium confusum* Jacquelin du Val, lesser grain borer, *Rhyzopertha dominica* (F.), and Indianmeal moth, *Plodia interpunctella* (Hübner), in stored wheat compared to 83 and 36% reduction by malathion and *Bt* (Solomon 1985). Fenoxycarb significantly delayed the developmental times of larvae from the stage treated to adult emergence (Liu and Chen 2001).

The benzoylphenylurea (BPU) insecticides, such as diflubenzuron and flucycloxuron, are called chitin synthesis inhibitors (CSI). These compounds prevent the molting cycle of insects leading to abnormal endocuticular deposition and abortive molting (Dhadialla et al. 2005), ecdysial failure and formation of globular bodies between the endocuticle and epidermis (Ren et al. 1988). They also affect reproduction by causing a reduction in egg hatch (Ishaaya et al. 1990). CSIs retarded larval growth of the red flour beetle, *T. castaneum* (Herbst), and hence increased larval life. CSI-treated larvae were found to eat less and grew consequently at a slower rate (Parween 1996). Diflubenzuron incorporated into the diet of *Diaprepes abbreviatus* (L.) at 10, 100, 500 or 1000 ppm resulted in mortality of 56.5, 73.9%, 65.2% and 65.2%, respectively (Beavers et al. 1976). The 3 d old larvae of the khapra beetle, *Trogoderma granarium* Everts, treated with diflubenzuron at 0.8, 1.6 and 12.8 mg ai /kg upon eclosing into the adult stage produced significantly less offspring (Rajendran and Shivaramaiah 1983). The highest dose of 12.8 mg a.i/kg diet resulted in 94, 80 and 76% mortality in 3, 7 and 14 d old larvae, respectively.

The cancellation of most indoor organophosphates and carbamate insecticides has reduced the number of residual insecticides for lesser mealworm control and the remaining products are facing the potential of developing insect resistant populations (Lambkin 2005). Fenitrothion was used for 20 y before the adoption of cyfluthrin, which is currently considered the industry standard in Australia. Unfortunately resistance to both fenitrothion and cyfluthrin has been found in populations of lesser mealworms in broiler houses in Australia resulting in failure of control measures (Lambkin 2005). Tetrachlorvinphos and cyfluthrin have been reported to lose effectiveness against lesser

mealworms in poultry operations in the US (Hamm et al. 2006). Due to environmental and resistance concerns related to the use of conventional insecticides, the research needs to be focused toward more selective compounds with different modes of action (Dhadialla et al. 2005). Insect growth regulators (IGRs) that interfere with insect growth and development have a great potential in pest management (Ishaaya et al. 1990).

Lesser mealworm beetles in poultry houses are exposed to insecticides by three different ways: direct spray contact, tarsal residual contact with treated surfaces and through feeding on treated litter and chicken feed. There have been no published reports of laboratory bioassays of lesser mealworm larvae, pupae and adults exposed to insecticides in these three ways that mimic conditions in poultry facilities. Little data exists on the efficacy of IGRs against lesser mealworm. The goals of the present study were to obtain mortality data on feeding, residual and topical toxicity of fenoxycarb, diflubenzuron and hydroxyecdysone to larvae, adults and pupae of lesser mealworm. Fenoxycarb, diflubenzuron and hydroxyecdysone were also compared to determine the differences in their toxicity levels to lesser mealworm. Topical, residual and systemic uptake methods were also compared to determine the most susceptible stage, percent pupation, mean pupation time and adult survival among fenoxycarb, diflubenzuron and hydroxyecdysone.

MATERIAL AND METHODS

Laboratory Colony of Lesser Mealworm

A large number of different stages of lesser mealworm were collected from the Applied Broiler Research Unit, in Savoy, AR, where pyrethroids, organophosphates and spinosad were used regularly over the last two years to reduce the beetle population.

Litter or manure was collected from these houses and beetles extracted by sieving samples through 2.8 and 2.0 mm screens. Beetles were transported to the laboratory and reared in covered plastic chambers maintained at 28 ^{o}C with 60% RH, and a photoperiod of 16:8 (L:D). These beetles were fed commercial starter chicken feed that contained corn and soybean meal along with vitamin and mineral supplements (Chick Starter, Herider Farms in Fayetteville, AR). Rolled cardboards (30 cm^2) (Northwest Arkansas Paper Company in Springdale, AR) were added to serve as shelter and pupation sites for mature larvae. Beetles were provided water weekly by placing a 6 cm^2 piece of water-saturated cotton on the litter surface.

To get the uniform age 7th instar for feeding, residual and topical bioassays, approximately ten thousand 6th instars (7 mm in length, according to Wilson and Miner 1969) were identified from field collected beetles and placed in a plastic chamber (27.5 long x 17.5 wide x 17.5 cm deep, Penn Plax Large Animal Carrier, Garden City, NY) with wood shavings. Chick starter feed and water were provided weekly to these larvae. After 7 d, the chambers were checked regularly for cast skins to confirm that larvae had molted to the 7th instar (8 mm in length, Wilson and Miner 1969). The newly molted 7th instars (light colored exoskeleton) were removed for bioassays. A piece of corrugated rolled cardboard (30 cm^2) was placed in each plastic rearing chamber that contained approximately ten thousand 7th instars. Chambers were checked weekly to confirm molting, and uniform aged pupae were collected for bioassays. The unused pupae were allowed to transform into adults and were used in bioassays. The newly emerged adults were removed, and held in groups of 100 per Petri dish (150 mm dia.). The adult exoskeleton was allowed to completely harden over a 5 d period (Hopkins et al. 1992),

during which time these adults were provided food (1 g finely ground chicken feed) and water (daily moistened 2 cm^2 cotton ball). Uniform aged 1^{st} instars were obtained by placing about one thousand newly emerged adults in plastic chambers containing water, food and wood shavings. Eggs were laid within a week, and the first instars were sieved from these chambers after two weeks.

Three different types of IGR's were used to develop their baseline responses on lesser mealworm. The IGR's included: juvenile hormone analog (JHA), fenoxycarb (98%); chitin synthesis inhibitors (CSI), diflubenzuron (99%); and ecdysone agonist, 20 hydroxyecdysone (98%) (Sigma-Aldrich Co., Milwaukee, WI).

Feeding Bioassay

Stock solutions of each treatment concentration were made by dissolving the insecticide in acetone. Aliquots of the stock solution were added to 100 g batches of chicken feed to achieve concentrations ranging from 0.01 to 100 ppm. Excess acetone was added to uniformly disperse the test compound. Some batches of food were treated with acetone alone which served as an untreated checks. The treated food mixtures were stirred and put in the fume hood for 24 h to evaporate the acetone completely. Batches of treated and check chicken feed were divided into aliquots of 2 g and one beetle was placed in each 30 ml plastic cup. Four day old first instars, newly eclosed 7^{th} instars and 1 wk old adults were used in all tests with hundred replicates for fenoxycarb and eighty replicates each for diflubenzuron and ecdysone. Insects were allowed to feed on treated feed for 10 d and, then transferred to normal diet. Water soaked cotton (2 cm^2) was placed on the surface of food and moistened twice weekly. Each beetle was observed at 10 d intervals to estimate percentage mortality and abnormal growth for each treatment.

Residual Contact Bioassay

IGR compounds were diluted in acetone and the concentrations ranged from 0.01 to 1000 ppm. Wood shavings (500 g) were sprayed uniformly and mixed with a specific IGR concentration in a glass container (40 cm long x 30 cm wide), and air dried for 12 h. Treated wood shavings (2 g) were transferred to 30 ml plastic cups and one beetle was added to each cup. Wood shavings treated with acetone alone served as checks. Newly eclosed 7^{th} instars and 1wk old adults were used in all tests with eighty replicates per treatment. Each beetle was placed in a treated cup and observations of percentage mortality and abnormal growth for each treatment were made at 10 d intervals.

Topical Bioassay

Newly eclosed 7^{th} instars and 1 wk old adults were used in all tests. Two day old pupae were used because it took about 1 to 2 days to further develop from prepupa to pupa. Before insecticide application, beetles were removed from rearing boxes and counted into batches of ten. Treatment concentrations in nanograms per milligram (ng/mg) or ppm were calculated using the mean weight of 7^{th} instars (15.55 ± 1.66 mg, N = 100) and adults (14.47 ± 1.52 mg, N = 100). Topically applied IGRs were reported to be more biologically active than when injected (Bowers et al. 1965). The 7^{th} instars or adult beetles were transferred to a small glass sheet (5 cm long x 4 cm wide) where 1 µl of an insecticide concentration (ranging from 0.01 to 100 ng/mg) were individually applied to the thoracic dorsum with a Hamilton micro syringe (Hamilton Co., Reno, NV). Check beetles were similarly treated with 1 µl of acetone. One beetle was placed in each 30 ml plastic cup with the normal diet. Sixty replicates per treatment were used for pupa and adults whereas eighty replicates were used for 7^{th} instars.

Cups were placed in the laboratory conditioned at 28 ^{o}C with 60% RH, and a photoperiod of 16:8 (L:D). The lesser mealworm was considered morbid or dead if it was unable to right itself or walk, or there was no movement when they were prodded with a metal probe. Pupae were regarded as dead if they turned black with no abdominal gyrating movement, or showed signs of desiccation. Initial and final weights of 7th and 1st instars treated with fenoxycarb were observed at 10 d intervals. Abnormalities or deformities in larvae, pupae and adults were also described and photographed.

Weight Gain

The weight gains were observed at 10 d intervals for the 7th or 1st instar fed on fenoxycarb treated diet, and 7th instar topically treated or exposed to the fenoxycarb treated wood shavings. The weights of 7th instars used were approximately identical at the beginning of the bioassays. However, weight gain was calculated as the difference between the final weight on the sampling date and the initial weight of the larva to eliminate the variations in the initial weights on analysis. The final weights were used to determine weight gains in the analysis for the 1st instars since initial weights were extremely low.

Experimental Plan and Statistical Analyses

Each experiment was a completely randomized block (CRB) design with each cup representing a replicate. Each IGR treatment with each concentration was replicated 10 times in a block with one beetle per replicate.Ten blocks were used in feeding bioassays whereas 8 blocks were used for residual bioassays. Eight blocks were used in topical bioassays for 7th instar and 6 blocks for pupae and adults.

The percent mortality (± SE) was calculated for each concentration for all IGRs. For percent mortality tests, original data were corrected for the check larval mortality by Abbott's (1925) formula. Due to wide ranges in percentage mortality, data were arcsine-square root transformed before analysis of variance (ANOVA). Treatment means were compared using Tukey test (PROC GLM, SAS Institute 2004).

Concentration-mortality responses were estimated with the PROC PROBIT option of SAS (SAS Institute 2004). Mortality in treated beetles was corrected for natural mortality in the check beetles using Abbott's formula (Abbott 1925). Corrected data from bioassays were analyzed by probit analysis to estimate: Probit $Y = a + b \text{ Log}_{10}$ (IGR concentration), where 'a' is the intercept and 'b' is the slope associated with concentration response curve and then calculated the IGR concentration that killed 50 or 90% of lesser mealworms (LC_{50} and LC_{90}); regression coefficient (slope) and its standard error; Pearson's goodness of fit Chi-square; and 95% confidence limits for effective level of concentrations. Only pairs of LC_{50} values or pairs of LC_{90} values that did not have overlapping 95% confidence limits were considered significantly different. Weight gains, percent pupation, mean pupation times (days), mortality in each stage and adult survival were analyzed using ANOVA, and means were distinguished using Tukey test (SAS Institute 2004).

RESULTS

Weight Gain

Feeding Bioassay

7^{th} Instar: The mean larval weight gains of 7^{th} instars were significantly higher at concentrations up to 0.1 ppm, 0.1 ppm and 1 ppm fenoxycarb at 10 d, 20 d and 30 d post

treatment, respectively, than at higher concentrations ($P < 0.05$) (Table 5.1). At 40 d, the larvae fed a dose of 10 ppm attained maximum weight gain of 13.7 mg (Photograph 5.1). The weight gains of untreated check (0 ppm) larvae were significantly lower than all the concentrations with the exception of 100 ppm, where check and 100 ppm had similarly low weight gains ($P < 0.05$). Many of the untreated check larvae developed to pupae within 10 d so thereafter weight gains for this group were not reported. The 86.5 % untreated check larvae that reached pupation did so in a mean of 13.7 d. Each value for the check larvae versus that for 7th instar fed on fenoxycarb treated diet was significantly greater than the overall mean percent pupation (33%) or significantly less than the mean pupation time of 26.3 d on treated diet ($P < 0.05$) (Table 5.2).

1st Instar: The first instars exhibited significantly higher weight gains to 0.1 ppm fenoxycarb at 10 d and 20 d, 1 and 10 ppm at 30 d, and at 10 ppm at 40 and 50 d post treatment ($P < 0.05$) (Table 5.1). The 1st instar weight gains were significantly greater at the concentration of 0.1 ppm at 10 and 20 d post treatment and at concentration of 10 ppm at 30, 40 and 50 d post treatment than at other concentrations ($P < 0.05$). The weight gain at concentration of 100 ppm was significantly lower than all other concentrations after 20, 30, 40 and 50 d post treatment with the exception where the concentration of 100 ppm was similar to the untreated check at 30 d, and to the concentration of 0.01 ppm at 50 d ($P > 0.05$) (Table 5.1). The fenoxycarb-treated 1st instars exhibited significantly lower percent pupation (~30%) than the untreated check (85%). The mean pupation time of fenoxycarb-treated larvae of 43 d was not statistically different from that of the check of 41 d ($P > 0.05$) (Table 5.2).

Residual Bioassay: The effect of the 7th instars exposed to fenoxycarb exhibited significantly higher weight gain to concentrations of 10, 100 and 1000 ppm fenoxycarb at 10 d, and to concentration of 100 ppm at 20 d as compared to other concentrations. The larvae in the untreated check or concentration of 1000 ppm either pupated or died at 20 d ($P < 0.05$) (Table 5.3). Comparisons of the weight gains showed that higher concentrations produced heavier larvae at 20 and 30 d post treatment, whereas lower concentrations of 0.1 and 1ppm delayed pupation of some 7th instars to 40 d (Table 5.4). Untreated check larvae attained significantly higher percent pupation (93%) than the 7th instar exposed to fenoxycarb (78%). The mean pupation time of 12 d for untreated check larvae was significantly lower than the 26.4 d recorded for larvae exposed to fenoxycarb ($P < 0.05$).

Topical Bioassay: The 7th instars in the untreated check or topically treated with 0.01 ppm fenoxycarb gained weight, whereas weight was reduced by all other concentrations after 10 d ($P < 0.05$) (Table 5.3). The mean larval weight gain was significantly higher to lower concentrations of 0.1 and 1 ppm at 20 d, and to concentration of 10 ppm at 30 d than other concentrations or the check ($P < 0.05$). The check larvae pupated at 10 d, whereas pupation was delayed to 40 d in larvae treated with lower concentrations of 0.1 ppm and 1 ppm fenoxycarb (Table 5.4). The percent pupation in untreated check (90.4%) larvae was significantly higher than fenoxycarb treated larvae (48.7%). Larvae treated with fenoxycarb resulted in significantly higher pupation time (22.8 d) than untreated check larvae (11.2 d) ($P < 0.05$) (Table 5.4).

Lethal Concentration (LC_{50})

Feeding Bioassay: Comparisons of fiducial limits for LC_{50} values within a life stage, showed that fenxoycarb (0.065 ppm) treated diet was significantly more toxic to 7^{th} instar followed by ecdysone (0.26 ppm), and then diflubenzuron (2.1 ppm). The LC_{50} values of diflubenzuron (0.06 ppm), ecdysone (0.11 ppm) and fenoxycarb (0.14 ppm) treated diet to 1^{st} instar were not significantly different. For adults, fenoxycarb (1,426 ppm) was most potent, followed by diflubenzuron (11,660 ppm) and then ecdysone (982,937 ppm). The LC_{50} values comparisons among life stages indicated that the fenoxycarb was significantly more toxic to 7^{th} instar than 1st instar whereas diflubenzuron was more toxic to 1^{st} instar than 7^{th} instar. The toxicity of ecdysone to 7^{th} and 1^{st} instar was not significantly different. Fenoxycarb, diflubenzuron or ecdysone were more toxic to 1^{st} or 7^{th} instar than adults among and across IGR's (Table 5.5).

Residual Bioassay: The LC_{50} values and fiducial limits within a life stage indicated a significantly greater toxicity of fenxoycarb (0.2 ppm) treated wood shavings to 7^{th} instar than diflubenzuron (7.1 ppm), which was then followed by ecdysone (112.4 ppm). The fenoxycarb (4,350 ppm) was more toxic to adults followed by ecdysone (12,201 ppm) and then diflubenzuron (117,829 ppm). The LC_{50} values for fenxoycarb, diflubenzuron or ecdysone were much lower and more toxic for 7^{th} instar than for adults (Table 5.6).

Topical Bioassay: The lowest LC_{50} values and fiducial limits within a life stage were recorded for topical application compared to feeding and residual bioassays. Diflubenzuron (0.003 ppm) and fenoxycarb (0.007 ppm) had significantly higher toxicity to 7^{th} instars than did ecdysone (1.1 ppm). The topical toxicity of fenoxycarb (0.07 ppm),

diflubenzuron (0.08 ppm) and ecdysone (0.1 ppm) to pupae were not significantly different. Likewise, adults susceptibility were not significantly different to fenoxycarb (18.0 ppm), diflubenzuron (15.2 ppm) and ecdysone (16.8 ppm).The topical LC_{50} values among life stages revealed that the fenoxycarb, diflubenzuron or ecdysone were more toxic to 7th instar or pupae than to adults among and across IGR's. Fenoxycarb and diflubenzuron were more toxic to 7th instar than pupae whereas ecdysone was more toxic to pupae than to 7th instar (Table 5.7).

Percent Pupation, Mean Pupation Time, Adult survival and Stage Mortality

Feeding Bioassays: Percent pupation attained in 7th instar was significantly different among (ranked in descending order): untreated check (86.5 %), diflubenzuron (67%), ecdysone (55%) and fenoxycarb (33%). Inversely, mean pupation times were statistically different and ranked in ascending order: untreated check (13.7 d), diflubenzuron (15.7 d), ecdysone (19.6 d) and fenoxycarb (26.3 d). Percent mortality in 7th instar were significantly higher in fenoxycarb (67%) followed by ecdysone (45%), diflubenzuron (33%) and untreated check (13.4%) ($P < 0.05$). The diflubenzuron (13%), ecdysone (14.7%) and fenoxycarb (17%) caused similar pupal mortality that was all significantly higher than untreated check. Adult mortality for larvae that fed on diflubenzuron (14%) or ecdysone (10.7%) were significantly higher than for fenoxycarb (4.2%) or check (3.4%). Percent survival of emerged adults were significantly higher in untreated check (80%) followed by diflubenzuron (40%), ecdysone (29%) and then fenoxycarb (12%) ($P < 0.05$) (Table 5.2).

The IGR-fed 1st instars had significantly lower percent pupation than did the untreated check. No significant difference in mean pupation times were observed for 1st

instars among all treatments. The 1st instars treated with diflubenzuron (67.5%), ecdysone (70%) and fenoxycarb (69.4%) were more susceptible in the larval stage than untreated checks (15%) ($P < 0.05$). Percent mortality in the pupal stage was significantly higher in fenoxycarb (10.2%) and diflubenzuron (10%) than in ecdysone (5%) or check (5%). Adults eclosed from pupae had greater survival in untreated check (80%) than in pupae treated with diflubenzuron (22.5%), ecdysone (24.0%) or fenoxycarb (20.4%) ($P < 0.05$) (Table 5.2).

Residual Bioassays: Percent pupation in untreated 7th instars (93%) was significantly higher than larvae exposed to wood shavings treated with diflubenzuron (79.3%), fenoxycarb (78%) or ecdysone (75.4%). Fenoxycarb delayed the pupation as mean pupation times were statistically higher in fenoxycarb (28.4 d) than that in diflubenzuron (20 d), ecdysone (20 d) or untreated check (15 d) ($P < 0.05$). Mortality in larval stage was greater in fenoxycarb, diflubenzuron or ecdysone treated larvae than in untreated check. Pupal mortalities were significantly higher in fenoxycarb (34.3%) treated larvae than both diflubenzuron (17%) and ecdysone (11.6%), which were not significantly different from each other but were significantly higher than untreated check larvae (5.4%) ($P < 0.05$). Fenoxycarb (10.2%) and diflubenzuron (13.7%) treated larvae suffered significantly higher mortality in adult stage than ecdysone (6.2%) or untreated check larvae (5%). Survival of adults were significantly higher in untreated check larvae (80.4%), followed by ecdysone (59%), diflubenzuron (48.5%) and fenoxycarb (34.4%) ($P < 0.05$) (Table 5.4).

Topical Bioassays: Percent pupation was significantly greater in 7th instars in untreated check (90.4%) than larvae topically treated with ecdysone (62.2%), fenoxycarb

(48.7%) or diflubenzuron (42.2%). However, percent pupation in fenoxycarb and diflubenzuron treated larvae were lower than ecdysone although not significantly different from each other. Mean pupation times were significantly higher in fenoxycarb (22.8 d) than diflubenzuron (16.7 d), ecdysone (16.5 d) which were similar but significantly greater than the untreated check (11.2 d) ($P < 0.05$). Higher larval mortality was recorded in 7th instars topically treated with fenoxycarb (52%) or diflubenzuron (58.5%) than ecdysone (36.5%) which was greater than the untreated check (10%). Significantly more larvae treated with fenoxycarb (25.2%) and diflubenzuron (26%) died in the pupal stage than did ecdysone (9%) or untreated check larvae (8%) that had similarly low mortality ($P < 0.05$). Mortality in the adult stage was higher in larvae treated with ecdysone than in untreated check, diflubenzuron or fenoxycarb. Adult survival was greater in untreated check (79.5%) than ecdysone (46%), diflubenzuron (35%) or fenoxycarb treated larvae (22.5%). More adults from ecdysone treated larvae survived than adults from larvae treated with fenoxycarb ($P < 0.05$) (Table 5.4).

Percent Mortality in Fenoxycarb

Feeding Bioassay: The percent mortality of 1st and 7th instars and adults fed the fenoxycarb treated diet increased in dose dependent manner with highest mortality of 100%, 93% and 41%, respectively, to 100 ppm at 50 d post treatment. The 100 ppm at 10 d, 1 ppm and 100 ppm at 20 and 30 d, 10 ppm and 100 ppm at 40 d and 50 d produced significantly higher mortalities in 7th instars than other treatments ($P < 0.05$). Untreated check larvae had significantly lower mortalities than larvae fed on fenoxycarb treated feed on all the days (Table 5.6). Percent mortalities in 1st instar to 10 and 100 ppm fenoxycarb treated diet were significantly higher at 10, 20 and 40 d whereas 100 ppm

produced significantly higher mortalities at 30, 50 and 60 d post treatment ($P < 0.05$). The untreated check larvae had significantly lower mortalities than other concentrations on all days (Table 5.6). Higher mortalities in adults were found when the treatment for larvae was 10 ppm at 10 and 20 d and 100 ppm at 30, 40 and 50 d ($P < 0.05$). The mortalities in untreated check larvae were significantly lower than other concentrations with the exception of 0.01 ppm, where both had similar mortalities on all days (Table 5.8).

Residual Bioassays: There was a concentration dependent relationship in the rate of mortality of 7th instar and adults exposed to fenoxycarb treated wood shavings. The highest mortality of 86% and 48%, occurred in 7th instar and adults, respectively, treated with 1000 ppm at 50 d. There were significantly higher mortalities in 7th instar at 10 d to 10 ppm, at 20 d to 1000 ppm and at 30, 40 and 50 d to 100 and 1000 ppm ($P < 0.05$). The untreated check larvae had significantly lower mortality than all concentrations with the exception to 0.01 at 10 d where both had similar mortality. Percent mortality in adults was significantly higher to 1000 ppm on all days. The untreated check larvae had significantly lower mortalities to all concentrations except to 0.01, 0.1 and 1 ppm at 10, 20 and 30 d, and to 0.01 ppm at 40 and 50 d, where check and these treatments had significantly similar percent mortalities in adults ($P < 0.05$) (Table 5.9).

Topical Bioassays: Percent mortality in 7th instar, pupae and adults topically treated with fenoxycarb increased in dose response fashion with highest mortality of 94, 100 and 67%, respectively, to 100 ppm at 50 d post treatment ($P < 0.05$). Higher mortalities were reported to 1, 10 and 100 ppm at 20 and 30 d, to 10 ppm and 100 ppm at 40 d, and to 100 ppm at 50 d in 7th instar. Significantly higher mortalities were recorded

in pupae treated to 10 and 100 ppm at 10 and 20 d and to 100 pm at 30, 40 and 50 d ($P < 0.05$). Percent mortalities in adults were significantly higher for the 10 and 100 ppm treatments at 10, 20, 30 and 40 d and to 100 ppm at 50 d. Check mortalities were significantly lower than all other concentrations at all days for 7th instar, pupae and adults (Table 5.10).

Percent Mortality in Diflubenzuron

Feeding Bioassay: The highest mortality of 77.5%, 87.5% and 35.0% occured in 7th and 1st instar and adults, respectively, to 100 ppm diflubenzuron treated diet at 50 d. The 7th instars were significantly more susceptible to 10 and 100 ppm at 40 and 50 d, and to 10 ppm at 30 d with the exception of 100 ppm. Concentrations of 10 ppm and 100 ppm at 30 d produced significantly similar percent mortality of 7th instars ($P < 0.05$). A concentration of 100 ppm diflubenzuron was significantly more toxic to 1st instars than other treatments at 20, 30, 40 and 50 d. The 7th and 1st instars in the untreated check had statistically lower mortality than other concentrations at all the days. Adults were significantly more susceptible to 10 and 100 ppm at 20 d, to 100 ppm at 30 d, and to 10 and 100 ppm at 40 and 50 d ($P < 0.05$). Untreated check larvae had statistically lower mortality than other treatments with the exception of 0.01 ppm at 40 d and 50 d where check and 0.01 ppm had similar mortalities ($P < 0.05$) (Table 5.11).

Residual bioassays: Diflubenzuron treated wood shavings exposed to 7th instar and adults responded in a concentration dependent manner resulting in the highest mortalities of 74% and 35.6% recorded for 1000 pm at 50 d post treatment. Significantly higher numbers of 7th instars were killed by 1000 ppm than all other concentrations except by 100 ppm at 50 d when 100 ppm and 1000 ppm killed similar number of larvae

($P < 0.05$). Untreated check larvae had statistically lower mortality to all other concentrations at 10 and 50 d but had had similar mortalities as 0.01 ppm at 20, 30 and 40 d. Adults had significantly higher mortalities at 10 and 20 d, whereas 100 and 1000 ppm produced the similar results at 30, 40 and 50 d ($P < 0.05$). All the concentrations had statistically higher mortality, with the exception of 0.01 at 30, 40 and 50 d, than that observed in untreated check (Table 5.12).

Topical Bioassays: The 7th instars topically treated to 100 ppm diflubenzuron had significantly higher mortality at 20 d. However, 10 ppm and 100 ppm killed significantly more larvae at 30, 40 and 50 d ($P < 0.05$). Mortality in untreated check larvae was lower at 20, 30, 40 and 50 d than all other concentrations. 1, 10 and 100 ppm caused significantly higher toxicity to pupae at 10 d, whereas 10 and 100 ppm caused more mortality at 20, 30, 40 and 50 d ($P < 0.05$). Adult treated to 100 ppm suffered significantly higher mortality at 10, 30, 40 and 50 d. Pupae and adults subjected to check treatment had significantly less mortality than all other concentrations on all days (Table 5.13).

Percent Mortality in Ecdysone

Feeding Bioassays: When fed the ecdysone treated diet, mortality in 7th and 1st instar and adults increased in concentration dependent manner with highest mortality of 92.5, 92.5 and 21%, respectively, to 100 ppm at 50 d post treatment. Percent mortality in 7th instar to 100 ppm was significantly higher at 30, 40 and 50 d. Untreated check larvae had statistically lower mortality, with the exception of 0.01 ppm at 10, 20 and 30 d, than all other concentrations at all days. First instars fed 100 ppm ecdysone treated diet exhibited significantly higher mortalities at 10, 20 and 30 d, and at 40 d, 10 and 100 ppm

killed significantly more larvae ($P < 0.05$). At 50 d, 1 ppm in addition to 10 and 100 ppm caused significantly higher mortalities than all other concentrations ($P < 0.05$). Check mortalities were significantly lower than all other concentrations for all the days. There was no significant effect of concentration on adult mortality at 10, 20 and 30 d ($P > 0.05$). Adults fed 100 ppm of the ecdysone treated diet suffered significantly higher mortality than adults fed 0.1, 0.01 ppm diets. Check mortalities were significantly lower than all other concentrations at 40 and 50 d (Table 5.14).

Residual Bioassays: The highest concentration of 1000 ppm attained the maximum mortality of 65% and 40% at 50 d in 7^{th} instar and adults, respectively, when exposed to ecdysone treated wood shavings. Untreated check larvae had significantly lower mortality, with the exception to 0.01 at 20, 30, 40 and 50 d post treatment, than all other concentrations on all days. Adults exposed to 100 and 1000 ppm diflubenzuron suffered significantly higher mortalities than all other concentrations on all days ($P < 0.05$). Untreated check larvae had statistically lower mortality than other concentrations on all days but 0.01, 0.1 ppm and untreated check had similar percent mortalities at 40 and 50 d (Table 5.15).

Topical Bioassays: There was a dose dependent relationship in the rate of mortality of 7^{th} instar, pupae and adults topically treated with ecdysone with highest mortality of 80, 88 and 61%, respectively, to 100 ppm at 50 d post treatment. At 10 d, 10 and 100 ppm topically applied to 7^{th} instars caused significantly higher mortality and after that 100 ppm at 20, 30, 40 and 50 d, produced significantly higher mortality than all other concentrations ($P < 0.05$). Untreated check, 0.01 ppm and 0.1 ppm had similar mortalities at 10 d. However untreated check larvae had statistically lower mortality than

all other concentrations at 20, 30, 40 and 50 d. Pupae treated topically with 1, 10 and 100 ppm ecdysone had significantly higher percent mortality at 10 d, whereas 100 ppm caused significantly higher mortality at 20, 30, 40 and 50 d with the exception to 10 ppm at 30, 40 and 50 d ($P < 0.05$). Adults topically treated with 10 and 100 ppm ecdysone had significantly higher mortality on all days. Untreated check larvae or adults had statistically lower mortality than other concentrations on all days (Table 5.16).

DISCUSSION

The lower IGR concentrations caused larvae to gain more weight in the first few days and then started gaining weight faster at higher concentrations. The higher IGR concentrations inhibited weight gain initially but prolonged the larval development times and elevated the weights gains later. Similarly, *T. confusum* larval period was found to increase with increased dose of fenoxycarb and methoprene (Smet et al. 1989). Pupation was inhibited in fenoxycarb treated 7th instars that continued to molt and gain weight. The same trend was reported in *T. castaneum*, where JHAs at the mature larval stage inhibited pupation of the treated larvae, which continued to molt and produce 'giant' or 'supernumerary larvae' (Pallos et al. 1971).

The mean pupation time in fenoxycarb treated 7th instars lesser mealworm was higher than the untreated check, diflubenzuron or ecdysone in feeding, residual and topical bioassays. The heavier larvae resulted in deformed larvae, abnormal pupae or intermediate larval-pupal and pupal-adult forms. Prolonged larval development periods up to 120 and 150 d were reported, when mature instars of *Tribolium* species were treated with fenoxycarb and hydroprene, respectively, whereas, untreated larvae pupated in 25 d (Loschiavo 1975, 1976). Last instars of *T. confusum* and *T. granarium* treated with JH

resulted in either abnormal pupae or intermediate larval-pupal and pupal-adult forms (El-Sayed 1987). Fenoxycarb exhibited more mortality in the pupal stage when 7th instars were exposed to fenoxycarb treated wood shaving than other IGRs.

There was reduced pupation and higher mortality in larval and pupal stages when 7th instars were treated topically with fenoxycarb or diflubenzuron. Ecdysone topically treated 7th instars had more mortality in the adult stage. The diflubenzuron topically treated against 2nd and 4th instars of the twenty eight spot lady bird, *Henosepilachna vigintioctopunctata* (F.), resulted in ecdysial failure (Rao et al. 1992). Diflubenzuron inhibited pupation totally when incorporated into the diet of *T. confusum* at 0.4 ppm (Ishaaya et al. 1981).

In feeding bioassays, fenoxycarb was more toxic to 7th instars than 1st instars or adults, whereas diflubenzuron was more toxic to 1st instars than 7th instars or adults. Diflubenzuron was more effective in inhibiting the growth and development of 1st instars of *T. castaneum* than the 4th instars (Ishaaya and Ascher 1977). The ecdysone was more toxic to 7th instars in feeding bioassays than was diflubenzuron, whereas diflubenzuron was more effective than ecdysone in residual bioassays. The adults were less susceptible to all the IGRs than any other life stage in feeding, residual and topical bioassays. The lower concentrations were also less toxic to adults initially in residual and feeding bioassays, whereas in topical bioassays the lower concentrations were significantly more toxic to adults when compared to untreated checks.

Fenoxycarb caused significant toxicity to 7th and 1st instars lesser mealworms in feeding bioassays causing 94% and 100% mortality, respectively. Pupae suffered 83% mortality at 10 d post treatment and 100% at 50 d post treatment. Also more than 87%

mortality was achieved in 7th instars when they were treated with the highest concentration of fenoxycarb in residual or topical bioassays. The 7th instars fed on fenoxycarb-treated diet suffered higher mortality in the larval stage and pupation was inhibited. Percentage pupation is normally reduced in IGR-treated larvae due either to larval death during metamorphosis or inhibition of pupation due to a prolonged juvenile period, when fed, injected or in contact with CSIs or JHAs (Loschiavo 1976).

Ecdysone caused 70% mortality of 1st instar lesser mealworms 10 d post treatment in feeding bioassays. Topically applied ecdysone resulted in mortality of 75% and 89% at 10 and 50 d in pupae, and adult mortality of 47% at 10 d. Tebufenozide (RH-5992), an agonist of 20E was tested topically and through the diet on 1st and 6th instars of African armyworm, *Spodoptera exempta* (Walker). It induced the premature larval molt within 24 h of treatment along with inhibition of weight gain and food intake, as well as loss of hemolymph and abnormal pupation (Smagghe and Degheele 1994).

The slope of the concentration-mortality line gives information about the phenotypic variation among the population including genetic and environmental variation. The probit analysis for all the bioassays yielded slope values of less than one suggesting the increased variation within the lesser mealworm population in response to the IGRs used (Plapp 1979).

The results of this study show that fenoxycarb, diflubenzuron and ecdysone exhibit feeding/ systemic (insecticide-treated diet), residual and topical toxicity to lesser mealworm. All three bioassay methods produced usable concentration-response curves and may be used for detecting changes in IGR susceptibility in lesser mealworm populations. Nevertheless, the bioassay using insecticide-treated diets produced tighter

confidence intervals and steeper slopes and generally a better fit of the data for the 1st and 7th instars. Residual contact also seems to be a promising testing method for 7th instars. However, residual bioassay tests provide data that more closely simulates the exposure of beetles to an insecticide on a substrate in field applications. This method can be used to detect IGR's susceptibility to later instars of lesser mealworm. But this method was not effective against adults and is not easy to use for 1st instars due to their small size. Adults responded to topical bioassay better than feeding or residual contact. Therefore, these three different bioassays can be used for different stages for future research and to determine temporal changes in susceptibility of local populations of lesser mealworms to various IGRs.

Data generated from these experiments comprise initial efforts in establishing baseline susceptibility to IGR's that can be used as reference points for future concentration-response bioassays for lesser mealworm. Although, all three IGRs mainly fenoxycarb prolonged the larval development but they also caused significant mortality of early instars through systemic feeding, and late instars and adults through feeding, residual contact or direct exposure. The spraying of fenoxycarb, diflubenzuron and ecdysone in poultry house during flock free period can kill mature larval stages as well as reduce progeny due to inability of mature larvae to pupate or pupae to transform into normal adult forms. Higher susceptibility and an insignificant effect on larval period of 1st instars suggest the potential of all three IGRs in reducing the numbers of early instars or inhibiting their development to complete life cycle. Moreover, diflubenzuron or ecdysone prolongs the larval development to a smaller extent as compared to fenoxycarb. The benefits of IGRs seem to outweigh their limitations and might evolve as an excellent

option in management of lesser mealworm where standard insecticides no longer provide adequate control. The use of selective pesticides is a major consideration in developing an integrated control program for lesser mealworm. The IGRs affect insect endocrine and energy production systems and their incorporation in established lesser mealworm management program will help to overcome resistance mechanisms for other conventional insecticides. The results from this laboratory study will help the insecticide manufacturing companies to register their product for the lesser mealworm if they are not currently on the insecticide label.

There have not been many published reports on the performance of IGRs on insecticide-resistant populations. Although, fenoxycarb was found to be more effective on a malathion resistant strain of *T. castaneum* than the malathion susceptible strains confirming no evidence of cross-tolerance to fenoxycarb in insecticide resistant strains (Thind and Edwards 1986). Future studies will include testing of IGRs on several insecticide-resistant lesser mealworm populations in laboratory bioassays. The combination of IGRs and recommended insecticides should be tested to find if both have any synergistic effect greater than either alone on lesser mealworm. Also, adults found to be less susceptible to IGRs may be controlled with an insecticide-IGR combination.

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Table 5.1 Mean weight gains (± SE) of 7th and 1st instar lesser mealworms after exposure to fenoxycarb-treated diet in feeding bioassays.

				Weight Gain (mg)			
Method	Stage	Conc.(ppm)	Initial Wt. (mg)	10 d	20 d	30 d	40 d
Feeding	7th	0.01	15.16 ± 0.17	3.61 ± 0.24b[a]	4.21 ± 0.31c	1.12 ± 0.18d	0.90 ± 0.18d
Feeding	7th	0.1	15.36 ± 0.66	4.80 ± 0.37a	8.80 ± 0.22a	4.90 ± 0.26b	2.72 ± 0.44c
Feeding	7th	1	15.26 ± 0.14	3.48 ± 0.28b	9.08 ± 0.19a	10.38 ± 0.10a	7.94 ± 0.21b
Feeding	7th	10	15.65 ± 0.15	1.62 ± 0.28c	7.53 ± 0.16b	9.98 ± 0.16a	13.66 ± 0.13a
Feeding	7th	100	15.54 ± 0.20	0.18 ± 0.15d	0.56 ± 0.15d	1.86 ± 0.11c	8.70 ± 0.19b
Feeding	7th	0 (check)	15.42 ± 0.18	0.58 ± 0.16d			

			Weight Gain (mg)				
	Stage	Conc.(ppm)	10 d	20 d	30 d	40 d	50 d
Feeding	1st	0.01	2.66 ± 0.18b	5.00 ± 0.31b	9.20 ± 0.38c	14.14 ± 0.38d	17.00 ± 0.30d
Feeding	1st	0.1	3.23 ± 0.21a	5.91 ± 0.37a	10.53 ± 0.49b	15.74 ± 0.34c	18.80 ± 0.32c
Feeding	1st	1	1.68 ± 0.12d	4.83 ± 0.15b	12.32 ± 0.39a	17.02 ± 0.39b	21.13 ± 0.25b
Feeding	1st	10	1.85 ± 0.13cd	4.76 ± 0.22b	11.90 ± 0.45a	18.18 ± 0.31a	25.14 ± 0.51a
Feeding	1st	100	1.84 ± 0.11cd	3.51 ± 0.18c	6.50 ± 0.27d	9.14 ± 0.60e	16.00 ± 0.57d
Feeding	1st	0 (check)	2.17 ± 0.10c	4.62 ± 0.15b	7.65 ± 0.21d	14.70 ± 0.12d	

[a] Means in same column with a similar letter are not significantly different ($P > 0.05$, Tukey's HSD).

Table 5.2 Stage specific percent mortality, percent pupation, adult survival and mean pupation time of 7^{th} and 1^{st} instars after exposure to insect growth regulator (IGR)-treated diet in feeding bioassays.

Method	IGR	Stage	N	% Dead Larvae	% Dead Pupae	% Dead Adults	% Alive Adults	Total % Pupated	Mean Pupation Time
Feeding	FXB	7^{th}	10	67.0 ± 3.3a[a]	17.0 ± 2.0a	4.2 ± 1.2b	12.0 ± 1.4d	33.0 ± 3.3d	26.3 ± 0.45a[a]
Feeding	ECD	7^{th}	8	44.7 ± 3.8b	14.7 ± 1.0a	10.7 ± 0.9a	29.0 ± 3.2c	55.2 ± 3.8c	19.6 ± 0.88b
Feeding	DFB	7^{th}	8	33.0 ± 1.9c	13.0 ± 1.5a	14.0 ± 1.6a	40.0 ± 2.9b	67.0 ± 1.9b	15.7 ± 0.37c
Feeding	0 (check)	7^{th}	8	13.4 ± 1.4d	4.2 ± 0.9b	3.4 ± 0.9b	80.0 ± 1.3a	86.5 ± 1.4a	13.7 ± 0.31d
Feeding	FXB	1^{st}	10	69.4 ± 3.0a	10.2 ± 1.2a	0b	20.4 ± 2.4b	30.6 ± 3.0b	43.0 ± 4.70a
Feeding	ECD	1^{st}	8	70.0 ± 3.3a	4.8 ± 0.8b	1.2 ± 0.5a	24.0 ± 3.5b	30.0 ± 3.3b	46.0 ± 5.50a
Feeding	DFB	1^{st}	8	67.5 ± 2.6a	10.0 ± 1.8a	0b	22.5 ± 1.7b	32.5 ± 2.6b	45.0 ± 6.60a
Feeding	0 (check)	1^{st}	8	15.0 ± 1.2b	5.0 ± 0.9b	0b	80.0 ± 5.7a	85.0 ± 1.2a	41.0 ± 3.80a

[a] For each stage, means in the same column with a similar letter are not significantly different ($P > 0.05$, Tukey's HSD). Percentages (± SE) were transformed [arcsin(sqrt)] before analysis, but actual values are presented. FXB: fenoxycarb; DFB: diflubenzuron; ECD: ecdysone; and 0: check.

Table 5.3 Mean weight gains (± SE) of 7th instar lesser mealworms exposed to fenoxycarb either in residual (treated wood shavings) or topical bioassays.

				Weight Gain (mg)			
Method	Stage	Conc. (ppm)	Initial Wt.(mg)	10 d	20 d	30 d	40 d
Residual	7th	0.01	16.69 ± 0.13	3.33 ± 0.24e[a]	5.88 ± 0.57c		
Residual	7th	0.1	16.08 ± 0.14	3.85 ± 0.25de	9.69 ± 0.40b	3.82 ± 0.45a	
Residual	7th	1	16.34 ± 0.15	4.44 ± 0.23c	9.46 ± 0.36b	3.06 ± 1.01a	
Residual	7th	10	16.71 ± 0.14	4.89 ± 0.16b	9.29 ± 0.46b		
Residual	7th	100	16.81 ± 0.09	5.91 ± 0.16a	10.90 ± 0.42a		
Residual	7th	1000	16.57 ± 0.12	5.65 ± 0.24ab			
Residual	7th	0 (check)	16.68 ± 0.13	4.00 ± 0.20cd			
Topical	7th	0.01	15.46 ± 0.12	1.39 ± 0.41a	2.16 ± 0.32c		
Topical	7th	0.1	15.70 ± 0.11	-0.35 ± 0.13b	3.72 ± 0.22a	10.74 ± 0.93b	3.96 ± 0.47a
Topical	7th	1	15.60 ± 0.10	-0.71 ± 0.27b	4.11 ± 0.45a	10.96 ± 0.88b	5.73 ± 0.24a
Topical	7th	10	15.70 ± 0.10	-1.34 ± 0.14b	2.80 ± 0.64b	13.96 ± 0.55a	
Topical	7th	100	15.50 ± 0.11	-1.33 ± 0.23b	2.30 ± 0.22bc	10.15 ± 0.32b	
Topical	7th	0 (check)	15.20 ± 0.25	1.83 ± 0.19a			

[a] Means in same column with a similar letter(s) are not significantly different ($P > 0.05$, Tukey's HSD).

Table 5.4 Stage specific mortality, percent pupation, mean pupation time and adult survival in 7th instars exposed to insect growth regulators (IGR) in either residual or topical bioassays.

Method	IGR	Stage	N	% Dead Larvae	% Dead Pupae	% Dead Adults	% Alive Adults	Total % Pupated	Mean Pupation Time
Residual	FXB	7th	8	22.5 ± 1.6a [a]	34.3 ± 2.3a	10.2 ± 1.2a	34.3 ± 2.8d	77.9 ± 1.6b	28.4 ± 0.64a[a]
Residual	ECD	7th	8	23.7 ± 2.1a	11.6 ± 0.9b	6.2 ± 0.7b	59.1 ± 2.0b	75.4 ± 2.1b	20.0 ± 0.43b
Residual	DFB	7th	8	20.6 ± 2.3a	17.0 ± 1.9b	13.7 ± 1.0a	48.5 ± 2.3c	79.3 ± 2.3b	19.8 ± 0.55b
Residual	0 (check)	7th	8	5.4 ± 1.0b	8.3 ± 0.7c	5.0 ± 1.0b	80.4 ± 1.2a	93.3 ± 1.1a	12.0 ± 0.37c
Topical	FXB	7th	8	52.0 ± 2.4a	25.2 ± 1.7a	0.3 ± 0.1b	22.5 ± 2.3c	48.7 ± 2.4c	22.8 ± 0.39a
Topical	ECD	7th	8	36.5 ± 3.0b	9.0 ± 0.8b	8.0 ± 0.6a	46.0 ± 2.7b	62.2 ± 3.1b	16.5 ± 0.31b
Topical	DFB	7th	8	58.5 ± 3.7a	26.0 ± 2.0a	0.7 ± 0.4b	34.7 ± 5.5bc	42.2 ± 3.6c	16.7 ± 0.61b
Topical	0 (check)	7th	8	10.0 ± 1.3c	7.9 ± 1.5b	2.5 ± 0.9b	79.5 ± 1.2a	90.4 ± 1.4a	11.2 ± 0.27c

[a] For each bioassay, means in the same column with a similar letter are not significantly different ($P > 0.05$, Tukey's HSD). Percentages (± SE) were transformed [arcsin(sqrt)] before analysis, but actual values are presented. FXB: fenoxycarb; DFB: diflubenzuron; ECD: ecdysone; and 0: check.

Table 5.5 Slope, lethal concentrations (LC_{50} or LC_{90}) and 95% fiducial limits (FL) for insect growth regulator (IGR) to field-collected 7th and 1st instars and adult lesser mealworms exposed to treated diet in feeding bioassays.

IGR	Method	Stage	Slope (±SE)	LC_{50} (95% FL)	LC_{90} (95% FL)	χ^2	*P*	DF
FXB	Feeding	7th	0.45 ± 0.05	0.065 (0.024 - 0.11)f[a]	42.06 (15.33 - 182.22)	42.5	0.7	48
DFB	Feeding	7th	0.36 ± 0.05	2.13 (0.83 - 5.82)d	7307 (893.94 - 304030)	33.8	0.6	38
ECD	Feeding	7th	0.50 ± 0.05	0.257 (0.11 - 0.51)e	90.59 (30.79 - 449.45)	22.1	0.9	38
FXB	Feeding	1st	0.72 ± 0.08	0.144 (0.067 - 0.25)e	8.58 (4.50 - 21.07)	33.6	0.8	48
DFB	Feeding	1st	0.33 ± 0.05	0.062 (0.012 - 0.18)e	385.05 (69.15 - 8203)	18.0	0.9	38
ECD	Feeding	1st	0.54 ± 0.06	0.105 (0.045 - 0.20)e	23.47 (9.52 - 83.95)	40.4	0.3	38
FXB	Feeding	Adult	0.40 ± 0.09	1426 (233.71 - 154906)c	2127372 (37797 - $1.8x10^{10}$)	12.5	1.0	48
DFB	Feeding	Adult	0.30 ± 0.10	11660 (461.05 - $2.2x\ 10^{11}$)b	$2.1x10^{8}$ (177287 - $2.1x10^{25}$)	12.5	0.9	38
ECD	Feeding	Adult	0.27 ± 0.13	982937 (2530 - $5.2x10^{10}$)a	$90x10^{8}$ ($4.8\ x10^{10}$ - $6.7x10^{37}$)	20.6	1.0	38

[a] Means in same column with a similar letter are not significantly different. FXB: fenoxycarb; DFB: diflubenzuron, ECD: ecdysone.

Table 5.6 Slope, lethal concentrations (LC_{50} or LC_{90}) and 95% fiducial limits (FL) for insect growth regulator (IGR) in field-collected 7th instar and adult lesser mealworms exposed to treated wood shavings in residual bioassays.

IGR	Method	Stage	Slope (±SE)	LC_{50} (95% FL)	LC_{90} (95% FL)	χ^2	*P*	DF
FXB	Residual	7th	0.33 ± 0.04	0.20 (0.054 - 0.53)f[a]	1477 (315.55 - 16202)	25.8	0.9	46
DFB	Residual	7th	0.25 ± 0.04	7.11 (2.11 - 25.64)e	842379 (39050 - 2.5 x10^{8})	10.2	1.0	46
ECD	Residual	7th	0.23 ± 0.04	112.41 (26.53 - 1107)d	42012772 (563120 - 3.4 x10^{11})	13.0	1.0	46
FXB	Residual	Adult	0.38 ± 0.07	4350 (901.86 - 109869)c	10325030 (284512 - 4.1 x10^{10})	17.0	1.0	46
DFB	Residual	Adult	0.28 ± 0.07	117829 (4581 - 4.3 x10^{9})a	4.4 x10^{8} (4864912 - 6.9 x10^{19})	11.4	1.0	44
ECD	Residual	Adult	0.35 ± 0.08	12201 (1649 - 1856511)b	57708795 (623966 - 1.5 x10^{13})	9.8	1.0	46

[a] Means in same column with a similar letter are not significantly different. FXB: fenoxycarb; DFB: diflubenzuron, ECD: ecdysone.

Table 5.7 Slope, lethal concentrations (LC_{50} or LC_{90}) and 95% fiducial limits (FL) for insect growth regulators (IGR) in field-collected larval, pupal and adult lesser mealworms in topical bioassays.

IGR	Method	Stage	Slope (±SE)	LC_{50} (95% FL)	LC_{90} (95% FL)	χ^2	*P*	DF
FXB	Topical	7^{th}	0.35 ± 0.05	0.0067 (0.00062 - 0.020)d[a]	32.39 (8.24 - 345.83)	11.7	1.0	38
DFB	Topical	7^{th}	0.48 ± 0.07	0.0031 (0.00039 - 0.01)d	1.49 (0.60 - 5.70)	18.4	0.9	38
ECD	Topical	7^{th}	0.34 ± 0.05	1.11 (0.389 - 3.06)b	6996 (750.75 - 435143)	11.3	1.0	38
FXB	Topical	Pupa	0.61 ± 0.08	0.072 (0.027 - 0.151)c	9.20 (3.64 - 37.16)	12.0	0.9	28
DFB	Topical	Pupa	0.47 ± 0.06	0.080 (0.023 - 0.198)c	42.96 (12.68 - 306.31)	10.4	0.9	28
ECD	Topical	Pupa	0.43 ± 0.06	0.10 (0.026 - 0.262)c	100.35 (24.70 - 1062)	8.9	0.9	28
FXB	Topical	Adult	0.41 ± 0.07	18.01 (6.29 - 84.97)a	22414 (1843 - 3885212)	9.0	0.9	28
DFB	Topical	Adult	0.40 ± 0.07	15.18(5.27 - 69.83)a	23098 (1884 - 3636354)	11.5	0.9	28
ECD	Topical	Adult	0.36 ± 0.06	16.84 (5.19 - 102.70)a	63654 (3348 - 34927190)	8.9	0.9	28

[a] Means in same column with a similar letter are not significantly different. FXB: fenoxycarb; DFB: diflubenzuron, ECD: ecdysone.

Table 5.8 Percent mortality (± SE) of 7th and 1st instar and adult lesser mealworms to fenoxycarb-treated diet in feeding bioassays.

			Percent Mortality					
Method	Stage	Conc. (ppm)	10 d	20 d	30 d	40 d	50 d	60 d
Feeding	7th	0.01	25.0 ± 5.2bc [a]	38.0 ± 6.1b	42.0 ± 6.8b	47.0 ± 4.5c	51.1 ± 2.6c[a]	
Feeding	7th	0.1	22.0 ± 3.2cd	47.0 ± 3.6b	56.0 ± 2.6b	64.0 ± 4.7bc	70.0 ± 2.9b	
Feeding	7th	1	35.5 ± 5.6b	66.6 ± 5.8a	73.0 ± 5.1a	75.0 ± 3.0b	79.0 ± 3.6b	
Feeding	7th	10	34.5 ± 1.6b	50.0 ± 3.6b	56.0 ± 4.2b	70.0 ± 4.7b	88.1 ± 3.3a	
Feeding	7th	100	48.3 ± 6.4a	63.0 ± 3.6a	82.0 ± 1.3a	90.0 ± 2.6a	94.0 ± 3.0a	
Feeding	7th	0 (check)	10.0 ± 1.2d	10.5 ± 1.6c	15.0 ± 1.6c	15.5 ± 1.6d	20.0 ± 1.6d	
Feeding	1st	0.01	13.0 ± 1.5c	28.0 ± 1.3c	38.0 ± 3.0d	40.0 ± 3.3c	42.6 ± 2.9d	61.6 ± 5.0d
Feeding	1st	0.1	27.0 ± 1.5b	40.0 ± 2.6b	52.0 ± 1.3c	52.0 ± 1.3b	65.6 ± 1.5c	68.0 ± 4.1d
Feeding	1st	1	33.0 ± 1.5b	44.0 ± 2.6b	47.5 ± 3.6c	53.5 ± 2.6b	71.0 ± 3.7c	79.0 ± 3.4c
Feeding	1st	10	57.0 ± 4.4a	67.0 ± 1.5a	72.0 ± 1.3b	78.0 ± 1.3a	85.6 ± 1.6b	90.5 ± 0.1b
Feeding	1st	100	56.0 ± 1.6a	70.5 ± 2.1a	79.0 ± 2.3a	82.0 ± 1.3a	100.0 ± 0a	100.0 ± 0a
Feeding	1st	0 (check)	10.0 ± 0.1c	15.0 ± 1.6d	15.5 ± 1.6e	17.0 ± 1.7d	20.0 ± 1.6e	20.0 ± 1.6e
Feeding	Adult	0.01	0c	2.5 ± 1.3d	5.5 ± 1.6d	11.5 ± 2.7de	11.5 ± 2.7de	
Feeding	Adult	0.1	4.5 ± 1.6b	6.0 ± 1.6c	10.0 ± 1.2c	15.0 ± 1.6cd	15.0 ± 1.6cd	
Feeding	Adult	1	5.0 ± 1.6b	10.0 ± 1.1b	12.0 ± 1.3c	19.0 ± 1.2c	19.0 ± 1.2c	
Feeding	Adult	10	9.5 ± 1.8a	17.0 ± 1.5a	21.0 ± 1.8b	27.0 ± 1.5b	27.0 ± 1.5b	
Feeding	Adult	100	9.0 ± 1.8a	13.0 ± 1.5b	29.0 ± 2.7a	39.0 ± 2.3a	41.0 ± 2.45a	
Feeding	Adult	0 (check)	0c	0d	3.0 ± 1.5d	9.0 ± 1.2e	10.0 ± 1.3e	

[a] Values for each stage in the same column with a similar letter(s) are not significantly different (P > 0.05, Tukey's HSD). Percentages were transformed [arcsin(sqrt)] before analysis, but actual values are presented.

Table 5.9 Percent mortality (± SE) of 7th instars and adults lesser mealworm exposed to wood shavings treated with fenoxycarb in residual bioassays.

			Percent Mortality				
Method	Stage	Conc.	10 d	20 d	30 d	40 d	50 d
Residual	7th	0.01	8.7 ± 1.25c[a]	23.7 ± 3.3d	27.5 ± 2.5c	31.2 ± 1.2e	40.0 ± 3.3d[a]
Residual	7th	0.1	18.7 ± 3.5b	33.7 ± 6.5d	36.2 ± 7.5c	41.2 ± 6.1d	49.0 ± 6.4d
Residual	7th	1	7.5 ± 1.6c	31.2 ± 1.2d	38.7 ± 2.9c	53.7 ± 4.2c	64.0 ± 3.3c
Residual	7th	10	33.7 ± 2.6a	47.5 ± 2.5c	58.7 ± 2.2b	67.5 ± 1.6b	76.0 ± 2.6b
Residual	7th	100	22.5 ± 2.5b	58.7 ± 4.4b	68.7 ± 2.9ab	80.0 ± 1.8a	87.5 ± 2.5a
Residual	7th	1000	25.0 ± 4.6b	73.7 ± 4.2a	77.5 ± 4.5a	84.0 ± 2.6a	86.0 ± 2.6a
Residual	7th	0 (check)	5.0 ± 1.8c	10.0 ± 2.7e	10.0 ± 2.7d	15.0 ± 3.3f	17.5 ± 1.6e
Residual	Adult	0.01	0c	2.5 ± 1.6c	5.0 ± 1.8d	10.0 ± 1.3e	10.0 ± 1.3e
Residual	Adult	0.1	0c	5.0 ± 1.8c	7.5 ± 1.6d	12.5 ± 1.6de	12.5 ± 1.6de
Residual	Adult	1	2.5 ± 1.6bc	5.0 ± 1.8c	6.2 ± 1.8d	20.0 ± 3.2cd	20.0 ± 3.2cd
Residual	Adult	10	5.0 ± 1.8b	10.0 ± 1.2b	15.0 ± 3.2c	25.0 ± 2.6bc	25.0 ± 2.6bc
Residual	Adult	100	5.0 ± 1.8b	10.0 ± 1.3b	26.2 ± 1.8b	29.3 ± 3.0b	31.2 ± 4.0b
Residual	Adult	1000	11.2 ± 1.2a	16.2 ± 2.6a	36.2 ± 1.8a	45.5 ± 4.3a	47.5 ± 4.5a
Residual	Adult	0 (check)	0c	2.5 ± 0.11c	5.0 ± 1.8d	6.2 ± 1.8e	6.2 ± 1.8e

[a] For each stage, means in the same column with a similar letter(s) are not significantly different (P > 0.05, Tukey's HSD). Percentages were transformed [arcsin(sqrt)] before analysis, but actual values are presented.

Table 5.10 Percent mortality (± SE) of 7th instars, pupae and adult lesser mealworms treated with fenoxycarb in topical bioassays.

Method	Stage	Conc. (ppm)	Percent Mortality 10 d	20 d	30 d	40 d	50 d
Topical	7th	0.01	23.7 ± 1.8b[a]	40.0 ± 3.2b	47.5 ± 1.6b	53.7 ± 1.8c	56.2 ± 1.8d[a]
Topical	7th	0.1	22.5 ± 3.6bc	43.7 ± 8.2b	53.7 ± 4.9b	57.0 ± 3.6c	70.0 ± 3.2c
Topical	7th	1	18.7 ± 2.9c	60.0 ± 5.5a	70.0 ± 3.2a	70.0 ± 3.2b	81.2 ± 2.9b
Topical	7th	10	30.0 ± 3.2a	62.5 ± 3.6a	72.5 ± 3.6a	79.2 ± 1.8a	86.2 ± 1.8b
Topical	7th	100	32.5 ± 1.6a	65.0 ± 3.2a	75.0 ± 4.9a	81.2 ± 2.9a	93.9 ± 1.8a
Topical	7th	0 (check)	3.7 ± 1.8d	10.0 ± 2.2c	13.7 ± 1.8c	17.5 ± 1.6d	17.5 ± 1.6e
Topical	Pupa	0.01	31.6 ± 3.0c	36.6 ± 3.3 c	43.3 ± 3.3e	43.3 ± 3.3e	43.3 ± 3.3e
Topical	Pupa	0.1	31.6 ± 1.6c	40.0 ± 5.1c	55.0 ± 4.2d	55.0 ± 4.2d	55.0 ± 4.2d
Topical	Pupa	1	50.0 ± 3.6b	60.0 ± 2.5b	71.6 ± 1.6c	71.6 ± 1.6c	71.6 ± 1.6c
Topical	Pupa	10	81.6 ± 3.0a	83.3 ± 2.1a	91.6 ± 3.0b	91.6 ± 3.0b	91.6 ± 3.0b
Topical	Pupa	100	83.3 ± 3.3a	90.0 ± 2.5a	100.0 ± 0a	100.0 ± 0a	100.0 ± 0a
Topical	Pupa	0 (check)	10.0 ± 2.5d	13.3 ± 2.1d	13.3 ± 2.1d	13.3 ± 2.1d	15.0 ± 2.2e
Topical	Adult	0.01	8.3 ± 2.1b	12.0 ± 3.6c	16.0 ± 3.6c	20.0 ± 3.6c	22.0 ± 3.6d
Topical	Adult	0.1	10.0 ± 3.6b	12.2 ± 3.6c	20.0 ± 3.6c	20.0 ± 3.6c	25.0 ± 3.4d
Topical	Adult	1	14.0 ± 3.6b	21.6 ± 1.6b	31.6 ± 1.6b	31.6 ± 1.6b	35.0 ± 3.4c
Topical	Adult	10	28.0 ± 3.6a	36.3 ± 3.07a	38.6 ± 3.07ab	41.6 ± 3.07ab	52.0 ± 3.0b
Topical	Adult	100	31.6 ± 4.7a	40.0 ± 5.7a	50.0 ± 5.7a	50.0 ± 5.7a	67.0 ± 3.3a
Topical	Adult	0 (check)	3.3 ± 2.1c	6.6 ± 2.1d	6.6 ± 2.1d	6.6 ± 2.1d	10.0 ± 2.6e

[a] For each stage, means in the same column with a similar letter are not significantly different (P > 0.05, Tukey's HSD). Percentages were transformed [arcsin(sqrt)] before analysis, but actual values are presented.

Table 5.11 Percent mortality (± SE) of 7th and 1st instars and adults lesser mealworm exposed to diflubenzuron-treated diet in feeding bioassays.

			Percent Mortality					
Method	Stage	Conc. (ppm)	10 d	20 d	30 d	40 d	50 d	60 d
Feeding	7th	0.01	22.5 ± 3.1bc [a]	27.5 ± 3.1c	30.0 ± 2.7d	32.5 ± 1.6c	35.0 ± 1.9c[a]	
Feeding	7th	0.1	27.5 ± 4.1abc	45.0 ± 5.7ab	57.5 ± 3.1bc	60.0 ± 3.8b	60.0 ± 3.8b	
Feeding	7th	1	17.5 ± 4.1c	35.0 ± 1.9bc	50.0 ± 4.6c	52.5 ± 4.9b	52.5 ± 4.9b	
Feeding	7th	10	35.0 ± 4.2a	50.0 ± 7a	67.5 ± 3.1a	75.0 ± 4.2a	75.0 ± 4.2a	
Feeding	7th	100	32.5 ± 4.9ab	50.0 ± 2.7a	62.5 ± 1.6ab	77.5 ± 1.6a	77.5 ± 1.6a	
Feeding	7th	0 (check)	5.7 ± 2.1d	5.7 ± 2.1d	12.8 ± 3.6e	18.6 ± 3.4d	20.0 ± 3.3d	
Feeding	1st	0.01	27.5 ± 2.5bc	41.2 ± 3.5b	42.5 ± 3.1b	43.7 ± 3.2c	49.0 ± 3.9c	56.2 ± 4.6d
Feeding	1st	0.1	32.5 ± 3.1ab	45.0 ± 5.6b	45.0 ± 5.6b	45.0 ± 5.6c	50.0 ± 4.6c	65.0 ± 3.3c
Feeding	1st	1	20.0 ± 4.6c	37.5 ± 5.6b	56.2 ± 5.3b	60.0 ± 5.3b	62.5 ± 5.6b	77.5 ± 1.6b
Feeding	1st	10	30.0 ± 5.9abc	50.0 ± 7.31b	57.5 ± 9.5b	61.0 ± 7.4b	72.5 ± 3.6b	82.5 ± 3.1ab
Feeding	1st	100	40.0 ± 4.6a	75.0 ± 3.3a	82.5 ± 1.6a	82.5 ± 1.6a	87.5 ± 1.6a	90.0 ± 0a
Feeding	1st	0 (check)	0d	7.5 ± 1.63c	17.5 ± 1.6c	20.0 ± 2.6d	20.0 ± 2.6d	20.0 ± 2.6e
Feeding	Adult	0.01	0b	0c	15.0 ± 1.8c	16.2 ± 1.8b	16.2 ± 1.8b	
Feeding	Adult	0.1	0b	0c	13.7 ± 3.2c	16.2 ± 2.6b	16.2 ± 2.6b	
Feeding	Adult	1	2.5 ± 1.6ab	5.0 ± 1.8b	13.7 ± 1.8c	15.0 ± 1.8b	15.0 ± 1.8b	
Feeding	Adult	10	5.0 ± 1.8a	7.5 ± 1.6a	22.5 ± 2.5b	27.5 ± 3.1a	27.5 ± 3.1a	
Feeding	Adult	100	5.0 ± 1.8a	8.8 ± 2.9a	30.0 ± 2.6a	30.0 ± 2.4a	35.0 ± 2.6a	
Feeding	Adult	0 (check)	0b	1.2 ± 1.2c	3.7 ± 2.6d	6.2 ± 3.0b	8.7 ± 3.5b	

[a] For each stage, means in the same column with a similar letter(s) are not significantly different (P > 0.05, Tukey's HSD). Percentages were transformed [arcsin(sqrt)] before analysis, but actual values are presented.

Table 5.12 Percent mortality (± SE) of 7th instars and adults lesser mealworms exposed to diflubenzuron-treated wood shavings in residual bioassays.

			Percent Mortality				
Method	Stage	Conc. (ppm)	10 d	20 d	30 d	40 d	50 d
Residual	7th	0.01	7.5 ± 3.6b[a]	13.7 ± 4.9cd	16.2 ± 4.9e	19.0 ± 5.4e	30.0 ± 3.2d[a]
Residual	7th	0.1	10.0 ± 0b	25.0 ± 1.8b	32.5 ± 1.6cd	42.5 ± 1.6cd	42.5 ± 1.6c
Residual	7th	1	9.0 ± 1.2b	20.0 ± 2.6bc	29.0 ± 2.9d	32.5 ± 3.6d	45.0 ± 2.6c
Residual	7th	10	14.0 ± 1.8b	29.0 ± 4.4b	40.0 ± 3.2c	46.0 ± 4.9c	55.0 ± 2.6b
Residual	7th	100	12.5 ± 1.6b	26.0 ± 2.6b	50.0 ± 0b	59.0 ± 2.9b	66.0 ± 1.8a
Residual	7th	1000	35.0 ± 4.6a	41.0 ± 5.4a	60.0 ± 3.2a	70.0 ± 3.2a	74.0 ± 3.2a
Residual	7th	0 (check)	0c	5.0 ± 1.8d	10.0 ± 3.7e	15.0 ± 1.8e	17.5 ± 1.6e
Residual	Adult	0.01	0b	5.0 ± 1.8d	8.8 ± 2.2cd	10.0 ± 1.8cd	10.0 ± 1.8cd
Residual	Adult	0.1	0b	7.5 ± 1.6cd	11.2 ± 2.9c	12.5 ± 2.5c	12.5 ± 2.5c
Residual	Adult	1	0b	7.5 ± 1.6cd	20.0 ± 1.1b	20.0 ± 1.1b	20.0 ± 1.1b
Residual	Adult	10	0b	12.5 ± 4.1bc	20.0 ± 2.6b	21.2 ± 2.2b	21.2 ± 2.2b
Residual	Adult	100	0b	17.5 ± 1.6ab	28.7 ± 2.2a	28.7 ± 2.2a	28.7 ± 2.2a
Residual	Adult	1000	3.3 ± 2.1a	20.0 ± 3.6a	31.6 ± 4.7a	33.3 ± 4.2a	35.6 ± 4.7a
Residual	Adult	0 (check)	0b	1.2 ± 1.2d	3.7 ± 1.8d	3.7 ± 1.8d	3.7 ± 1.8d

[a] For each stage, means in the same column with a similar letter(s) are not significantly different (P > 0.05, Tukey's HSD). Percentages were transformed [arcsin(sqrt)] before analysis, but actual values are presented.

Table 5.13 Percent mortality (± SE) of 7th instars, pupae and adults lesser mealworms treated with diflubenzuron in topical bioassays.

			Percent Mortality				
Method	Stage	Conc. (ppm)	10-d	20-d	30-d	40-d	50-d
Topical	7th	0.01	15.0 ± 4.6c[a]	57.5 ± 3.6e	60.0 ± 1.8c	66.0 ± 1.8c	66.0 ± 1.8c[a]
Topical	7th	0.1	34.0 ± 4.9b	69.0 ± 2.9d	75.0 ± 2.9b	78.0 ± 2.9b	79.0 ± 2.9b
Topical	7th	1	45.0 ± 4.6ab	78.0 ± 2.9c	82.0 ± 3.2b	85.0 ± 3.2b	85.0 ± 3.2b
Topical	7th	10	44.0 ± 4.9b	86.6 ± 1.8b	92.2 ± 1.8a	94.0 ± 1.8a	96.2 ± 1.8a
Topical	7th	100	57.5 ± 3.6a	100.0 ± 0a	100.0 ± 0a	100.0 ± 0a	100.0 ± 0a
Topical	7th	0 (check)	10.0 ± 3.7c	10.0 ± 2.2f	16.0 ± 2.2d	20.0 ± 2.2d	20.0 ± 2.2d
Topical	Pupa	0.01	28.3 ± 3.0c	30.0 ± 3.0d	32.6 ± 3.0d	33.3 ± 3.0d	38.3 ± 3.0d
Topical	Pupa	0.1	50.0 ± 6.8b	50.0 ± 5.0c	52.6 ± 4.2c	56.6 ± 4.2c	56.6 ± 4.2c
Topical	Pupa	1	70.0 ± 2.6a	70.0 ± 2.2b	72.0 ± 2.2b	75.0 ± 2.2b	75.0 ± 2.2b
Topical	Pupa	10	80.0 ± 3.6a	83.3 ± 3.3a	84.0 ± 2.1a	86.6 ± 2.1a	86.6 ± 2.1a
Topical	Pupa	100	80.0 ± 5.1a	86.6 ± 2.1a	88.6 ± 3.0a	91.6 ± 3.0a	91.6 ± 3.0a
Topical	Pupa	0 (check)	8.3 ± 1.6d	10.0 ± 2.1e	12.0 ± 2.10e	13.3 ± 2.10e	13.3 ± 2.10e
Topical	Adult	0.01	10.0 ± 0d	13.3 ± 2.1b	15.2 ±1.6c	18.3 ±1.6c	18.3 ±1.6c
Topical	Adult	0.1	13.3 ± 3.3d	20.0 ± 4.4 b	21.6 ± 3.0c	21.6 ± 3.0c	21.6 ± 3.0c
Topical	Adult	1	23.3 ± 2.1c	30.0 ± 5.7ab	42.0 ± 2.6b	50.0 ± 2.6b	50.0 ± 2.6b
Topical	Adult	10	35.0 ± 3.4b	40.0 ± 5.7a	43.6 ± 3.3b	46.6 ± 3.3b	46.6 ± 3.3b
Topical	Adult	100	48.3 ± 4.0a	50.0 ± 5.1a	55.0 ± 4.0a	65.0 ± 4.0a	66.2 ± 4.0a
Topical	Adult	0 (check)	1.6 ± 1.6e	1.6 ± 1.6c	2.0 ± 2.1d	3.3 ± 2.1d	3.3 ± 2.1d

[a]For each stage, means in same column with a similar letter(s) are not significantly different (P > 0.05, Tukey's HSD). Percentages were transformed [arcsin(sqrt)] before analysis, but actual values are presented.

Table 5.14 Percent mortality (± SE) of 7th and 1st instars and adults lesser mealworm exposed to ecdysone-treated diet in feeding bioassays.

			Percent Mortality					
Method	Stage	Conc. (ppm)	10 d	20 d	30 d	40 d	50 d	60 d
Feeding	7th	0.01	3.7 ± 1.8cd [a]	13.7 ± 1.8c	23.7 ± 1.8d	30.0 ± 1.8d	42.5 ± 1.6d[a]	
Feeding	7th	0.1	13.7 ± 1.8ab	32.5 ± 3.6b	36.2 ± 1.8c	46.2 ± 1.8c	56.2 ± 1.8c	
Feeding	7th	1	11.2 ± 2.9bc	33.7 ± 4.9b	53.7 ± 4.9b	63.7 ± 4.9b	76.2 ± 4.2b	
Feeding	7th	10	12.5 ± 3.6abc	40.0 ± 3.2ab	56.2 ±1.8b	71.2 ± 4.4b	82.5 ± 4.1b	
Feeding	7th	100	21.2 ± 6.4a	47.5 ± 5.9a	71.2 ± 4.4a	85.0 ± 3.3a	92.5 ± 1.6a	
Feeding	7th	0 (check)	0d	10.0 ± 0 c	16.2 ± 1.8d	16.2 ± 1.8e	18.7 ± 2.9e	
Feeding	1st	0.01	20.0 ± 1.8c	25.0 ± 3.2e	30.0 ± 2.6e	36.2 ± 3.7d	39.0 ± 2.9c	40.0 ± 2.6c
Feeding	1st	0.1	20.0 ± 1.8c	34.0 ± 3.2d	50.0 ± 3.7d	58.7 ± 3.9c	66.0 ± 4.9b	69.0 ± 5.1b
Feeding	1st	1	45.0 ± 3.7b	57.5 ± 3.6c	66.0 ± 3.2c	75.0 ± 2.6b	84.0 ± 2.6a	86.0 ± 1.8a
Feeding	1st	10	50.0 ± 3.7b	70.0 ± 2.6b	79.0 ± 2.2b	83.7 ± 3.2a	89.0 ± 3.5a	90.0 ± 3.2a
Feeding	1st	100	70.0 ± 3.7a	82.5 ± 2.5a	89.0 ± 2.2a	90.0 ± 1.8a	92.5 ± 1.6a	92.5 ± 1.6a
Feeding	1st	0 (check)	6.2 ± 1.8d	9.0 ± 1.2f	9.0 ± 1.2f	11.2 ± 1.2e	20.0 ± 1.8d	20.0 ± 2.6d
Feeding	Adult	0.01	2.5 ± 1.6ab	5.0 ± 2.6ab	7.5 ± 2.5ab	9.0 ± 2.9c	9.0 ± 2.9cd	
Feeding	Adult	0.1	0b	2.5 ± 2.5b	9.0 ± 2.2ab	12.5 ± 1.6bc	12.5 ± 1.6bc	
Feeding	Adult	1	4.0 ± 2.6ab	9.0 ± 4.4a	12.5 ± 4.1a	16.0 ± 3.2ab	16.0 ± 3.2abc	
Feeding	Adult	10	7.5 ± 3.6a	9.0 ± 5.8 a	15.0 ± 4.6a	19.0 ± 3.9a	19.0 ± 3.9ab	
Feeding	Adult	100	5.0 ± 3.7ab	7.5 ± 4.9a	12.5 ± 4.1a	19.0 ± 2.9a	21.0 ± 2.9a	
Feeding	Adult	0 (check)	0b	0b	2.5 ± 1.64b	4.0 ± 1.8d	5.0 ± 1.6e	

[a] For each stage, means in the same column with a similar letter(s) are not significantly different (P > 0.05, Tukey's HSD). Percentages were transformed [arcsin(sqrt)] before analysis, but actual values are presented.

Table 5.15 Percent mortality (± SE) of 7th instars and adults lesser mealworm exposed to diflubenzuron-treated wood shavings in residual bioassays.

			Percent Mortality				
Method	Stage	Conc. (ppm)	10 d	20 d	30 d	40 d	50 d
Residual	7th	0.01	0d	15.0 ± 1.8ef [a]	15.0 ± 1.8d	20.0 ± 0d	25.0 ± 1.8d[a]
Residual	7th	0.1	0d	20.0 ± 3.7de	30.0 ± 3.7c	35.0 ± 1.8c	35.0 ± 1.8c
Residual	7th	1	0d	25.0 ± 1.8cd	30.0 ± 0c	35.0 ± 1.8c	40.0 ± 2.6c
Residual	7th	10	5.0 ± 1.8c	30.0 ± 3.7bc	30.0 ± 3.7c	35.0 ± 5.6c	40.0 ± 4.2c
Residual	7th	100	10.0 ± 1.1b	35.0 ± 1.8b	45.0 ± 1.8b	45.0 ± 1.8b	55.0 ± 3.2b
Residual	7th	1000	27.5 ± 3.1a	50.0 ± 2.6a	57.5 ± 1.6a	62.5 ± 3.1a	65.0 ± 1.8a
Residual	7th	0 (check)	0d	10.0 ± 0f	17.5 ± 1.6 d	17.5 ± 1.6d	18.7 ± 2.2d
Residual	Adult	0.01	10.0 ± 2.6b	12.5 ± 1.6b	12.5 ± 1.6b	12.5 ± 1.6cd	12.5 ± 1.6cd
Residual	Adult	0.1	10.0 ± 2.6b	12.5 ± 1.6b	12.5 ± 1.6b	12.5 ± 1.6cd	12.5 ± 1.6cd
Residual	Adult	1	15.0 ± 1.8b	15.0 ± 1.8b	17.5 ± 1.6b	17.5 ± 1.6bc	17.5 ± 1.6bc
Residual	Adult	10	10.0 ± 0b	12.5± 1.6b	12.5 ± 1.6b	20.0 ± 2.6b	20.0 ± 2.6b
Residual	Adult	100	25.0 ± 1.8a	25.0 ± 1.8a	29.0 ± 1.6a	35.0 ± 1.8a	35.0 ± 1.8a
Residual	Adult	1000	27.0 ± 2.6a	30.0 ± 1.8a	32.5 ± 1.6a	37.5 ± 2.6a	40.0 ± 2.6a
Residual	Adult	0 (check)	0c	2.5 ± 1.6c	5.0 ± 1.8c	8.7 ± 1.2d	8.7 ± 1.2d

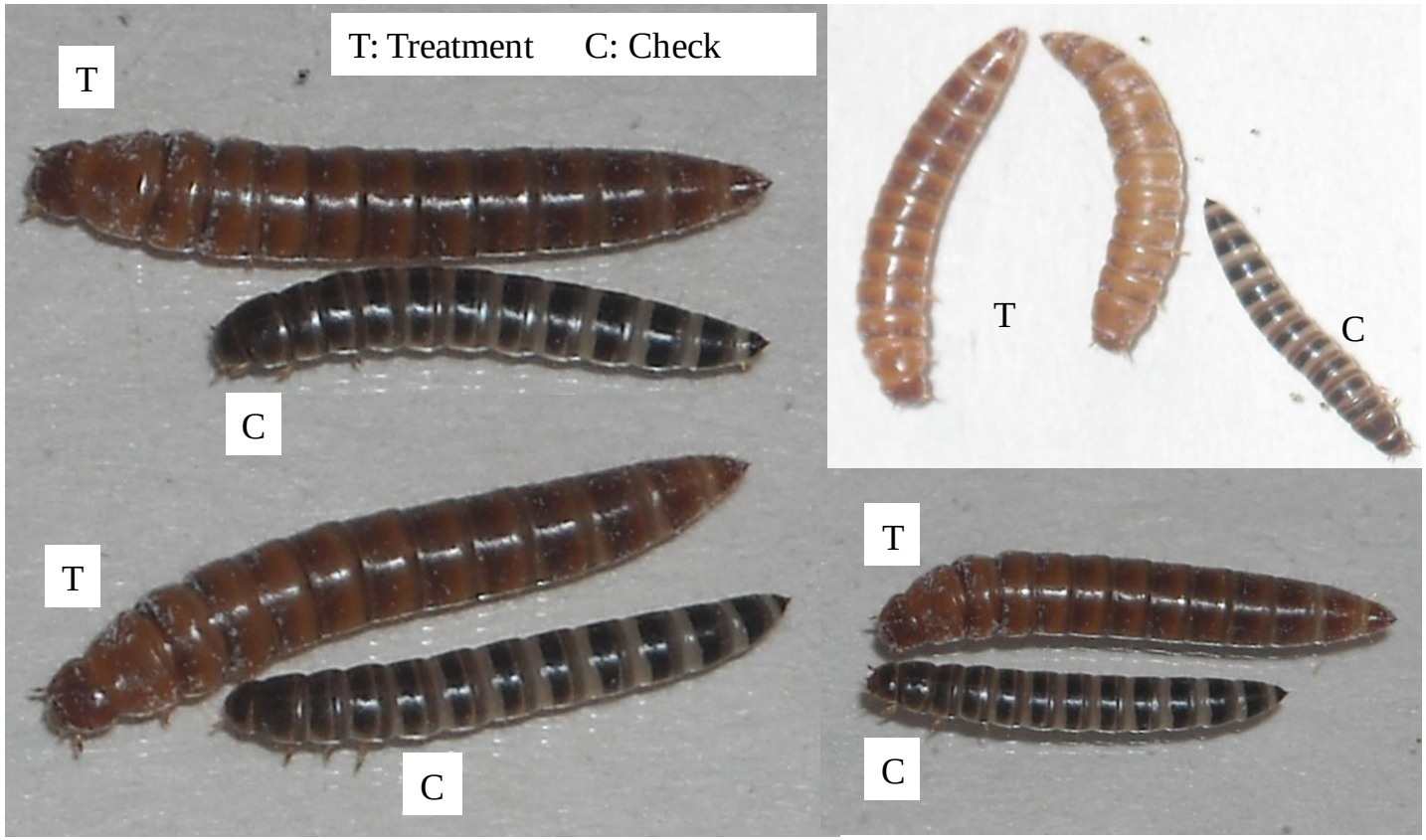
[a] For each stage, means in the same column with a similar letter(s) are not significantly different (P > 0.05, Tukey's HSD). Percentages were transformed [arcsin(sqrt)] before analysis, but actual values are presented.

Table 5.16 Percent mortality (± SE) of lesser mealworm 7th instars, pupae and adults treated with ecdysone in topical bioassays.

			Percent Mortality				
Method	Stage	Conc. (ppm)	10 d	20 d	30 d	40 d	50 d
Topical	7th	0.01	10.0 ± 0c [a]	20.0 ± 0d	25.0 ± 1.8d	27.5 ± 1.6d	35.0 ± 1.8d[a]
Topical	7th	0.1	10.0 ± 0c	20.0 ± 0d	35.0 ± 1.8c	37.5 ± 1.6c	42.5 ± 1.6cd
Topical	7th	1	20.0 ± 2.6b	32.5 ±1.6c	40.0 ± 2.6c	42.5 ± 1.6c	50.0 ± 2.6c
Topical	7th	10	40.0 ± 2.6a	47.5 ± 3.1b	55.0 ± 4.2b	60.0 ± 4.6b	65.0 ± 4.2b
Topical	7th	100	45.0 ± 4.2a	65.0 ± 4.2a	72.5 ± 3.1a	75.0 ± 3.2a	80.0 ± 2.6a
Topical	7th	0 (check)	8.7 ± 1.2c	11.2 ± 2.2e	16.2 ± 1.8e	16.2 ± 1.8e	20.0 ± 2.6e
Topical	Pupa	0.01	28.3 ± 3.0c	33.3 ± 2.1d	33.0 ± 2.1d	35.0 ± 2.2d	35.0 ± 2.2d
Topical	Pupa	0.1	40.0 ± 5.7b	42.0 ± 4.3c	50.6 ± 4.2c	52.6 ± 4.2c	56.6 ± 4.2c
Topical	Pupa	1	64.3 ± 4.7a	68.3 ± 4.2b	70.6 ± 2.1b	76.6 ± 2.1b	76.6 ± 2.1b
Topical	Pupa	10	70.3 ± 4.7a	81.6 ± 3.0a	81.6 ± 3.0ab	82.6 ± 3.0ab	81.6 ± 3.0ab
Topical	Pupa	100	75.0 ± 3.6a	82.0 ± 2.2a	86.3 ± 1.6a	88.3 ± 1.6a	88.3 ± 1.6a
Topical	Pupa	0 (check)	13.3 ± 2.1d	14.6 ± 2.1e	16.6.0 ± 0e	20.0 ± 2.2e	20.0 ± 2.2e
Topical	Adult	0.01	16.6 ± 3.3cd	16.6 ± 3.3d	18.3 ± 3.0c	18.3 ± 3.0c	19.6 ± 3.0c
Topical	Adult	0.1	25.0 ± 4.3bc	26.6 ± 3.3c	28.6 ± 3.0b	29.6 ± 3.0b	31.6 ± 3.0b
Topical	Adult	1	30.3 ± 4.9b	33.6 ± 3.3b	36.3 ± 3.0b	38.3 ± 3.0b	38.3 ± 3.0b
Topical	Adult	10	48.6 ± 4.7a	53.3 ± 4.2a	56.6 ± 4.2a	56.6 ± 4.2a	58.7 ± 4.2a
Topical	Adult	100	46.6 ± 4.9a	50.6 ± 3.0a	52.6 ± 3.0a	57.6 ± 3.0a	61.6 ± 3.0a
Topical	Adult	0 (check)	6.6 ± 2.1d	10.0 ± 2.6d	10.0 ± 2.6c	13.3 ± 2.1c	13.3 ± 2.1c

[a] For each stage, means in the same column with a similar letter(s) are not significantly different (P > 0.05, Tukey's HSD). Percentages were transformed [arcsin(sqrt)] before analysis, but actual values are presented.

Photograph 5.1 Weight gains of lesser mealworm larvae after exposure to fenoxycarb-treated diet in feeding bioassays.



CHAPTER VI

BASELINE SUSCEPTIBILITY AND CROSS-RESISTANCE IN ADULT AND LARVAL LESSER MEALWORM, *ALPHITOBIUS DIAPERINUS* (PANZER) (COLEOPTERA: TENEBRIONIDAE) COLLECTED FROM POULTRY HOUSES IN ARKANSAS

ABSTRACT

The development of resistance in lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) may pose a serious threat on their successful management in broiler chicken facilities in Arkansas. Residual bioassay tests were conducted on adults and 7th instars collected from two broiler farms with different insecticide application histories. House M in Fayetteville, AR was treated with insecticides for a long time, whereas house S in Savoy, Arkansas had limited insecticide use. A probit analysis found the lesser mealworm population in broiler house M to be resistant to cyfluthrin and tetrachlorvinphos but lesser mealworms in broiler house S were susceptible to both compounds. The M population showed no cross-resistance to imidacloprid, spinosad and chlorfenapyr. The M and S populations were similar in their susceptibility to imidacloprid, spinosad and chlorfenapyr. The suitability of imidacloprid and spinosad, and testing of chlorfenapyr as a potential candidate for lesser mealworm control is explained.

Key Words: broiler, residual, spinosad, chlorfenapyr, probit, resistance

INTRODUCTION

The cancellation of most of the recommended insecticides in broiler production facilities and the development of insecticide resistant lesser mealworm, *Alphitobius diaperinus* (Panzer), populations to many of the remaining available products has posed a serious threat to management of mealworms (Lambkin 2005). Resistance to both fenitrothion and cyfluthrin has been found in populations of lesser mealworm in broiler houses in Australia resulting in failure of control measures (Lambkin 2005). Tetrachlorvinphos and cyfluthrin were reported to lose effectiveness against lesser mealworm in poultry facilities in eastern United States (Hamm et al. 2006; Ronda et al. 2006). Steelman (2009) confirmed resistance to carbaryl, methoxychlor, DDT, cyfluthrin, cypermethrin, tetrachlorvinphos, and chlorpyrifos in adult and larval lesser mealworm beetles collected from poultry facilities in Arkansas in laboratory topical and residual bioassays.

Cyfluthrin, tetraclorvinphos, imidacloprid and spinosad are currently recommended to control lesser mealworm populations in broiler production farms in Arkansas. No studies have been completed so far that evaluate the cross-resistance to imidacloprid and spinosad in lesser mealworm from broiler farms in Arkansas. Understanding the mode of action of each insecticide is very crucial in developing insecticide-resistant management programs for lesser mealworm. Cyfluthrin is a sodium channel modulators whereas tetraclorvinphos is acetyl cholinesterase inhibitor (IRAC 2010). Imidacloprid mimic the agonist action of acetylcholine at nicotinic acetylcholine receptors and spinosad allosterically activate nicotinic acetylcholine receptors (IRAC

2010). Spinosad was found to be very effective against cyfluthrin (pyrethroid) resistant lesser mealworm populations in Australian broiler houses (Lambkin and Rice 2007).

Research is needed to determine the efficacy of novel insecticides with different modes of action. Chlorfenapyr uncouples oxidative phosphorylation through disruption of proton gradient and interferes with metabolic processes and energy production in mitochondria (Brown 2005). The comparative susceptibility to insecticides of populations collected from two chicken broiler houses with different histories of insecticide use is presented. The preexisting resistance and cross-resistance in pyrethroid and organophosphate resistant population to imidacloprid, spinosad and chlorfenapyr were also investigated.

MATERIAL AND METHODS

Adult and larval beetles were collected from two broiler houses in Arkansas. Farm M was located 20 km north of Fayetteville, and had been treated with organochlorine, organophosphate, carbamate and pyrethroid insecticides over the last 10 y. Farm S was located 20 km northwest of Fayetteville, and had minimal insecticidal usage history. Beetles collected from this farm were considered as susceptible population to compare with a potentially resistant population at the farm M. Beetles were collected and reared with the procedures described in the 5th chapter. Five different classes of insecticides were used: a pyrethroid, cyfluthrin (99% pure); organophosphate, tetrachlorvinphos (99%); neonicotinoid, imidacloprid (99%) (Chem Service, West Chester, PA); spinosad (44.2%) (Elanco Animal Health, Indianapolis, IN); and a pyrrole, chlorfenapyr (99.6%) (Sigma-Aldrich Co., Milwaukee, WI). Five concentrations were made in acetone including 0.05, 0.5, 5, 50, and 500 ppm. The insecticide concentrations

were applied by pipetting 1 ml into center of glass Petri dish (9 cm dia and 1.5 cm high) (VWR International, Suwanne GA), following the procedures of Plapp (1971). The Petri dish was rotated manually so that an even layer of insecticide dried on its inner surface and the acetone evaporated completely. Untreated check Petri dishes were prepared by dispensing 1 ml of acetone. Petri dishes were then placed for 1 h in fume hood to make them completely dry.

Ten adults or 7^{th} instars were placed in the bottom portion of Petri dish, and then covered. The tests were repeated 10 times with 10 beetles for each concentration. The Petri dishes were placed in laboratory conditions at 28 ^{o}C with 60% RH and a photoperiod of 16:8 (L:D). Mortality counts were taken at 24 and 48 h post exposure. The lesser mealworm was considered morbid or dead if it was unable to right itself or walk, or there was no movement when it was prodded with a metal probe.

Statistical analyses

Concentration-mortality responses were estimated with the PROC PROBIT option of SAS (SAS Institute 2004). Mortality in treated beetles was corrected for natural mortality in the check beetles using Abbott's formula (Abbott 1925). Insecticide concentration that killed 50 or 90% of lesser mealworm (LC_{50} and LC_{90}); regression coefficient (slope) and its standard error; Pearson's goodness of fit Chi-square; and 95% confidence limits for effective level of concentrations were calculated. Only pairs of LC_{50} values or pairs of LC_{90} values that did not have overlapping 95% confidence limits were considered significantly different. Resistance ratios (RR_{50} or RR_{90}) were calculated by dividing the LC_{50} or LC_{90} of the presumed resistant M population to LC_{50} or LC_{90} of the presumed susceptible S population.

RESULTS AND DISCUSSION

Larvae treated with cyfluthrin, tetrachlorvinphos, imidacloprid, spinosad or chlorfenapyr, and adults treated to cyfluthrin and chlorfenapyr yielded slope values of less than one suggesting increased variation within M and S populations in response to these insecticides (Tables 6.1 and 6.2). The slope values of greater than one in adults exposed to tetrachlorvinphos, imidacloprid and spinosad suggested increasingly homogeneous adult population within two farms in response to these two insecticides. The non-significant *P* values in the goodness of fit table for the Pearson's chi-square for all the bioassays indicated an adequate fit for the model with the normal distribution (Tables 6.1 and 6.2).

The results from the probit analysis at 48 h of exposure are presented and discussed. The adult beetles from farm M were significantly more resistant to cyfluthrin (LC_{50} = 2.2 ppm) than the beetles from farm S (LC_{50} = 0.2 ppm) (Table 6.1). Similarly larvae collected from farm M exhibited higher resistance to cyfluthrin (LC_{50}= 0.23 ppm) than did larvae from farm S (LC_{50} = 0.016 ppm) (Table 6.2). Less susceptibility to tetrachlorvinphos was reported in adult beetles collected from farm M (LC_{50} = 3.4 ppm) as compared to populations from farm S (LC_{50} = 0.5 ppm) (Table 6.1). The larvae from farm M were more resistant to tetrachlorvinphos (LC_{50} = 0.45 ppm) than were larvae from farm S (LC_{50} = 0.06 ppm) (Table 6.2). The RR_{50} values of 11 and 14 in adults and larvae, respectively, collected from the farm M suggest that the populations from this farm had the ability to detoxify and survive a treatment of cyfluthrin. The RR_{90} values of 20 and 22 in adults and larvae, respectively, collected from the farm M indicated that a very high concentration of cyfluthrin was needed to achieve 90% mortality (Tables 6.1

and 6.2). Twenty cyfluthrin applications in 4 y resulted in 22 fold resistance to cyfluthrin in lesser mealworms in Australia (Lambkin and Rice 2007). The adults and larvae collected from farm M were 6.4 and 7.5 folds, respectively, more resistant to tetrachlorvinphos than those collected from farm S (Tables 6.1 and 6.2).

The susceptibility of adults or larvae exposed to imidacloprid, spinosad or chlorfenapyr were not significantly different between farm M or S suggesting no cross-resistance to previously used insecticides. However, higher concentrations of imidacloprid were needed to kill 50% of adults or larvae as compared to other insecticides. For chlorfenapyr, the concentration ranges from 1-3 ppm caused 50% to 90% mortality in adults and larvae. The results indicate the loss of field efficacy of pyrethroids and organophosphates in field collected beetles. The use of pyrethroids and organophosphates for controlling poultry pests such as house flies may also contribute to build up resistance in litter beetles. Development of new chemistry insecticides and development of resistance management based integrated program for lesser mealworm beetles is required. Insecticides to which resistance has not yet developed should be carefully used with non chemical techniques and other classes of insecticides.

Additional studies should be conducted to evaluate the effectiveness of imidacloprid and chlorfenappyr in the poultry facilities. A discriminating concentration ($LC_{99.9}$) needs to be determined for cyfluthrin, tetrachlorvinphos, imidacloprid, spinosad or chlorfenapyr to separate insecticide-susceptible and resistant populations. Beetles from different poultry facilities in Arkansas should be subjected to a discrimination dose to check the resistance levels in these populations.

There seems to be no preexisting resistance and no cross-resistance to imidacloprid, spinosad or chlorfenapyr in cyfluthrin/tetrachlorvinphos resistant M population. The absence of cross-resistance to imidacloprid, spinosad and chlorfenapyr is not surprising due to different modes of action of these three insecticides. The results of the current study confirm the suitability of imidacloprid and spinosad in controlling lesser mealworm. Chlorfenapyr seemed to be good potential candidate for testing in laboratory and poultry houses against lesser mealworm.

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Table 6.1 Susceptibility of field collected adult lesser mealworm (M = pesticide treated farm in Fayetteville, AR; and S = no pesticide use farm in Savoy, AR) exposed to cyfluthrin, tetrachlorvinphos, imidacloprid, spinosad and chlorfenapyr for 48 h in residual bioassays.

		48 h							
Farm	Insecticide	LC_{50} (95% FL) ppm	LC_{90} (95% FL) ppm	Slope (±SE)	RR_{50}	RR_{90}	χ^2	DF	*P*
M	Cyfluthrin	2.2 (0.8-5.9)a	271.5 (77.4-527.0)a	0.5 ± 0.009	11.0 [b]	20.0 [c]	11.3	48	0.8
S	Cyfluthrin	0.2 (0.001-0.3)b	13.46 (0.8-7.4)b	0.5 ± 0.009			10.6	48	0.9
M	Tetrachlorvinphos	3.4 (1.4-8.8)a	14.7 (5.6-25.2)a	1.4 ± 0.02	6.4	7.4	14.0	48	0.8
S	Tetrachlorvinphos	0.5 (0.3-1.0)b	2.0 (1.2-4.2)b	2.0 ± 0.03			19.5	48	0.6
M	Imidacloprid	20.3 (10.7-34.0)a	188.2 (91.0-496.0)a	1.4 ± 0.02	1.1	1.2	20.2	48	0.3
S	Imidacloprid	17.4 (8.0-31.7)a	158.3 (81.3-393.0)a	1.3 ± 0.02			20.2	48	0.3
M	Chlorfenapyr	0.5 (0.2-1.0)a	3.0 (1.2-7.2)a	0.8 ± 0.01	1.3	1.3	9.0	48	0.8
S	Chlorfenapyr	0.4 (0.1-0.9)a	2.2 (1.0-5.6)a	0.7 ± 0.01			7.9	48	0.9
M	Spinosad	3.3 (1.2-8.4)a	17.4 (11.0-49.0)a	1.1 ± 0.02	1.1	1.0	17.2	48	0.6
S	Spinosad	2.4 (0.9-5.2)a	16.5 (9.0-32.0)a	1.3 ± 0.02			15.2	48	0.5

[a] Between M and S, means in same column with different letter are significantly different based on no overlap of 95% FL.
[b] RR_{50}: Resistance ratio at LC_{50} (i.e. LC_{50} farm M population/LC_{50} farm S population).
[c] RR_{90}: Resistance ratio at LC_{90} (i.e. LC_{90} farm M population/LC_{90} farm S population).

Table 6.2 Susceptibility of field collected larval lesser mealworm (M = pesticide treated farm in Fayetteville, AR; and S = no pesticide use farm in Savoy, AR) exposed to cyfluthrin, tetrachlorvinphos, imidacloprid, spinosad and chlorfenapyr for 48 h in residual bioassays.

		48h							
Farm	Insecticide	LC_{50} (95% FL) ppm	LC_{90} (95% FL) ppm	Slope (±SE)	χ^2	RR_{50}	RR_{90}	DF	*P*
M	Cyfluthrin	0.23 (0.07-0.6)a[a]	112.6 (22.2-129.0)a	0.5 ± 0.08	5.6	14[b]	22.0[c]	18	0.9
S	Cyfluthrin	0.016 (0.001-0.02)b	5.11 (0.7-7.3)b	0.5 ± 0.09	9.6			18	0.9
M	Tetrachlorvinphos	0.45 (0.2-1.4)a	17.6 (5.7-39.5)a	0.6 ± 0.08	16.9	7.5	8.4	22	0.7
S	Tetrachlorvinphos	0.06 (0.03-0.1)b	2.1 (0.9-4.2)b	0.8 ± 0.09	24.1			22	0.3
M	Imidacloprid	8.0 (6.0-25.0)a	110.0 (40.4-340.6)a	0.7 ± 0.11	15.9	1.3	0.8	18	0.6
S	Imidacloprid	6.2 (4.2-16.6)a	132.0 (50.0-310.2)a	0.8 ± 0.11	18.5			18	0.4
M	Chlorfenapyr	0.5 (0.2-1.1)a	2.8 (1.2-14.5)a	0.9 ± 0.12	9.2	1.2	1.1	18	0.8
S	Chlorfenapyr	0.4 (0.15-0.9)a	2.6 (1.0-11.5)a	0.7 ± 0.10	6.1			18	0.9
M	Spinosad	2.6 (1.1-7.2)a	15.2 (9.0-42.0)a	1.0 ± 0.03	11.1	1.2	1.04	18	0.8
S	Spinosad	2.2 (0.6-4.2)a	14.5 (6.0-30.0)a	1.1 ± 0.02	13.2			18	0.7

[a] Between M and S, means in same column with different letter are significantly different based on no overlap of 95% FL.
[b] RR_{50}: Resistance ratio at LC_{50} (i.e. LC_{50} farm M population/LC_{50} farm S population).
[c] RR_{90}: Resistance ratio at LC_{90} (i.e. LC_{90} farm M population/LC_{90} farm S population).

CHAPTER VII

SUSCEPTIBILITY OF LESSER MEALWORM, *ALPHITOBIUS DIAPERINUS* (PANZER) LARVAE AND ADULTS TO IMIDACLOPRID, METAFLUMIZONE AND DIFLUBENZURON

ABSTRACT

The use of selective and reduced risk pesticides is a major consideration in developing an integrated control program for the lesser mealworm. Baseline susceptibility of lesser mealworm to imidacloprid, metaflumizone and diflubenzuron were tested in residual bioassays. Higher percent mortality was observed in larvae treated with insecticides than the adults on 5, 7 and 10 d post exposure. Solutions of 0.25% imidacloprid + 0.25% metaflumizone and 0.25% imidacloprid + 0.50% metaflumizone were both more effective against adult beetles. Although, these combinations took 21 or 28 d to achieve > 80% mortality. Diflubenzuron was more effective against larvae than adults at 5, 7 or 10 d post exposure. Solutions of 0.50% imidacloprid and 0.25% imidacloprid alone were also found to be effective against lesser mealworm larvae. The testing of imidacloprid with other insect growth regulators is discussed.

Key Words: baseline susceptibility, solution, residual, metaflumizone, diflubenzuron

INTRODUCTION

The cancellation of most indoor organophosphates and carbamate insecticides has reduced the number of residual insecticides for lesser mealworm control and the remaining products are facing the problem of insect resistance. The ecological and resistance concerns necessitate the research to be focused toward more selective compounds with different modes of action. A few novel compounds available in the market such as imidacloprid, metaflumizone, and insect growth regulators have not been tested in combinations on lesser mealworm beetles. The use of selective and reduced risk pesticides is a major consideration in developing an integrated control program for the lesser mealworm.

Metaflumizone is a new chemistry (semicarbazone) insecticide that is very effective against insect-pests from different orders including Lepidoptera, Coleoptera, Hymenoptera, Isoptera, Siphonaptera and Diptera (BASF 2007). Metaflumizone blocks the sodium channel of the nervous system causing relaxed paralysis of the insect. It is relatively safe to applicators and non-target organisms including natural enemies and pollinators. Gradish et al. (2010) found no sub-lethal effects of metaflumizone to the bumble bee, *Bombus impatiens* (Cresson) colonies suggesting this chemical safe use with pollinators, and at the same time achieving the desired pest control in greenhouse. It has also been designated a Reduced Risk Candidate by the US EPA. Its novel mode of action and lack of cross-resistance to other classes of insecticides makes it an ideal candidate for insect resistance management programs for urban pests (Klein and Oloumi 2005).

Baited formulations of metaflumizone were highly potent on nuisance ants (*Crematogaster* spp., *Linepithema humile* Mayr, and *Camponotus floridanus* (Buckley)),

subterranean termites, *Reticulitermes flavipes* (Kollar), red imported fire ant, *Solenopsis invicta* Buren, and German cockroach, *Blatella germanica* L., in laboratory and field studies. The metaflumizone granular fly bait can be used effectively in and around livestock production facilities as well as in residential settings for the housefly, *Musca domestica* L. control (Ahmad and Zurek 2009).

Imidacloprid is less toxic and safer to mammals. The insects have higher number of nicotinic acetylcholine receptors than vertebrates. Also, selective binding affinity of imidacloprid is higher for insect nicotinic acetylcholine receptors than for mammalian receptors (Boyd and Boethel 1998). Imidacloprid was found effective against bed bug *Cimex lectularius* L. (Steelman et al. 2008). The workers of subterranean termites, *Reticuliternes virginicus* (Banks), died when exposed to 100 ppm imidacloprid-treated sand, and those survived tunneled less than did their unexposed nestmates (Thorne and Breisch 2001). Bait matrix containing 0.001 and 0.1% imidacloprid destroyed all laboratory colonies of Pharaoh's ant, *Monomorium pharaonis* (L.) in a dose-related time span (Rupes et al. 2008). Diflubenzuron prevents the formation of chitin, a molecule required for making insect cuticle, resulting in death during molting. The absence of chitin in humans makes this compound safer to use (U.S. EPA 1997).

Little data exists on the efficacy of modern insecticides for the lesser mealworm. Efficacy studies in the laboratory on lesser mealworm collected from poultry facilities provided baseline susceptibility data on the toxicity of imidacloprid, metaflumizone and diflubenzuron. This data will provide valuable information on the potential use of modern insecticides which have a lower toxicity to the environment.

MATERIAL AND METHODS

The lesser mealworm adults and larvae for all bioassays were collected from the Applied Broiler Research Unit, Savoy, AR, and reared with the procedures described in 5th chapter. Imidacloprid, metaflumizone and diflubenzuron used in the bioassays were provided by BASF Corporation, Research triangle Park, NC. Insecticides were tested alone or in combinations (Table 7.1). A 1 ml aliquot of an insecticide concentrations made in acetone was applied to circular filter papers (14 cm dia.) and placed under a fume hood for 4 h until completely dry. The filter papers were placed in plastic Petri dish (14 cm dia. and 2 cm high) (Becton Dickinson and Co., Franklin Lakes, NJ). Untreated check filter papers were treated with acetone. A group of 50 adults or 5th instars were exposed to insecticide treated filter papers in the Petri dish. A 2 cm^2 water soaked cotton ball was placed in the Petri dish and moistened twice weekly. The Petri dishes were placed in laboratory conditioned at 28 ^{o}C with 60% RH, and a photoperiod of 16:8 (L:D). Mortality counts were taken starting at 24 h post exposure and continued until untreated check mortality exceeds 20%. Adults were considered dead if they were unable to right themselves or walk, or there was no movement when they were prodded with a metal probe. The tests were replicated 3 times with 50 beetles for each insecticide concentration /combination.

Statistical analyses

The percent mortality of lesser mealworms was calculated for each concentration for all insecticides. Original mortality data were corrected for the check mortality by Abbott's (1925) formula. Due to wide ranges in mortality percentages, data were arcsine-square root transformed before analysis of variance (ANOVA). Two way ANOVA was

used to demonstrate interaction effects between treatments and exposure time. When interactions were significant, the effects of one factor were analyzed at each level of the interacting factor. If significantly different, treatment means were compared using Tukey test (PROC GLM, SAS Institute 2004).

RESULTS AND DISCUSSION

The concentrations of 0.1, 0.25 and 0.5% represent 100, 250 and 500 ppm, respectively. No significant differences in percent mortality were found among all single or two combination insecticide solutions at 3 or 5 d post exposure. Adults were significantly more susceptible to a solution of 0.25% imidacloprid + 0.25% metaflumizone at 10 (39%) and 14 d (47%) as compared to other insecticide treatments with the exception of solutions of 0.25% or 0.50% imidacloprid. The solution of 0.25% imidacloprid + 0.25% metaflumizone again caused significantly higher mortality (84%) at 21 d than other insecticide treatments except for a solution of 0.25% imidacloprid + 0.50% metaflumizone (71.6%). A solution of 0.25% imidacloprid + 0.25% metaflumizone was more effective against adult beetles at 7, 10, and 14 d post treatment. Higher percent mortality were found in adults exposed to solutions of 0.25% imidacloprid + 0.25% metaflumizone (97%), 0.25% imidacloprid + 0.50% metaflumizone (93%), 0.50% imidacloprid (84%), and 0.50% imidacloprid + 0.25% metaflumizone (84%) as compared to other insecticides/combined solutions at 28 d post exposure. However, all these combinations took 21 or 28 d to achieve > 80% mortality (Table 7.1). In similar studies, imidacloprid-treated wheat produced 100% mortality of book lice, *Liposcelis bostrychophila* Badonnel, and *L. entomophila* (Enderlein), at 21 or 28 d post exposure (Nayak and Daglish 2006). Imidacloprid bait formulations killed less

than 50% adults of brown-banded cockroach, *Supella longipalpa* (F.), after 21 d in laboratory trials (Savoldelli and Suss 2005).

The 5th instars were significantly less susceptible to a solution of 0.25% imidacloprid + 0.10% diflubenzuron than any other insecticide concentrations /combinations at 3 d post treatment. Percent mortality of larvae to 0.50% imidacloprid (39%) was significantly higher than 0.25% metaflumizone + 0.10% diflubenzuron (4.4%) at 5 d post treatment. Percent mortality in larvae to all insecticides/combinations was significantly higher than to a solution of 0.25% metaflumizone + 0.10% diflubenzuron at 7 and 14 d post treatment. Solutions of 0.50% imidacloprid and 0.25% imidacloprid caused 69% and 60% mortality, respectively, at 10 d post treatment. A solution of 0.10% diflubenzuron was also effective against larvae producing 60% percent mortality at 10 d. A solution of 0.25% metaflumizone + 0.10% diflubenzuron combination was the least toxic to larvae (Table 7.2).

Larvae were significantly more susceptible than adults to all insecticides/combinations with the exception to 0.25% metaflumizone + 0.10% diflubenzuron at 5 d post exposure. Higher percent mortality was observed in treated larvae than the adults to all insecticides/combinations on 5, 7 and 10 d post exposure except 0.25% metaflumizone + 0.10% diflubenzuron and 0.25% imidacloprid + 0.25% metaflumizone. Both of these combination solutions caused significantly similar percent mortalities in adults and larvae. A solution of 0.10% diflubenzuron caused significantly higher mortality of 23%, 40 % and 60% mortality at 5, 7 and 10 d, respectively, in larvae as compared to 0.007 % mortality in adults at 5, 7 and 10 d post exposure (Table 7.3).

The results from this experiment provide baseline data for the susceptibility of adult and larval lesser mealworm beetles to imidacloprid, metaflumizone and diflubenzuron. Combinations of imidacloprid and metaflumizone were effective against adults although metaflumizone alone did not have significantly different potency than other insecticides/combinations. These insecticides/combinations seemed to be slow acting in their effectiveness against lesser mealworn adults and larvae. The susceptibility of adult and larval lesser mealworm might not be accurately represented in this data due to the limited replications used in the study. Future studies should involve testing various combinations of imidacloprid with juvenile hormone analogs such as fenoxycarb, methoprene or hydroprene. Efficacy of imidacloprid or metaflumizone in combination with chlorfenapyr should also be determined.

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Table 7.1 Percent mortality (± SE) of adults lesser mealworm exposed to different percent concentrations or combinations of imidacloprid, metaflumizone and diflubenzuron in residual bioassays (N=3).

% insecticide concentration	3 d	5 d	7 d	10 d	14 d	21 d	28 d
0.10% Diflubenzuron	0a[a]	0.007 ± 0.6a	0.007 ± 0.7b	0.007 ± 0.7b	-1.4 ± 1.4d	1.8 ± 3.8e	4.1 ± 5.3e
0.25% Imidacloprid	0.6 ± 0.6a	0.007 ± 0.6a	12.7 ± 5.8ab	17.1 ± 5.9ab	29.4 ± 4.2ab	50.1 ± 6.5bc	54.5 ± 3.4bc
0.25% Imidacloprid + 0.10% Diflubenzuron	0.6 ± 0.6a	0.007 ± 0.6a	8.0 ± 6.8ab	10.9 ± 6.5b	17.5 ± 8.5bcd	40.1 ± 5.8cd	58.6 ± 2.6bc
0.25% Imidacloprid + 0.25% Metaflumizone	4.0 ± 1.1a	3.4 ± 1.1a	28.2 ± 11.7a	39.0 ± 11.9a	46.8 ± 8.7a	83.9 ± 1.3a	97.0 ± 1.7a
0.25% Imidacloprid + 0.50% Metaflumizone	2.6 ± 0.6a	2.0 ± 0.6a	10.0 ± 2.9ab	11.7 ± 3.5b	23.8 ± 3.0bc	71.6 ± 5.4ab	92.9 ± 1.0a
0.50% Imidacloprid	2.0 ± 2.0a	4.0 ± 1.7a	15.4 ± 3.0ab	19.9 ± 2.3ab	28.0 ± 1.3ab	54.7 ± 4.3bc	83.8 ± 3.6a
0.50% Imidacloprid + 0.25% Metaflumizone	0.6 ± 0.6a	0.007 ± 0.7a	3.3 ± 1.1b	5.5 ± 2.0b	9.0 ± 1.8bcd	42.4 ± 3.9cd	83.8 ± 1.0a
0.50% Imidacloprid + 0.50% Metaflumizone	1.3 ± 0.6a	0.7 ± 0.7a	6.0 ± 2.9ab	11.6 ± 3.1b	20.3 ± 6.4bcd	52.4 ± 8.4bc	76.8 ± 8.2ab
0.25% Metaflumizone	0a	1.3 ± 1.1a	3.3 ± 1.1b	6.8 ± 2.7b	9.8 ± 3.2bcd	18.6 ± 3.0de	17.2 ± 4.0d
0.25% Metaflumizone + 0.10% Diflubenzuron	0a	0.7 ± 1.3a	0.7 ± 1.3b	1.4 ± 2.0b	0.007 ± 1.4cd	2.8 ± 3.3e	7.3 ± 1.7e
0.50% Metaflumizone	0.6 ± 0.6a	1.4 ± 2.0a	1.3 ± 2.0b	2.0 ± 1.8b	4.2 ± 3.0cd	30.2 ± 9.0cd	39.4 ± 9.0cd

[a] Means in the same column with a similar letter(s) are not significantly different ($P > 0.05$, Tukey's HSD). Mortality data were corrected for the check larval mortality. Percentages were transformed [arcsin(sqrt)] before analysis, but actual values are presented.

Table 7.2 Percent mortality (± SE) of 5th instar lesser mealworm exposed to different percent concentrations or combinations of imidacloprid, metaflumizone and diflubenzuron in residual bioassays (N=3).

% insecticide concentration	3 d	5 d	7 d	10 d
0.10% Diflubenzuron	9.7 ± 3.0ab[a]	22.7 ± 2.6ab	37.6 ± 4.1a	59.8 ± 3.0a
0.25% Imidacloprid	13.9 ± 2.5a	31.4 ± 4.4ab	46.6 ± 3.9a	59.8 ± 5.7a
0.25% Imidacloprid + 0.10% Diflubenzuron	0.7 ± 1.8b	22.7 ± 4.0ab	36.9 ± 2.2a	41.4 ± 3.9a
0.25% Imidacloprid + 0.25% Metaflumizone	11.8 ± 3.4ab	31.4 ± 5.2ab	43.6 ± 5.9a	50.6 ± 11.6a
0.25% Imidacloprid + 0.50% Metaflumizone	9.0 ± 2.5ab	24.1 ± 9.5ab	37.6 ± 4.1a	48.3 ± 8.7a
0.50% Imidacloprid	8.3 ± 3.6ab	39.4 ± 9.3a	55.6 ± 3.2a	69.0 ± 7.1a
0.50% Imidacloprid + 0.25% Metaflumizone	3.5 ± 3.6ab	26.3 ± 6.9ab	34.6 ± 3.4a	34.5 ± 12.1a
0.50% Imidacloprid + 0.50% Metaflumizone	9.7 ± 3.4ab	29.9 ± 7.6ab	38.4 ± 9.4a	31.0 ± 17.3a
0.25% Metaflumizone	3.5 ± 0.6ab	13.2 ± 0.7ab	36.9 ± 3.4a	45.9 ± 7.0a
0.25% Metaflumizone + 0.10% Diflubenzuron	4.2 ± 2.4ab	4.4 ± 5.1b	7.5 ± 3.9b	13.8 ± 9.1b
0.50% Metaflumizone	4.9 ± 2.5ab	18.3 ± 3.8ab	33.9 ± 0.7a	41.4 ± 1.9a

[a] Means in the same column with a similar letter(s) are not significantly different ($P > 0.05$, Tukey's HSD). Mortality data were corrected for the check larval mortality. Percentages were transformed [arcsin(sqrt)] before analysis, but actual values are presented.

Table 7.3 Comparison of percent mortality (± SE) between 5th instar and adult lesser mealworms exposed to imidacloprid, metaflumizone and diflubenzuron in residual bioassays (N=3).

% insecticide concentration	5 d		7 d		10 d	
	Adult	Larvae	Adult	Larvae	Adult	Larvae
0.25% Imidacloprid	0.007 ± 0.6b[a]	31.4 ± 4.4a	12.8 ± 5.8b	46.6 ± 3.9a	17.1 ± 5.9b	59.8 ± 5.7a
0.50% Imidacloprid	4.0 ± 1.7b	39.5 ± 9.3a	15.4 ± 3.0b	55.6 ± 3.2a	19.9 ± 2.3b	68.9 ± 7.1a
0.25% Metaflumizone	1.3 ± 1.1b	13.2 ± 0.7a	3.4 ± 1.1b	36.9 ± 3.4a	6.9 ± 2.7b	45.9 ± 6.9a
0.50%Metaflumizone	1.3 ± 2.0b	18.3 ± 3.8a	1. 4 ± 2.0b	33.9 ± 0.7a	2.0 ± 1.8b	41.4 ± 1.9a
0.25% Imidacloprid + 0.25% Metaflumizone	3.4 ± 1.1b	31.4 ± 5.2a	28.2 ± 11.7a	43.6 ± 5.9a	39.0 ± 11.9a	50.6 ± 11.6a
0.25% Imidacloprid + 0.50% Metaflumizone	2.0 ± 0.6b	24.1 ± 9.5a	10.0 ± 2.9b	37.6 ± 4.1a	11.6 ± 3.5b	48.3 ± 8.6a
0.50% Imidacloprid + 0.25% Metaflumizone	0.007 ± 0.6b	26.3 ± 6.9a	3.4 ± 1.1b	34.6 ± 3.4a	5.5 ± 2.0b	34.5 ± 12.1a
0.50% Imidacloprid + 0.50% Metaflumizone	0.7 ± 0.6b	29.9 ± 7.6a	6.0 ± 2.9b	38.4 ± 9.4a	11.6 ± 3.1b	31.0 ± 17.3a
0.10% Diflubenzuron	0.007 ± 0.6b	22.7 ± 2.6a	0.007 ± 0.6b	37.1 ± 4.1a	0.007 ± 0.6b	59.8 ± 3.0a
0.25% Imidacloprid + 0.10% Diflubenzuron	0.007 ± 0.6b	22.7 ± 4.0a	8.0 ± 6.8b	36.9 ± 2.2a	10.9 ± 6.5b	41.4 ± 3.9a
0.25% Metaflumizone + 0.10% Diflubenzuron	0.7 ± 1.3a	4.4 ± 5.1a	0.7 ± 1.3a	7.5 ± 3.9a	1.4 ± 2.0a	13.8 ± 9.1a

[a] Within each day, means in the same with a similar letter(s) are not significantly different (P > 0.05, Tukey's HSD). Mortality data were corrected for the check larval mortality. Percentages were transformed [arcsin(sqrt)] before analysis, but actual values are presented.

CONCLUSIONS

My dissertation research provides information about the densities of lesser mealworm beetles in poultry litter that has been applied to the pastures in Savoy, Arkansas. This study determined 3.5, 2.2 and 0.4 million larvae, adults and pupae, respectively, per hectare of pasture land. This indicates the potential of pasture applied litter as a source of reinfestation in the poultry houses. One recommendation is to harvest the birds during the coldest part of the winter, remove and spread the poultry litter outside and open the house to expose beetles to sub-freezing temperatures. A more likely alternative would be to cover and hot compost the litter. Future research should involve estimating the numbers of adult beetles leaving field applied litter and find the relationship between their density and dispersal. Survivability of all stages of lesser mealworm spread on pastures during summer and winter needs to be studied.

Knowledge of lesser mealworm movement patterns in response to temperature and presence/absence of chicks inside the poultry house is very important for decision making regarding sampling and pesticide use. There was a huge reduction in beetle movement and numbers when the chicks were first introduced into the house but the beetle counts increased after a few days in field experiments using pitfall traps. More adults and larvae were captured when the temperatures were raised inside the house. Insecticides or sampling should be recommended when heat is turned on during flock free period because more numbers of beetles are present on surface of litter. There are higher chances of beetles to come in contact with the insecticides under warmer conditions.

Laboratory two-choice pitfall bioassay was found to be a useful and convenient tool for evaluating the potential attractants for lesser mealworm adults and larvae before testing them in the poultry house. Greater attraction of adults and larvae to a dose of 20-30 µg synthetic lesser mealworm aggregation lure were reported in laboratory pitfall bioassays. A combination of fresh Chicken droppings (CD) and pheromone lure was observed to be very attractive to adults and larvae in laboratory two-choice pitfall bioassays. Significantly higher numbers of beetles were found in traps treated with pheromone lure as compared to untreated checks in all field experiments. Our results generally indicate that there is a potential for combining the pheromone lure with the potential attractive CD compounds to enhance trap efficacy. Eight compounds were identified from gas chromatography/mass spectrometer analysis of fresh chicken droppings volatiles (CDV) including: 2-methyl-propanoic acid, 2, 3-butanediol, butanoic acid, 2-pentanone, 1-octen-3-ol, 2-chlorocyclohexanol, pentanoic acid and dodecanal. Attractiveness of these compounds to lesser mealworm larvae and adults need to be determined in electroantennogram and laboratory choice bioassays. The identified CDV compounds that are attractive to lesser mealworm adults and larvae should be tested in different combinations with the pheromone lure. The combinations that attract more beetles can be used to monitor the lesser mealworm populations and also combined with insecticide baits(s) to manage populations in the poultry house. An important outcome of my research was confirmation of larval attraction to adult produced pheromones. The larvae may have adapted and evolved the behavioral responses to male-produced aggregation pheromones for exploiting food resources and shelter occupied by adults.

There might be possibility of larval production of aggregation compounds in lesser mealworm.

Due to lack of insect growth regulators (IGR's) efficacy studies on lesser mealworm, baseline dose-response bioassays were conducted with three different classes of IGR's including fenoxycarb, diflubenzuron, and 20-hydroxyecdysone on 1st and 7th instars, pupae, and adult beetles. Beetles were exposed to these IGR's through topical application, residual contact with treated wood shavings, and feeding on treated chicken feed that simulated the possible ways insecticide was absorbed by beetles in poultry facilities. The mean pupation times increased and pupation was delayed in fenoxycarb treated 7th instar in feeding, residual and topical bioassays. The fenoxycarb was demonstrated to be significantly toxic to 1st and 7th instars in feeding bioassays causing 100% and 94% mortality, respectively. The feeding bioassay was more suitable for the 1st and 7th instars, although the residual contact bioassay can also be used for 7th instar. Adults succumbed to lower concentrations in a topical bioassay than in feeding or residual contact bioassays. These IGR's might evolve as an excellent option in management of lesser mealworm where standard insecticides no longer provide adequate control. All three bioassay methods produced usable dose-response curves and may be used for surveying temporal changes in the IGR susceptibility to lesser mealworm. The results from this laboratory study will help the insecticide manufacturing companies to register their product for the lesser mealworm if they are not currently on the insecticide label.

The comparative susceptibility and cross-resistance to selected insecticides were determined in beetles collected from broiler chicken production farms having different

insecticide application history. Residual bioassays were conducted on 7th instar and adults collected from House M with a long history of insecticide use and house S with limited insecticide use. Both larvae and adults from house M confirmed resistance to cyfluthrin and tetrachlorvinphos but lesser mealworms in broiler house S were susceptible to both compounds. There seems to be no preexisting resistance and cross-resistance to imidacloprid, spinosad or chlorfenapyr in cyfluthrin/tetrachlorvinphos resistant M population. The results indicate the loss of field efficacy of pyrethroids and organophosphates in field collected beetles. The results of the current study confirm the suitability of imidacloprid and spinosad in controlling lesser mealworm and chlorfenapyr as a potential candidate for testing in laboratory and poultry houses against lesser mealworm.

The use of selective and reduced risk pesticides is a major consideration in developing an integrated control program for the lesser mealworm. Baseline susceptibility of lesser mealworm to imidacloprid, metaflumizone and diflubenzuron were tested in laboratory residual bioassays. Higher percent mortality was observed in larvae treated with insecticides than the adults on 5, 7 and 10 d post exposure. Solutions of 0.25% imidacloprid + 0.25% metaflumizone and 0.25% imidacloprid + 0.50% metaflumizone were both more effective against adult beetles. Diflubenzuron was more effective against larvae than adults at 5, 7 or 10 d post exposure. This data provide valuable information on the potential use of modern insecticides which have a lower toxicity to the environment.

An integrated resistance management based approach is required for successful management of lesser mealworm in poultry houses. Cultural practices such as cleaning

spilled feed in feed storage areas or outside the poultry houses, checking the waterer's for leaks, keeping the manure dry, and removal of litter in winter months could help in keeping the populations low inside the poultry house. The pheromones can be used to detect early infestations and to attract and kill beetles in spot treatments rather than spraying the whole poultry house. Pheromones can be used in traps around the poultry houses to catch any beetles coming towards the house. Development of new chemistry insecticides and rotating different classes of insecticides will also help in delaying the resistance in these beetles against insecticides.