ABSTRACT

CALIBEO, DAWN RENEE. Role and Mitigation of Two Vectors of Turkey Coronavirus, *Musca domestica* L. and *Alphitobius diaperinus* Panzer. (Under the direction of D. Wes Watson.)

The darkling beetle, *Alphitobius diaperinus*, is a cosmopolitan pest of stored products and commercial poultry facilities. Considered the most important pest in North Carolina poultry production, darkling beetles damage poultry house insulation, affect bird performance when consumed by poultry and have been implicated in the transmission of over thirty avian diseases including turkey coronavirus (TCV). Common litter management practices include the removal of used poultry litter for use as organic fertilizer. Land applied litter may serve as a source of re-infestation or dispersal of darkling beetles to nearby farms or residential areas. This study evaluated the impact of mechanical incorporation of used poultry litter into field soil on darkling beetle emigration from land applied litter. Mechanical incorporation of poultry litter into field soil was shown to reduce beetle emergence from land applied litter. Mechanical incorporation was effective in both clay and sandy soil types. Application of litter in cold weather had a negative impact on beetle survival. Another common premise pest in poultry facilities is the house fly, *Musca domestica*. Turkey coronavirus was isolated from house fly crops up to nine hours after flies imbibed virus infected media. TCV was not isolated from house fly mid- and hindgut tissues. Turkey poults tested positive by indirect immunofluorescent assay and serology after exposure for 24 hours to TCV infected house flies, demonstrating the potential of the house fly to act as a mechanical vector of TCV. The house fly and the darkling beetle are both inhabitants of poultry

facilities and demonstrate the potential to act as mechanical vectors of TCV. Incidences of the darkling beetles dispersing by flight, although possible, are rare while the house fly is capable of dispersing several kilometers. The role of the darkling beetle in transmission of TCV remains a concern, however, the scope of the transmission appears limited to the poultry facility. The house fly is likely an important vector of TCV between facilities.

Role and Mitigation of Two Vectors of Turkey Coronavirus, *Musca domestica* L and *Alphitobius diaperinus* Panzer.

by

DAWN RENEE CALIBEO

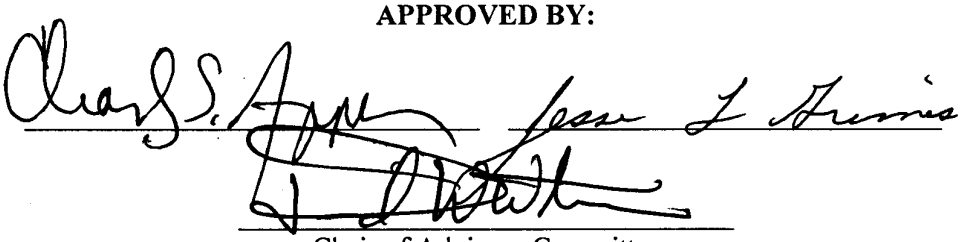
A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

ENTOMOLOGY

Raleigh

2002

APPROVED BY:



Chair of Advisory Committee

The miracle isn't that I finished...

The miracle is that I had the courage to start.

- John "The Penguin" Bingham

Biography

The author was born in Ft. Lauderdale, Florida at the Holy Cross Catholic Hospital on September 20, 1970 to Patricia Darlene Pace. Dawn grew up in and around Citrus County, known as the "Nature Coast" of Florida and winter residence of the manatee, and endangered aquatic mammal.

After graduating from Citrus High School in 1988, Dawn attended classes part-time at Central Florida Community College in Ocala, Florida and worked full-time at various jobs to support her educational endeavors. In 1993, Dawn earned an Associate of Arts degree in biology.

Dawn was accepted at the University of Florida and began taking courses in 1997 while working at the USDA Agricultural Research Service in Gainesville, Florida. Dawn majored in Zoology/Pre-Med and minored in Entomology and earned her Bachelor of Science degree in 1999.

Graduate studies leading to a Master's degree in Entomology were begun in 2000 at North Carolina State University in Raleigh. Dawn married Thomas W. Hayes in Chapel Hill, North Carolina in July 2001. A Master of Science degree in Entomology was conferred in 2002.

Acknowledgements

My deepest gratitude goes to my advisor, Dr. D. Wes Watson who took the job of "advisor" literally. Through Wes's guidance and assistance on this project I learned to be a better researcher. Wes shared his experiences and ideas, gave advice and described goals. I understand now that this one project is part of a larger body of work and one that will potentially define a career. I understand that the answers we find only lead to more questions. Through Wes's mentorship I learned to be a better scientist.

I would also like to thank the members of my committee, Dr. Charles Apperson and Dr. Jesse Grimes for their advice, support and valuable time and Dr. James Guy for inviting me to collaborate on the TCV project.

The technical assistance provided by Steve Denning is very appreciated and he should be applauded for his skill at constructing almost any piece of lab equipment from PVC.

Thank you Mike Stringham, Ellen Honeycutt, and James Barnes for help with the field work even when the manure was up to our knees, we were in danger of suffering heatstroke or had to use a pick ax to install the pitfall traps in frozen ground. Thanks also to the farm crew who prepared the field site each season.

Thanks to those who lent an ear, told a joke or bought a beer: Clyde Sorenson, Fred Hain, Felton Hastings, Dan Mott and Ron Stinner. Thanks to the support staff; Noreen Smith, Donna Nye, Pat Robertson and Gene Dupree for making students' lives a

little easier. Thanks to my fellow students for encouragement and critique and for setting such high standards.

Thanks to my family from whom I drew my inspiration of higher education.

A very special thanks to my husband, Tom who helped me find the courage to begin and the strength to finish.

Table of Contents

List of Tables

List of Figures

Chapter One

Statement of the Problem

North Carolina is a leading producer of poultry in the United States. Annual turkey production is approximately 1.37 billion pounds. Other important poultry commodities include broilers and table eggs with a combined income of $2.3 billion in 1993, comprising about 30% of the total value of agricultural products in North Carolina. Poultry related industry employs over 25,000 workers and generates an annual income of nearly $800 million (Vukina and Roka, 1995).

In response to the increasing demand for poultry products, poultry production is increasing worldwide. Poultry meat production is about 52 million metric tons and egg production is about 707 billion per year (Axtell, 1999). These increases involve the development of large-scale, man-made, highly managed production systems. High density, confined poultry production systems are stable environments with warm temperatures, high humidity and large accumulations of poultry manure that provide an ideal habitat for arthropod pests (Axtell and Arends, 1990).

Two major premise pests associated with poultry production are the house fly, *Musca domestica* (L.) and the lesser mealworm or darkling beetle, *Alphitobius diaperinus* (Panzer). Both insects are associated with the accumulated manure in the pit of a high-rise caged-layer house or the litter of a broiler or turkey growout house. The feeding habits of both insects make them likely candidates for disease transmission. The house fly and the darkling beetle have been implicated in the transmission of several avian diseases.

Recently, two diseases have impacted the North Carolina turkey industry, turkey coronavirus (TCV) and Poult Enteritis Mortality Syndrome (PEMS). TCV is an enteric

disease of turkeys characterized by reduced appetite, weight loss, diarrhea and dehydration. TCV is highly contagious and exposure to contaminated feces is considered a primary route of infection. TCV is sometimes associated with Poult Enteritis Mortality Syndrome (PEMS), an emerging disease of turkeys that originated in North Carolina. PEMS infection results in sudden, high mortality. Surviving chicks are unthrifty, stunted, immunocompromised and unable to survive other infectious agents. In laboratory studies, the darkling beetle was shown to have limited vector competency in the mechanical transmission of TCV and PEMS associated TCV (Watson et al., 2000). Turkey embryos inoculated with whole body and gut homogenates of beetles exposed to TCV tested positive for TCV by indirect immunofluorescent assay for at least one hour but not more than twelve hours. The darkling beetle may be involved in transmission of TCV in poultry facilities where the birds have access to the litter inhabiting beetles. The role of the darkling beetle and the house fly in the transmission and dissemination of diseases is an important consideration.

It is important to recognize the differences in poultry production systems and how they may contribute to pest and disease management. Axtell (1999) characterized modern commercial poultry production facilities as broiler-breeder, turkey-breeder, growout, caged-layers, or pullets. Broiler-breeder facilities are for the production of fertile eggs to hatch into broiler chicks. The birds are maintained in a one-story house with a dirt or concrete floor covered with wood shavings. Raised slats on the outer one-third of each side of the house provide areas for the birds to lay eggs and obtain food and water. Birds defecate while eating, allowing the manure to accumulate under the slats.

Turkey-breeder facilities are also for the production of fertile eggs and are similar to broiler-breeder facilities except there are no raised slats; rather, nest boxes are placed on the floor. Broilers or turkeys to be sold for meat are raised in growout houses. These facilities are single story with a dirt or concrete floor covered with wood shavings or some other absorbent material. Young birds are confined to a portion of the house by temporary partitions that are moved, as the birds require more space. Caged-layer facilities are for the production of table eggs. Birds are housed in cages arranged in tiers elevated above the floor. In single story houses, manure accumulates on the floor directly below the cages and is removed daily to monthly. High-rise deep pit caged-layer houses are two stories with the birds in the upper level. Manure accumulates in the lower level to be removed every one–four years. Pullets (chickens) or poults (turkeys) are grown to replace broilers, laying hens, or turkey growouts. Pullets may be produced using any of the above methods.

In North Carolina, the typical turkey house has an earthen floor to which approximately 8-10 cm of wood shavings or similar material is added. Wood shavings mixed with manure and feed are known as "litter". At the end of the growing season this litter may be re-used or refreshed by the addition of 4-8 cm of additional shavings. Periodically the litter is removed and may be used as an organic fertilizer. Land applied litter infested with darkling beetles and house flies is a potential source of dispersal, re-infestation and disease transmission. Mechanical incorporation of poultry manure into field soil has been shown to negatively impact house fly survival in land applied manure.

Little is known about the survival and movement of darkling beetle adults in land applied litter, and the associated potential disease transmission risk.

The objectives of this study were:

1. Define the risk factors associated with land application of used poultry litter in the inter- and intrafacility transmission of TCV.

2. Evaluate the impact of mechanical incorporation of used poultry litter onto field soil on darkling beetle survival.

3. Evaluate the vector competency of the house fly in mechanical transmission of TCV and compare against darkling beetle adults.

Chapter Two

Review of the Literature

Taxonomy

Alphitobius diaperinus, the lesser mealworm, (Appendix II, Fig. 1) belongs to the order Coleoptera and the family Tenebrionidae (Arnett, 1963). The generic name was published in 1832 by Stephens and the species name described by Panzer in 1794 (Spilman, 1966). Later it was determined that Panzer had described the generic name in 1829 with the species name *picipes*. *Helops picipes*, a tenebrionid named by Panzer in 1794, was later determined to be a synonym of *Alphitobius* and Panzer was credited with both the generic and species names (Spilman, 1966). The adult form of the lesser mealworm is known by the 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 (Swatonek, 1970; Nolan Jr., 1982; Guatam, 1989). *Alphitobius laevigatus* closely resembles *A. diaperinus* and inhabits similar environments. Characters for separating these two species are given by Preiss and Davidson (1970). Karyometrical analysis suggests that the darkling beetle arose from *Tribolium castaneum* and *Sphenariopsis imploita* by loss of a sex chromosome (Sharma et al., 1977).

Biology, Physiology and Ecology

Egg Stage

The egg of the darkling beetle is creamy white and elliptical with an average length of 1.0-1.4 mm and an average width of 0.4-0.5 mm. Egg hatch requires 3-13 days (Lancaster and Simco, 1967; Barke and Davis, 1969; De Las Casas, 1970) and is

temperature dependent (Preiss 1971). Preiss and Davidson (1968) reported that at temperatures below 21.1º C and above 37.8º C eggs failed to hatch. The highest rate of egg hatch occurred at 26.7º C. Rueda and Axtell (1996) reported that no egg hatch occurred at 17º C and for temperatures 20, 25, 30, 35, and 38º C, average egg development times were 13.4, 6.0, 4.4, 2.6, and 2.6 days, respectively. Egg hatch is also affected by relative humidity. At 26.6º C, eggs hatched at relative humidities that ranged from 7-81%. Greatest hatch percentage occurred at 68-71% relative humidity (Barke and Davis, 1969, Preiss 1971). Eggs are deposited in cracks and crevices and are attached to the substrate with a clear, sticky substance (Wilson and Miner, 1969).

Larval Stage

Larvae are elateriform, elongate, and cylindrical. Newly eclosed larvae are white but darken to a brownish color as they develop. Larvae progress through 6-12 instars and increase in length from 1.5 mm in the first instar to 10 mm in later instars. The number of instars through which a particular larva will pass is temperature dependent. Thus, the larval period varies from 23-133 days (Lancaster and Simco, 1967; Wilson and Miner, 1969; Ichinose et al., 1980; Rueda and Axtell, 1996). Larval transitions may be quantified by precise measurements of the larval head capsule. Mean head capsule widths for eight larval instars reared at 27º C and 70% relative humidity were: 0.228, 0.228, 0.348, 0.478, 0.721, 1.061, 1.208, and 1.339 mm, respectively (Fransisco and Prado, 2001).

Pupal Stage

The pupae are white in color and exarate. Female pupae average 5.9 mm in length and males average 5.5 mm in length. Both sexes average 2.2 mm in width (Preiss, 1971). The sex of the pupae may be determined by observing the developing genitalia under magnification. Females possess second valvifers on the ventral posterior of the abdomen. Males lack these appendages (Barke and Davis, 1967). The pupal stage generally lasts from 4-17 days and is temperature dependent (Rueda and Axtell, 1996). Preiss (1971) reported that pupae did not survive temperatures below 15.6° C or above 43.3° C. Pupation may be extended by short term (2-4 days) exposure to temperatures of 0-1° C (Preiss, 1971). Pupae may be found in the soil of the floor of a poultry house or in tunnels in the insulation in the walls. Pupation may also occur on the surface of the soil or concrete floor under the litter layer or in cracks and crevices in the house structure itself.

Adult Stage

The adult darkling beetle is a small, black and shiny. The pronotum is textured with a fine stipple and the elytra are finely grooved (Sarin, 1980b; De Grisse and Bohyn, 1985). Teneral adults are initially creamy white and change gradually to a red-brown, dark brown and finally black over a period of 7 days. There is a 1-1 sex ratio of males to females (Preiss, 1971). The males and females are nearly identical in appearance and range in size from 5.1- 6.1 mm in length (Wilson and Miner, 1969; Pospischil, 1996). The sexes may be separated by observing the meso- or metathoracic tibial spines. Both

sexes have a pair of spines. In females, both spines are straight, while in the male one spine is curved and the other straight (Hewlett, 1958). This is a difficult method of separating the sexes as it must be performed under magnification and live beetles move their legs about to rapidly to easily see the tibial spines. Another method to separate the sexes is to observe the posterior edge of the eighth sternite. In males, this edge will be deeply emarginate. The females' sternite will be straight (Barke and Davis, 1967). Preiss (1971) applied gentle pressure to the abdomen with forceps and extruded the genitalia.

Mating

"Calling", in insects, is communication of sexual receptivity by means of visual or chemical cues. Calling behavior in darkling beetles consists of headstands, wing fanning, wiping the tip of the abdomen with hind legs and dragging the abdomen on the substrate (Falomo, 1986). Falomo observed that darkling beetle mating was characterized by a specific behavioral sequence. In males, searching and calling were followed by recognition and/or excitation, mate grooming and/or antennation, then copulation. In females, the sequence was calling, mate recognition and/or acquiescence, mate grooming and/or antennation, and copulation (Falomo, 1986). Copulation varied from 15 seconds to 34 minutes and averaged 11.4 minutes. A single pair of adults can mate more than once and a receptive female will mate with more than one male (Falomo, 1986).

Reproduction

De Las Casas (1970) reported that males reach sexual maturity 48 hours after becoming adults yet Hopkins et al. (1994) found male beetles require seven days to reach sexual maturity. Female mating activity corresponds with an increase in neurosecretory cells in the brain, which may initiate oocyte maturation (Gangopadhyay et al., 1985). In the presence of males 7+ days old, females were able to mate as early as 3 days old. Otherwise, females became receptive to mating at 7 days (Hopkins et al., 1992).

Preoviposition for adult beetles averaged 12.7 days at 21.1°-23.9° C (Preiss and Davidson, 1971). The average number of eggs deposited by the female was 3.5 per day or 104.7 per month at room temperature (Preiss, 1971). Fecundity was positively correlated with increase in temperature with optimum fecundity at 32.2° C. Development from egg to adult occurred most rapidly at 32.2° C. At this temperature, development occurred in 45.6 days (Wilson and Miner, 1969).

Pheromones

Darkling beetle adults and larvae tend to form large aggregations suggesting an aggregation pheromone. Adult darkling beetles possess a pair of eversible posterior abdominal glands (Wilson and Miner, 1969) described as follows: "Adults of both sexes possess a pair of large, fleshy scent glands that protruded when pressure was applied to the posterior region of the abdomen. The fluid obtained by puncturing the extruded gland produced a musky odor and caused sexual excitement in both sexes." These observations suggested the presence of a sex and/or aggregation pheromone. In experiments using live

beetles and a convection current olfactometer, no evidence of a sex pheromone was found (Preiss, 1971). Tseng et al. (1971) described the morphology of the paired glands and found the glands to be equally well developed and morphologically identical in both sexes. Each gland consisted of two structures: a large bifurcating sac that serves as a reservoir for the secretory fluid and the glandular secretory cells. Infrared analysis of the gland secretion resulted in identical spectra for males and females. Thin layer chromatography, gas chromatography and mass spectrophotography revealed the major components of the gland secretion to be quinones and hydrocarbons. Quinones are known to be defensive compounds and are common defensive secretions in tenebrionid beetles (Tseng et al., 1971).

Falomo (1986) observed that while calling, male and female adult beetles rubbed the seventh and eighth abdominal tergites with the hind tarsi in a wiping motion as if to disperse pheromone into the air. Scanning electron microscopy revealed the presence of setiferous patches on the dorsal abdomen. Adults of both sexes were attracted to solvent extracts of the patch secretions suggesting a possible pheromone source. Live adults were allowed to contact filter paper for prescribed periods of time. The papers were then washed in solvent, concentrated and subsequently used in olfactometer bioassays. Unmated males 30-32 days-old responded to extracts collected from females 3-4 weeks-old with behaviors consistent with sexual excitement (Falomo, 1986). Male beetles locomoted rapidly to the source of the extract, extruded genitalia, performed headstands, made rapid circular turns, flapped wings and initiated flight. Extracts of females 1-2 weeks old were attractive to both sexes and all age groups except males 12-14 days old,

an age at which males are considered to have reached sexual maturity. Extracts collected from 1-2 week-old males were attractive to both sexes but only significantly to females 12 to 14 days old. Falomo (1986) suspected a complicated pheromone system whereby both a sex pheromone and an aggregation pheromone are secreted by both male and female adult darkling beetles.

Diet

Food preferences of the darkling beetle have been the subject of several studies. The larvae feed on a wide variety of stored grains, feeds, fruits and rice (Vaughan, 1982). Using developmental time as a measure of food preference, Sarin and Saxena (1975) reported that larvae preferred broken and whole wheat to urd (mung bean), cowpea, barley and rice. Larvae failed to complete development on gram (chickpea), peanut and corn. Wilson and Miner (1969) reported increased survival in larvae that fed upon spoiled grains. Larvae readily consume manure, moribund and dead animals and, in conditions of starvation and overcrowding, become cannibalistic.

The diet of the adult darkling beetle has been described as omnivorous, granivorous, carnivorous, predaceous and cannibalistic. Adult darkling beetles have been found feeding on live chicks, pigeon squabs, snakes and rats (Harding and Bissell, 1958; Harris, 1966; Crook et al., 1980; Vaughan, 1982; Kumar, 1983). In a choice test of four grains, in order of preference, adult beetles preferred cowpea, broken wheat, urad (lentil), and whole wheat (Sarin and Saxena, 1975). Hulley and Pfleiderer (1988) found that adult darkling beetles will feed on manure, house fly (*Musca domestica*) eggs and small live

house fly larvae. Darkling beetles readily fed on freshly killed house fly larvae and adults if the cuticle was damaged. A study of the digestive enzymes present in the darkling beetle found protease, lipase, amylase, invertase, maltase, lactase, and cellulase (Sarin, 1973). The darkling beetle requires a diet high in protein (Sarin, 1972). Morphological studies of the digestive system revealed the lack of cecae or regenerative crypts, confirmation of an omnivorous or scavenging feeding habit (McAllister et al., 1995a).

Survival Under Adverse Conditions

In addition to the ability to survive on nearly any foodstuff, the darkling beetle can withstand short periods of starvation. Early and midstage larvae survived an average of 10.6 days without food and water (Rahman et al., 1991). Late stage larvae survived starvation by pupating. The alimentary canal in the darkling beetle is approximately three times the length of the body and may afford the beetle food storage capacity during periods of scarcity. Newly emerged adults survived an average of 19.5 days without food or water. Older adults survived a slightly longer period of time (Preiss, 1971).

The rectal intima of the darkling beetle has a thick laminar cuticle that aids in the conservation of water (McAllister et al., 1995a). At temperatures ≤ 40° C, darkling beetles were able to absorb water from the atmosphere, even in environments of 0% relative humidity (Salin et al., 1999). At temperatures > 40° C, the adults became very agitated and locomotive and subsequently entered a coma state. Increasing the temperature a few degrees resulted in death, presumably from the rapid evaporation of

water vapor in the spiracular system. This “thermostupor point” (TSP), the highest temperature at which insect movement is observed (Vannier, 1987), was higher in dry air than in moist air, 47.4° versus 46.6° C, respectively. Extreme tolerance to dry environments was illustrated by Salin (1999) who placed adult darkling beetles in a dessicator with silica gel (0 - 5% relative humidity). After 12 days the beetles had lost 25% of their initial water weight and survived. In a similar experiment, beetles reared on ground wheat and corn for eight months without water survived a 17% loss of water weight (Salin, 1999).

Chill-coma, the low temperature at which insect activity ceases, is 6° C (Renault et al., 1999). Insects may recover from a chill-coma after as many as 21 days. The supercooling temperature of the adult beetle is -14.5° to -10.3° C. Under starved conditions the supercooling temperature was reduced to -17° to -11.2° C. This may contribute to beetle survival during poultry house clean-out when the heat is turned off and no food is available (Salin et al., 2000a).

Habitat

Adult and larval darkling beetles are found in the pit of high rise poultry houses at the lower portion of the manure cone (Stafford III and Collison, 1988; Wills and Mullins, 1991) and in broiler houses and turkey growout houses in and under the litter and any objects that might be lying on the surface of the litter e.g. feeders, dead birds, nest boxes (Lancaster and Simco, 1967). In a sampling survey of a heavily infested turkey growout house in England, Cogan et al. (1996) found 90% of the beetles under or near the support

pillars and feeders. Strother and Steelman (2001) found darkling beetle population densities increased with addition of successive flocks in a growout facility. Beetle populations initially concentrated along the perimeter of the house and spread toward the center as population density increased. Caked manure, areas under the drinkers and the edges of the structure provide moist areas for the insects to inhabit (Salin et al, 2000b). However, the darkling beetle is not found in areas of high moisture content (Despins, 1987) and will migrate up the pit walls to avoid excessively wet manure (Despins et al., 1989). In laboratory assays the darkling beetle demonstrated a preference for 20% relative humidity (Victor and Ogonor, 1987). Chatterjee and Gangopadhyay (1989) suggested that in stored grain adult beetles congregate in "hot spots" in the substrate that are the result of metabolic heat produced by fungi. Due to the narrow range of temperatures and dry conditions this is unlikely in a poultry house.

Adult darkling beetles are extremely long lived. Preiss (1971) reported survival of 700+ days, with a potential lifetime production of 2400 offspring. Summer populations of beetles in poultry farms in North Carolina can reach densities of 10,000 to 20,000 individuals per square meter (Arends, 1987). In laboratory population studies simulating the environmental conditions found in grain storage areas, it was reported that adult populations peak in late August (Sarin, 1978, 1980a). Larval density, however, was greatest in May and there were no larvae present during the months December to March. Hulley and Pfleiderer (1988) reported similar findings from a survey of Coleoptera in poultry manure in a caged-layer house in South Africa. Populations of beetles were highest from March until June and much reduced in the winter months. The darkling

beetle population overwinters in the adult stage (Pfeiffer and Axtell, 1980). With a corresponding increase in temperatures and shortened developmental time, population densities increase in the summer (Voris et al., 1994). Schmitz and Wohlgemuth (1988) found a correlation between larval behavior and poultry flock rearing schedules. The authors reported that larvae migrated to the walls of the house approximately 13 days prior to clean out and remained hidden until the new flock was introduced and the house temperature was again increased. Strother and Steelman (2001) reported increased population size and movement within a house with each addition in a series of flocks.

When certain conditions are met (i.e. starvation, clean-out, high density, seasonal) the beetles may disperse by flight. Occasional flight in the laboratory and in the field has been observed. Unfortunately, the mechanism that induces flight in the darkling beetle is unknown.

Distribution

The darkling beetle is thought to have originated in Africa as an inhabitant of birds nests (Hopkins, 1990). Now cosmopolitan in distribution, the darkling beetle is known more as a stored products pest in grain storage facilities and a premise pest in commercial poultry operations.

A survey of insect fauna of birds' nests in Pakistan and found *A. diaperinus* present in nests of *Passer domesticus, Columbo domestica, Hirundo fluvicola, Corvus splendens, Streptopelia decaocta* and *Coracias benghalensis* (Mohammed et al., 1985) (Table. 2.1). Interestingly, the author notes that *A. diaperinus* is "rarely found in stored

grain products and is a minor pest". Similar work performed in India found adult and immature beetles in the nests of five species of birds in West Bengal (Table 2.1).

Yagi and Razig reported *A. diaperinus* infesting pigeon houses in large numbers in Sudan in 1971. The darkling beetle was reported as a premise pest in poultry houses in Budapest, Chile, New Zealand, Finland, Poland, South Africa, Brazil, and Nigeria (Table 2.1). Surveys of Coleoptera in caged-layer houses in the United States and South Africa showed *A. diaperinus* to be one of the most abundant species present (Pfeiffer and Axtell, 1980; Hulley and Pfleiderer, 1988; Rueda and Axtell, 1997; Banjo and Soyoye, 1999). Livestock facilities in England, Russia and Ireland also reported infestations of darkling beetles. The darkling beetle was reported infesting stored products in Belgium and India (Table 2.1) and as a new species in Italy and the Mediterranean.

Table 2.1. Worldwide distribution of *Alphitobius diaperinus*.

Location	Year	Habitat	Reference
Sudan	1971	pigeon houses	Yagi and Razig, 1972
India	1972	stored wheat	Pajni et al., 1976
Budapest	1973	poultry farms	Nemeseri, 1973
Chile	1973	poultry farms	Pena, 1973
New Zealand	1976	poultry farms	Dale et al., 1976
England	1979	livestock buildings	Le Torc'h, 1979
Finland	1979	broiler house	Silfverberg, 1979
United States	1980	poultry houses	Pfeiffer and Axtell, 1980
Poland	1985	poultry houses	Majchrowicz, 1985
Pakistan	1985	birds nests	Mohammad et al., 1985
Russia	1987	livestock facilities	Mishchenko, 1987
Italy	1986	wild	Ratti, 1986
Ireland	1987	piggery	O'Connor, 1987
Ireland	1988	livestock facilities	Good and Sleeman, 1988
South Africa	1988	poultry houses	Hulley and Pfleiderer, 1988
Brazil	1989	poultry houses	Filho et al., 1989
India	1991	dried milk	Basak et al., 1991
India	1995	birds nests	Bhattacharyya, 1995
Belgium	1996	stored grains	Casteels et al., 1996
Nicaragua	1998	wild	Merkl, 1998
Nigeria	1999	poultry houses	Banjo and Soyoye, 1999
Mediterranean	2000	wild	Fattorini and Leo, 2000

Pest Status

Prior to World War II there were few stored product pests (Yoshida, 1983). With the mechanization of farming, surplus grains and cereals were produced and large scale storage facilities needed. These environments, with controlled environmental conditions, proved ideal for the proliferation of populations of granivorous insects. Meanwhile, large-scale livestock and poultry production was being developed providing a constant and favorable environment for a wide variety of synanthropic insects. Introduced insects to animal production systems from infested grains and feeds successfully colonized the new habitat. Among these, *Alphitobius diaperinus* successfully established itself as a major premise pest in poultry production facilities. Pest status of the darkling beetle includes: 1) damage to the facility by tunneling larvae and adults; 2) reduced weight gains caused by poultry feeding on the darkling beetle; 3) nuisance complaints initiated by neighbors plagued with beetles from land applied litter; and 4) transmission of pathogenic agents (Eidson et al., 1966).

The larvae and adults of the darkling beetle can bore into many types of poultry house insulation materials including polyurethane foam, fiberglass, extruded polystyrene and expanded bead polystyrene (Appendix II, Fig. 2) (Vaughan et al., 1984; Ichinose et al., 1980). Le Torc'h (1983) investigated a variety of insulating materials and found no significant difference in damage caused by darkling beetle larvae. Damage by darkling beetle larvae and adult tunneling can reduce the effectiveness of the insulating material by as much as 20–30% (Vaughn et al., 1984; Despins et al., 1987). Darkling beetles can destroy extruded foam insulation in only 3-4 years (Hinkle and Hickle, 1999). Safrit and

Axtell (1984) observed that larvae bored directly into the wood of a trapping device suggesting that beetles may pose a significant threat to the poultry house structure. The hide beetle (*Dermestes maculatus* DeGeer), another poultry premise pest, has been observed boring into wooden poultry house support posts and is likely the cause of structural damage (Axtell, 1999). The darkling beetle is more likely to climb and bore into insulating materials when population densities are high and when the floor of the house is not comprised of soil (Geden and Axtell, 1987a).

Darkling beetle adults and larvae are active in the litter in poultry houses and populations may become large in number. Broiler chicks readily consume as much as 33 grams live weight of larvae per day (Despins and Axtell, 1995). Consumption of larvae is so great that the chicks' crops become distended and the larvae may be visible through the stretched skin (Appendix II, Fig. 3). Weight gain in chicks consuming only larvae was significantly less than that of chicks fed starter feed (Despins and Axtell, 1995). The difference in weight gain continued even after the chicks were returned a normal diet. The chicks also ingested large amounts of litter while scavenging for beetles and feed consumption was reduced. Skewes and Monroe (1991) attempted to correlate beetle populations in poultry houses with disease related production parameters but were not successful owing to the difficulty of estimating the beetle population at any time. Dry conditions compel the beetle to search for moisture. Moisture starved beetles will crawl onto the birds and chew on the skin at the base of the feathers (Savage 1992). The result is lesions that may be confused with skin leukosis. This annoyance of the birds causes

them to wander at night rather than rest, negatively effecting weight gain and feed conversion.

Land application of beetle infested poultry litter may serve as a dispersal mechanism for the adult beetle to nearby neighborhoods and businesses whereby they become a nuisance and a public relations problem. After one such incident in LaRue, Ohio, local residents filed a $25 million lawsuit against the poultry farm responsible for providing the litter (Jerrard and Wildey, 1980; Miller, 1997; Hinchey, 1997). Darkling beetle adults have been captured in suction traps placed several miles from the nearest granary or farm (White et al., 1995).

Occasionally, darkling beetles have been purposefully introduced into poultry facilities for control of other insect pests (Davidson, 1987; Wallace et al., 1985). Guatam (1989) observed that darkling beetle larvae readily consumed the eggs of the moth *Sitotroga cerealella*, a stored grains pest and Dass et al. (1984) observed adults and larvae feeding upon the eggs and larvae of *Corcyra cephalonica*. The darkling beetle has also been reported as an effective house fly predator (Despins et al., 1988; Armitage, 1986; Propp and Morgan, 1985; Legner et al., 1975). The afore mentioned incident in LaRue, Ohio, demonstrates how biological control using the darkling beetle may not produce the intended results (Miller, 1997). Watson et al. (2001) reported that the beetles actually have a negative impact on house fly predators such as *Carcinops pumilio*. In deep pit caged-layer houses, the tunneling activity of the beetles dried the manure and resulted in a less favorable substrate for house fly reproduction (Geden, 1990). However, the potential to cause structural damage and ability to vector avian disease precludes the

darkling beetle as a biological control agent (Axtell, 1999; Prudnikova, 1991; Geden, 1990).

Some individuals may develop allergy to darkling beetles. Falomo (1986) describes symptoms of allergic angioedema that manifested by severe swelling of the ankles, feet, hands, eyelids and tongue. The cause of the allergic reaction was determined to be exposure to the darkling beetle in the laboratory.

Disease Transmission

The first report of disease transmission involving the darkling beetle was Eidson et al. (1966) who described induction of tumors associated with Marek's disease by inoculation of chicks with suspensions of homogenized beetles. The darkling beetle has since been implicated as a potential vector of several avian bacterial, viral and fungal diseases.

Bacteria in the genera *Micrococcus, Streptococcus, Staphylococcus, Serratia, Klebsiella, Pseudomonas, and Salmonella* have been isolated from the darkling beetle (De Las Casas et al., 1968; De Las Casas, 1970, 1972; Olsen and Hammack, 2000). Harein et al. (1970) isolated 48 serotypes of *Escherichia coli*, 26 of which were pathogenic. Des Las Casas et al. (1973, 1974, and 1976) isolated fifteen serotypes of *E. coli* from the darkling beetle that were virulent to poultry. McAllister et al. (1996) found the darkling beetle to be a competent reservoir of pathogenic *E. coli.* After exposure to *E. coli*, bacteria were detected in and on the insect for 12 days. *Escherichia coli* was detected in feces 6-10 days post exposure but not following a larval molt. Broilers that

consumed the infected larvae were more likely to have cloacal swabs positive for pathogenic *E. coli* than control birds.

McAllister et al. (1994) found the darkling beetle to be a competent reservoir for *Salmonella typhimurium*, a leading cause of foodborne illness in humans. In larval and adult darkling beetles, *S. typhimurium* was detected from surface swabs or whole body homogenates 16 days post exposure and was isolated from insect feces for 24 days following a single 24 hour feeding. One-day-old broiler chicks tested positive for *S. typhimurium* by cloacal swab after consuming only one infected insect. *S. typhimurium* was detected in the feces of one insect after the insect molted into an adult, suggesting the possibility of transstadial transmission.

Darkling beetle larvae exposed to feces containing enteric pathogens and subsequently consumed by turkey poults caused symptoms indicative of enteritis (watery feces, inactivity, and reduced weight gain) within 2 days (Despins et al., 1994). Exposed larvae that were surface sterilized were also able to transmit pathogens.

Darkling beetles harbor reovirus 24, turkey coronavirus (TCV) and viruses that cause fowl pox, Newcastle disease, avian influenza and infectious bursal disease in poultry (Des Las Casas et al., 1973, 1976, Wilson et al., 1986; McAllister et al., 1995b, Watson et al., 2000).

Goodwin and Waltman (1996) isolated pathogens from beetles, including *Eimeria*, the causal agent of intestinal coccidosis. Coccidosis has been induced in poultry by feeding the birds infected adult darkling beetles (Reyna et al., 1982). The darkling

beetle is the intermediate host for *Hymenolepsis minutissima*, a causal agent of fowl cestodes (Gogoi and Chaudhuri, 1982).

Darkling beetles harbor fungi in the genera *Aspergillus* and *Candida* (Des Las Casas, 1970, 1972) and the mycotoxin, F-2 (Eugenio et al., 1970). F-2 is produced by *Fusarium roseum*, a grain fungus. The toxin is responsible for deformation of sex organs and abortions in swine.

Morphological studies of the darkling beetle digestive system revealed the insect lacks a crop (McAllister, 1995a), a condition indicative of continuous feeding. This, coupled with the omnivorous, scavenging feeding habits of the darkling beetle, has implications of potential vectorship of pathogenic organisms. Continuous feeding allows large populations of pathogenic organisms to increase in number without biological amplification.

Control

The initial step of any successful control program is accurate monitoring of the target insect population. Safrit and Axtell (1984) suggested two trapping devices to be used for monitoring *A. diaperinus* in poultry houses. The first is a "pan trap" consisting of small panels of an insulating material (i.e. styrofoam, foil covered polyisocyanurate) placed on the surface of the litter and covered with a metal pan. The pan must be staked to the substrate to prevent the birds from disturbing the trap. The authors report that this trap is the most effective at capturing the insects but that it can be awkward to use. The second alternative is the "Arends tube trap" suggested by J.J. Arends. This trap consists

of rolled-up corrugated cardboard inserted into a length of PVC pipe. The pipe is placed lengthwise on the surface of the litter and staked to the substrate. Successful and convenient sampling can be accomplished by placing the traps in the open center area of the poultry house.

Insecticide treatment is normally carried out in deep litter broiler and turkey growout houses during clean-out cycles after litter removal and before introduction of the new flock. Many insecticides have been banned or are no longer in use. For historical reference a brief discussion of their use follows.

Lancaster et al. (1969) tested several chemical insecticides in actual broiler house conditions. Dursban and ronnel at high doses were 100% effective for eight and three weeks respectively. Concerned the high rates were not economical or could result in residues, the authors repeated the test with lower doses. Dursban and ronnel were 100% effective for only one to two weeks, insufficient time to eliminate the beetle population. Fosfotion, a Czech product, was found to be effective against darkling beetles when applied topically (Blahutiak and Barus, 1969). Also tested were Soldep, lindane, DDT and aldrin but were found to be ineffective or too toxic to mammals to be used in poultry facilities.

As contact poisons, mevinphos, fenitrothion, pyrethrum, and lindane were found to be 22, 9.6, 2.8, and 2 times more toxic, respectively, than DDT to darkling beetles (Saxena and Sarin, 1972). In Germany, Heimbucher and Kutzner (1979) found applications of 2% Dursban (Gesektin K) and a 0.4% mixture of permethrin and pyrethrum (Permanent) to be effective at controlling darkling beetles on a laying-hen

farm. Methoprene and fenoxycarb, insect juvenile hormone analogs, have been found to be 100% effective for twelve weeks in laboratory assays against larval darkling beetles (Edwards and Abraham, 1985). Weaver and Kondo (1987; Weaver, 1996) reports 95 - 100% control with the insect growth regulators; hexafluron, triflumeuron and UC84572T, and the pyrethroids cyfluthrin and permethrin. In field trials, feed treated with benzoylphenylurea compounds eliminated larvae after eight weeks (Miller and Redfern, 1988). Miller (1990) found that a poultry feed treated at a rate of 2 ppm with ivermectin effectively reduced beetle populations in field trials. Ivermectin residues were not apparent in poultry liver tissue, however, the birds suffered reduced body weight, weight gain and feed efficiency. The botanical insecticide neem was found to be effective against beetles and has the added benefit of delayed resistance (Azmi et al., 1993). Larvacidal benzoylphenylureas were also effective against darkling beetles (DeMilo et al., 1995). Cogan et al. (1996) evaluated residual applications of four insecticides for control of *A. diaperinus* in turkey growout houses in England. Single applications of iodofenphos SC, fenitrothion WP and permethrin WP were ineffective. Azamethiphos reduced mean beetle counts from over 50 per sample to less than one over a four-month period. Toyoshima et al. (1996) performed bioassays regarding sensitivity of the imago stage to certain insecticides. In filter paper assays, the insects were highly sensitive to the following chemicals: naled, metrifonate, carbaryl, fenitrothion with permethrin, and fenitrothion with resmethrin and pipernyl butoxide. Tabassum et al. (1998) studied the toxicities of Danitol 10 EC (fenpropathin) and neem extract RB against darkling beetle adults. Concentrations of 1.98 µg/cm^2 Danitol in water and 117.80 µg/cm^2 neem RB in

methanol induced mortalities of 88 and 70%, respectively. Tetrachlorvinphos applied as a dust during clean out was effective for five weeks (Kahn et al., 1998).

Potential drawbacks to chemical control are restrictions imposed by Federal and State agencies on use of chemicals in the presence of live animals, the development of insecticide resistance and the adverse effect on beneficial arthropods that inhabit the litter. Another consideration is the behavior of the beetle. Pupae tunneled into the soil floor may not be affected by topical insecticide applications. Also, during clean out, when insecticide application is usually made, beetles may hide in cracks and crevices or migrate up the walls to areas that the insecticide cannot penetrate (Lancaster and Simco, 1967; Wohlgemuth, 1989). Proper application of chemical insecticides is essential. The common practice of combining insecticides with disinfectants in a single tank might reduce both insecticidal and bactericidal efficacy (Geden et al., 1987).

One possible alternative to chemical insecticide treatment for the control of the darkling beetle is microbial control. Several studies have been conducted to assess the efficacy of various bacterial and fungal agents against this pest.

Beauveria bassiana applied as an aqueous solution and as a starch dust formulation was effective against the darkling beetle in laboratory assays (Steinkraus et al., 1991; Geden et al., 1998; Crawford et al., 1998. Larvae were more susceptible than adults at comparable rates of conidia application. The environmental conditions in a poultry house; earthen floors, constant warm temperature and high humidity, are favorable conditions for *B. bassiana* propagation suggesting that this fungus may be an effective biocontrol agent (Steinkraus et al., 1991). Entomopathogenic fungi in the genus

Acremonium also had activity against the darkling beetle (Steenburg and Humbert, 1999).

Toxin from the bacterial strains *Bacillus thuringiensis* (BT) *finitimus, wratislaviensis, alesti, japonesis* and others has been tested against the darkling beetle. Ten field isolates of BT from Poland and two commercial microbial insecticides, Biobit® and Thuridan®, were tested against the darkling beetle in laboratory assays. The insects tested showed little susceptibility to any of the isolates or commercial preparations. The highest mortality (9.9 %) was induced by *B. thuringiensis finitimus* (Lonc et al., 2001).

Protozoan infections are common in darkling beetles collected in poultry houses in North Carolina. *Gregarina alphitobii* (Eugregarinorida, Gregarinidae) and *Farinocystis tribolii* (Neogregarinorida, Lipotrophidae) were isolated from both adult and larval darkling beetles (Steinkraus et al., 1992). An evaluation of these infections determined them to be chronic and not at levels high enough to illicit an epizootic. However, protozoan infection may increase the deleterious effect of a stressful condition (i.e. starvation, crowding) or infection by another pathogen (Apuya et al., 1994). Bala et al. (1990) found darkling beetles in Africa, Asia, Europe and the United States infected with the protozoans *G. alphitobii* and *Mattesia alphitobii*. *Mattesia alphitobii* is highly infective and pathogenic, destroying the insect's fat body and reducing fecundity and longevity.

Some control of darkling beetle populations may be attained by the use of entomopathogenic nematodes in broiler houses with earthen floors (Steenberg et al.,

1998). Three nematodes were evaluated for infectivity against the darkling beetle on the following substrates: petri dish lined with filter paper, artificial rearing medium, used poultry litter, manure, clay soil and sandy loam type soil. *Steinernema feltiae* was infective against all life stages of darkling beetle on and on all substrates (Geden et al., 1985). Subsequent laboratory assays resulted in mortality rates of 56 to 94% in darkling beetle larvae exposed to various strains of *S. feltiae*. In field studies, populations of darkling beetles increased more slowly for three weeks following litter clean out and application of nematodes. However, after ten to thirteen weeks, populations in treated houses were equal to those in control houses (Geden et al., 1987b). A similar study determined that nematodes did not survive longer than five weeks at temperatures greater than 24º C (Geden and Axtell, 1988) suggesting that high temperatures in the poultry house during the nematode study might have resulted in the elimination of the nematode population before control was achieved.

Acarophenax mahunkai, a predaceous mite, attacks eggs of *A. diaperinus*. In laboratory studies, the female mites parasitized 51% of the egg masses in a beetle colony (Steinkraus and Cross, 1993). The authors concluded this mite had little biological control significance.

Depopulation and thorough cleaning of the house may aid in reducing beetle populations (Geden, 1989). Litter treated with alum and shredded paper litter treated with boric acid reduced beetle populations in broiler houses (Worley et al., 1999, 2000). Insulation materials that were encased in aluminum foil or heavy paper and had tape sealed joints effectively reduced beetle damage (Wildey, 1983; Turner Jr., 1986).

Impregnating the insulating materials with insecticide or painting them with insecticide impregnated paint also afforded control against damage (Mekada et al., 1983, 1984; Vaughan and Turner, 1984; Despins et al., 1991). Constructing the pit of high-rise caged-layer houses from concrete reduced damage to insulation (Despins et al., 1989). Mechanical barriers of polyethylene terepthalate (PET) were 100% effective at preventing darkling beetle larvae from climbing to insulation (Geden and Carlson, 2001). Barrier effectiveness was reduced as fly fecal spots accumulated on the surface, however, removal of fecal spots restored barrier effectiveness. Stafford and Collison (1987) found a relationship between arthropod populations in high-rise caged-layer houses and the temperature of the house and the manure in the pit suggesting that darkling beetle control may be attained by maintaining the temperature in the house below that at which larval development occurs. Maintaining dry manure though adequate ventilation and attention to watering devices reduced beetle and house fly populations (Turner Jr., 1986). Treating infested feed with gamma radiation may aid in preventing the spread of the pest (Zaedee and Ignatowicz, 1993) or reduce occurrence in stored grain areas (Lorenzo, 1990).

Chapter Three

Evaluation of the Impact of Mechanical Incorporation of Poultry Litter into Field Soil on Darkling Beetle (*Alphitobius diaperinus*) Emergence

Abstract

Darkling beetle (*Alphitobius diaperinus* Panzer) survival and emergence from field soil was evaluated in a controlled experiment simulating land application of turkey litter and later in field studies. Under controlled conditions, adult darkling beetles were buried in Cecil red clay at depths of 0, 8, 15, 23, and 30 cm (0, 3, 6, 9, and 12 inches). Beetles emerging from the soil were counted 1, 3, 7, 10, 13, 17, 21, 24, and 28 days following burial. Fewer beetles emerged to the surface at depths of 23 and 30 cm (7.5 ± 0.01and 6.7 ± 2.31, respectively) as compared to 8 and 15 cm (9.5 ± 0.99 and 10 ± 2.62, respectively) but differences were not significant. Beetles survived at least 28 days buried in the soil at depths ≤ 30 cm. In seasonal field studies, darkling beetle emergence from clay soil was compared following disk, mulch and plow incorporation of poultry litter to no incorporation. Mechanical incorporation of poultry litter reduced beetle emergence (df = 3, F = 13.83, $p \leq 0.0001$), although no incorporation method was superior. Beetle activity was greatest following land application of litter during the warm season and reduced during colder months. Generally, darkling beetle emergence decreased with time and few beetles emerged from the soil 28 days after litter was applied. In a similar study, mechanical incorporation of poultry litter into sandy soil reduced beetle emergence (df = 3, F = 14.76, $p \leq 0.0001$). In sandy soil, disk and plow treatments significantly reduced beetle emergence as compared to control.

Introduction

The darkling beetle, *Alphitobius diaperinus* Panzer is often found in great numbers in poultry litter (Pfeiffer and Axtell, 1980; Rueda and Axtell, 1997). High beetle densities concern producers because they vector avian bacterial, viral and fungal pathogens. Darkling beetles harbor bacteria in the genera *Escherichia*, *Salmonella*, *Bacillus*, and *Streptococcus* and viruses that cause leukosis and infectious bursal disease in poultry (Despins et al., 1994; McAllister et al., 1994, 1995b, 1996; Goodwin and Waltman, 1996). Recently, transmission of turkey coronavirus by darkling beetles was demonstrated under laboratory conditions (Watson et al., 2000). Broiler chicks and poults readily consume darkling beetle adults and larvae aiding in the transmission of pathogenic organisms. Consumption of darkling beetles also negatively effects bird weight gains and feed conversion efficiency (Despins and Axtell, 1995).

Insulated poultry houses are built to maintain an optimal temperature range in the house and reduce heat loss. Tunneling beetle larvae, in search of pupation sites, reduce the insulating value of the material about 30% (Vaughn et al., 1984). The inability to maintain temperature within an optimal range results in higher costs associated with reduced feeding efficiency and poor production. In addition, producers incur replacement costs of the insulating material and lost time while the house is out of production (Vaughn et al., 1984; Geden, 1995).

Current litter management practices include the periodic clean out of the poultry litter and its use as an organic fertilizer. The North Carolina Department of Natural Resources requires litter be applied to a growing crop or to a field destined for planting

within 30 days (Zublena et al., 2002). Frequent litter removal reduces beetle populations within poultry houses but serves as a potential source of dispersal and re-infestation. Emigration of beetles to nearby homes and businesses may result in litigation and poor public relations (Miller, 1997; Hinchey, 1997). Mechanical incorporation of poultry manure into field soil negatively impacted house fly survival (Watson et al, 1998). The affect of this practice on darkling beetle survival had not been investigated. The objectives of this study were: (1) Evaluate survival of darkling beetles buried in soil simulating litter incorporated with plow, mulch till, and disk methods. (2) Monitor the emigration of darkling beetles from litter applied to field soil and evaluate the impact of mechanical incorporation of the poultry litter into field soil on beetle emergence in clay and sandy soils.

Materials and Methods

Experiment One: Simulated incorporation of beetle infested litter

Polyvinyl chloride (PVC) pipes, 14.16 cm (4") diameter were cut into varying lengths to allow addition of soil to 8, 15, 23, and 30 cm depths with 2.5 cm space between soil and cover and 2.5 cm of soil base for addition of litter and beetles (Appendix II, Fig. 4). One end of each pipe was sealed using a PVC end cap. Pipe lengths were placed upright on the sealed end and 2.5 cm (1") of lightly packed Cecil red clay soil (Buol, personal communication) was added to each pipe to form a soil base. Used turkey litter (60 cc) was added to form a litter layer above the soil base. Adult darkling beetles were collected from a turkey house and separated into groups of 20

individuals. Beetles were placed on the litter layer in each length of pipe. Soil was then added to the pipes to achieve the proposed burial depths of 8, 15, 23, and 30 cm (0, 3, 6, 9, and 12 inches). One pipe length received no additional soil (control). A moistened, crumpled paper towel was placed on the surface of the soil and the pipe was covered with mesh fabric secured with a rubber band. The paper towel was moistened with water as needed but no additional food was added. The number of beetles on the surface of the soil was counted on days 1, 3, 7, 10, 13, 17, 21, 24, and 28 post-burial. The experiment was conducted under controlled conditions at 25º C, 75% relative humidity and 18:6 L/D cycle and was replicated six times. Differences in beetle emergence based on soil depth were analyzed using ANOVA (Minitab, 1996).

Experiment Two: Field incorporation of darkling beetle infested litter

The 85 by 15-meter field selected for this study was located at the Lake Wheeler Field Experiment Station (North Carolina State University, Wake Co.). Treatments were randomized and replicated four times for a total of 16 treatment plots each measuring 3.5 x 15-m (Appendix II, Fig. 5). Treatments assigned to the plots included control (no incorporation), disk (incorporation to 8 cm depth), mulch till (15 cm) and moldboard plow (33 cm) (Appendix II, Figs. 6 A, B, and C).

Turkey litter containing all life stages of darkling beetle was collected from a poultry house and loaded onto a manure spreader (Appendix II, Fig. 7 A). Litter was spread at a rate of 0.013 cubic meters/square meter. Trays (45.1 x 35.6 cm) were used to collect pretreatment samples and estimate the number of beetles applied to the field

before incorporation (Appendix II, Fig. 7 B). One tray per treatment plot was placed on the field to capture litter from each pass of the manure spreader. The spreader made four passes for a total of 64 samples per block. The number of live beetles on each tray was counted and the density of beetles per tray was calculated (Appendix II, Fig. 7 C). The number of beetles applied to the field was then extrapolated from the sample density using the following formula: field area/tray area x mean number beetles collected per tray = mean number beetles/field. Differences in numbers of beetles applied to each treatment plot were analyzed using ANOVA (Minitab, 1996).

After pre-treatment samples were collected, the litter was incorporated into the soil. Litter in disk and mulch till treatment plots was incorporated with two passes of the implement and only one pass with the plow.

Ten each of three types of traps (cylinder, tile and pitfall) (Appendix II, Figs. 8 A, B and C) were randomly placed in each plot. In addition, a cylinder of alsynite (90 x30 cm) mounted on a 1.52 m length of conduit and covered with sticky transparent acetate sheet (Appendix II, Fig. 8 D) was placed at the ends of each treatment plot to monitor flying beetles (Broce, 1988). Traps were examined and beetles counted and removed on days 1, 3, 7, 10, 14, 17, 21, 24, and 28 following incorporation. The study was repeated seasonally and climatological data was recorded for each seasonal replicate.

This study was performed on soil characterized as Cecil (Piedmont red clay) under normal moisture conditions. Since soils in North Carolina vary regionally, a single replicated experiment was conducted during the summer of 2001 on sandy loam soil typical of the coastal plain. Litter from a commercial broiler house located in Duplin Co.

was used for this experiment. Analysis of variance (ANOVA) was used to compare differences in darkling beetle emergence between treatments and seasons (Minitab, 1996; SAS, 1987).

Results and Discussion

Experiment One: Simulated incorporation of beetle infested litter

Beetles emerged from and were present on the surface of the soil after burial at all depths (Table 3.1). The number of beetles emerging from the soil surface was $\leq$ 50% of the number applied, regardless of treatment (Table 3.1). Mean beetle emergence from the 23 and 30 cm depths (7.5 ± 0.01and 6.7 ± 2.31, respectively) was slightly lower than from the 8 and 15 cm depths (9.5 ± 0.99 and 10 ± 2.62, respectively). These differences were not significant ($F = 0.95$, $df = 4$, $p \leq 0.452$). Few beetles (4.8 $\pm$ 1.07) were observed on the surface of the control (Table 3.1). When densities are low, as in this experiment, exposed beetles rapidly bury themselves demonstrating negative phototaxis (Axtell, 1994). Beetles are rarely seen on the surface of the litter in poultry houses unless population densities are high (Geden and Axtell, 1987a).

Upon completion of the experiment, soil from each pipe length was examined for beetles. In every treatment, all beetles introduced to the pipe length were recovered alive. This clearly demonstrated that adult beetles survived being buried at depths $\leq$ 30 cm for 28 days. It is very likely these beetles could have lived significantly longer buried in soil with adequate food supply (litter) and favorable environmental conditions. Preiss (1971) recorded adult beetle survival of 700 days under favorable conditions suggesting great

potential for re-infestation of poultry houses through walking or flight. Adult darkling beetles may fly as much as a mile per day (Savage, 1992). Savage speculated that if litter was applied 0.4 km (0.25 miles) from the poultry house, and beetle dispersal was random, approximately 60,000 beetles of every one million beetles applied to the field would return to the poultry house. In this experiment, darkling beetle survival was not impacted by burial and implications of disease transmission are evident in the ability of the darkling beetle to harbor and transmit disease pathogens, the longevity of the beetle and ability to disperse over long distances. In experiment two, field studies were conducted to determine the impact of mechanical incorporation of land applied litter into field soil on beetle emergence

Experiment Two: Field incorporation of darkling beetle infested litter

The number of beetles applied to the field varied significantly from season to season, ($df = 3$, $F = 0.84$, $p \leq 0.475$), (Table 3.2). Most beetles were applied during the summer (62, 879) and winter (188, 606) seasons and least during the fall (21, 374) and spring (14, 658). There was no significant difference between the number of beetles applied to each treatment plot within a season (Table 3.2).

The mechanical action of the manure spreader was expected to kill a portion of the beetles inhabiting the litter. In a similar study using land applied fresh caged layer manure, Watson et al. (1998) observed 89% mortality of house fly larvae resulting from the flailing action of the manure spreader. Although many dead beetles were observed in spread litter, mortality rates were not as high. Physical properties of caged layer manure

differ from that of turkey litter several ways, particularly moisture and texture, and may affect post-spreading mortality. Manure spreader design may also affect mortality. Essentially, there are two basic designs, one is a chain driven rear delivery system with large paddles designed to throw the manure behind the spreader. The second design has an augur screw in the bottom of the hopper that feeds the litter into a delivery unit that crushes clumped litter before it is thrown from the side. Specific studies to examine manure spreader induced insect mortality have not been conducted.

Disk, mulch till and moldboard plow treatments significantly reduced mean beetle emergence compared to control (no treatment) ($df = 3$, $F = 13.83$, $p \leq 0.0001$) (Table 3.2). Trends in these data suggest that plow treatment had the greatest impact of the three methods of incorporation, regardless of season. However, these differences were not statistically significant ($df = 2$, $F = 4.01$, $p \leq 0.018$). Based on observations in the field, plow treatment was expected to have the least impact, because the plow produced deep furrows in the red clay that remained raised above the field surface throughout the study. It was expected these raised furrows and deeply textured surface would provide harborage for the beetles in the plow treatment rows. In contrast, disk and mulch treatments effectively covered the litter with a layer of soil and within a few days the soil settled and the treatment plots were relatively smooth.

The average soil temperature 15 cm (6 in.) below the surface was 25° C during the summer seasonal study. Treatment effects for the four summer replicates were similar to the combined data for all four seasonal studies. Disk, mulch and plow treatments significantly reduced beetle emergence from the soil but no treatment was

better than another (df = 3, F = 9.93, $p \leq 0.0001$) (Table 3.2). The number of beetles emerging from the soil declined sharply 14 days post incorporation (Fig. 3.1A). Between days 14 and 17, heavy rainfall saturated the treatment plots apparently forcing buried beetles to the surface resulting in increased beetle emergence days 17 to 21. In the laboratory study simulating mechanical incorporation, beetles that did not emerge to the surface after 28 days were found alive in the soil at the conclusion of the study. Presumably, saturating the soil with water would have driven these beetles to the surface.

Treatment differences from the fall experiment were inconsistent with those of the summer. Mean beetle emergence from the control and disk treatments were similar, 12.9 ± 3.4 and 11.9 ± 3.9, respectively and mean beetle emergence from the mulch treatment was 0 (Table 3.2). There are two possible explanations, 1) fewer beetles were applied to the field with the used poultry litter in this study (Table 3.2) and the percentage of beetles captured in the traps may have been to low to show treatment effects statistically; 2) temperature effects may have obscured treatment effects. The fall seasonal study was during a period of unusually cold weather. The average soil temperature 15 cm (6 in.) below the surface was 4° C. Renault et al. (1999) reported that chill coma in darkling beetle adults occurred at 6° C. Under these circumstances the beetles would not have survived the cold buried 15 cm in the soil. Although daily shifts in radiant heat in the control and disk treatments may have allowed some beetle survival.

The winter seasonal study was under only slightly milder temperatures than the fall seasonal study with an average soil temperature 15 cm below the surface of 6.2° C. Treatment effects were apparent (df = 3, F = 34.78, $p \leq 0.0001$) but no difference was

observed between treatments (Table 3.2). As expected, percent beetle emergence was effected by temperature (Fig. 3.2) and when mean temperatures were below 6° C beetle survival was reduced.

The average soil temperature, recorded at 15 cm depth, was 25.7° C for the spring replicate. Beetle emergence was not typical of the other seasonal studies. Fewer beetles emerged from the control plots (86.0 $\pm$ 14.4) and plow plots (99.0 $\pm$ 16.0), although the difference was not significant from the other treatments (Table 3.2). As in the fall study, relatively few beetles were applied to the field and the threshold may have been too low to elucidate statistical patterns given the number of traps per treatment (n = 130). Extremely heavy rain the second day of the study resulted in a field too wet to collect data on day 3 post-incorporation (Fig. 3.1D). Beetles in the control treatment had little refuge from the rains and may have been washed away. In contrast, the other treatments may have provided some protection from the rains.

In general, beetle emergence from the soil had nearly ceased by day 28 (Figs. 3.1 A, B , C, and D). Although beetles survived burial in soils 28 days under laboratory conditions, (experiment one), harsh field conditions, particularly temperature and moisture, probably limits beetle survival beyond 28 days.

Eastern North Carolina soils are characterized as coastal plain sands. It was suspected that soil type would have an effect on beetle emergence, however, trends observed on clay soils were consistent with sandy soils. As in clay soils, the mean number of beetles emerging from the sandy soil field declined and little or no beetle activity was observed after 28 days (Fig. 3.3). Mechanical incorporation of poultry litter

significantly reduced beetle emergence in sandy soil types (df = 3, F =18.83, $p \leq 0.0001$). Disk and plow treatments reduced emergence significantly as compared to control (Fig. 3.4). Plow treatment may be more effective in sandy soils than in clay type soils as the sand settles completely after treatment and does not provide crevices for harborage. Incorporation by mulch till treatment was not significantly different from the control in sandy soils. Presumably, this is due to greater spacing between the tines of the mulch till implement which do not completely turn the soil.

Conclusions

The potential for emigration of darkling beetles from land applied litter to poultry houses, residential areas and nearby businesses continues to be a concern. This study demonstrated that mechanical incorporation of poultry litter and/or application during cooler temperatures had a significant impact on darkling beetle emergence and serves to reduce the potential for disease transmission and nuisance complaints. However, requirement for applying litter to a growing crop or to fields planted within 30 days limits the practical application of litter during the cooler months as a beetle control strategy.

Table 3.1 Mean number of beetles that emerged from soil at depths 0, 8, 15, 23, and 30 centimeters.

Soil Depth (cm)	Mean Beetle Emergence	SE
0	4.8	± 1.07
8	9.5	± 0.99
15	10	± 2.62
23	7.5	± 3.01
30	6.7	± 2.3

Table 3.2. Estimated number of beetles applied to field and mean beetle emergence for each seasonal study and for all four combined studies.

Replicate	N	Treatment	Total Beetles Applied	Within Treatment Variation*			Between Treatment Variation**			Mean Emergence ± SE
				df	F	*p*	df	F	*p*	
Summer	4	control	16, 575	3	1.21	0.352	3	4.63	0.023	642.9 ± 70.4
		disk	12, 111	3	0.28	0.836				349.1 ± 41.0
		mulch	20, 597	3	2.97	0.079				430.1 ± 54.1
		plow	13, 596	3	0.95	0.456				257.0 ± 36.5
Fall	4	control	5, 568	3	0.58	0.637	3	0.39	0.759	12.9 ± 3.4
		disk	6, 736	3	2.93	0.077				11.9 ± 3.9
		mulch	4, 490	3	1.11	0.384				0.0 ± 0.0
		plow	4, 580	3	1.08	0.394				3.9 ± 2.2
Winter	4	control	41, 673	3	1.59	0.267	3	2.19	0.102	165.0 ± 19.4
		disk	27, 183	3	0.11	0.953				23.0 ± 4.9
		mulch	59, 875	3	1.38	0.318				32.0 ± 5.4
		plow	59, 875	3	0.89	0.488				19.8 ± 4.5

Table 3.2 cont'd

Replicate	N	Treatment	Total Beetles Applied	Within Treatment Variation*			Between Treatment Variation**			Mean Emergence ± SE
				df	F	*p*	df	F	*p*	
Spring	4	control	2, 874	3	1.12	0.380	3	3.61	0.018	86.0 ± 14.4
		disk	3, 521	3	2.27	0.133				110.0 ± 23.5
		mulch	4, 239	3	1.22	0.344				123.0 ± 20.0
		plow	4, 024	3	3.19	0.063				99.0 ± 16.0
Total	16	control	66, 690				3	0.84	0.475	907.2 ± 80.8
		disk	49, 551							494.2 ± 51.8
		mulch	89, 651							509.4 ± 62.0
		plow	82, 075							379.4 ± 42.7

*There was no significant difference in the number of beetles applied to each treatment within a replicate.
**There was no significant difference in the number of beetles applied to treatments within a study.

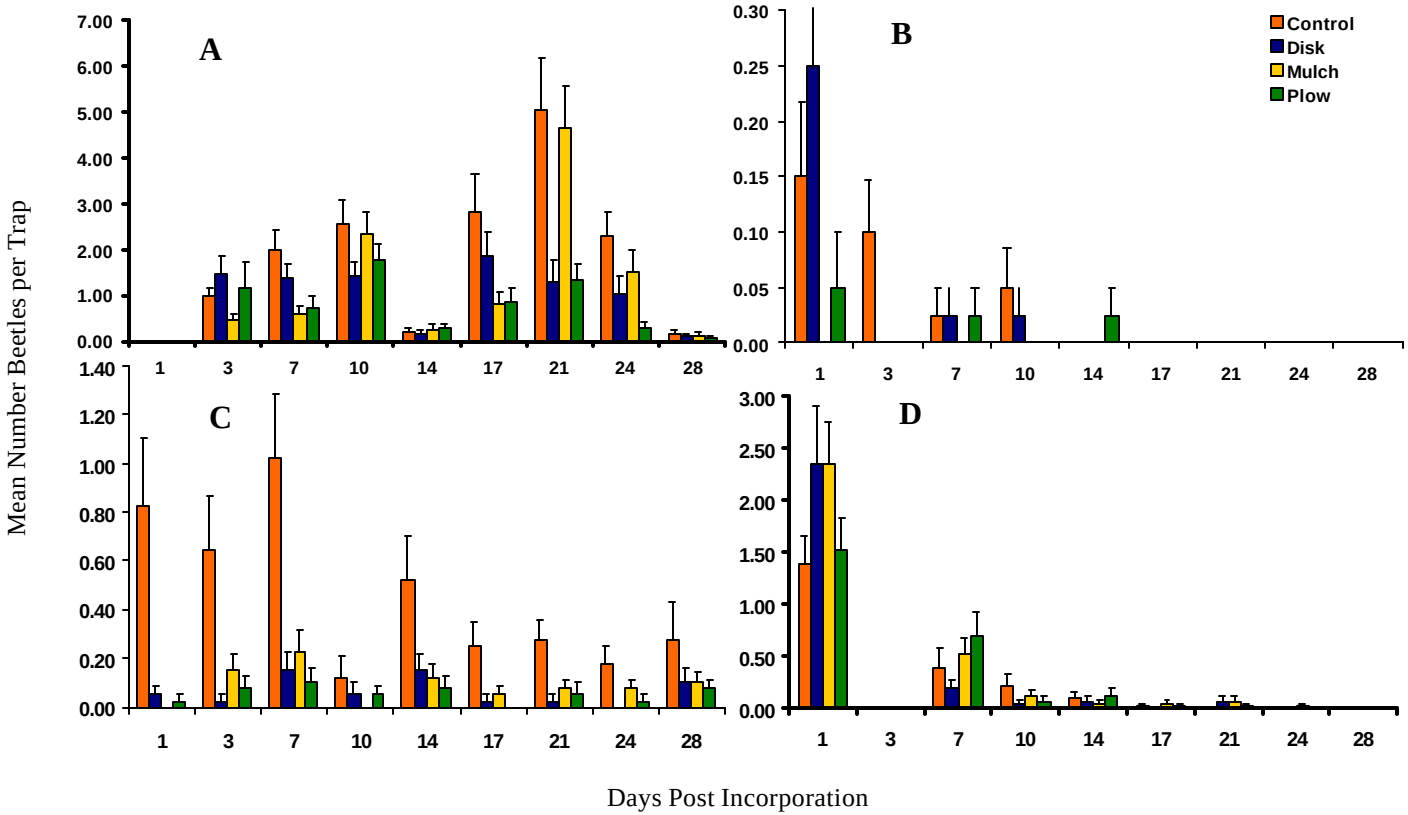


Fig. 3.1. Mean beetle emergence per trap on days 1, 3, 7, 10, 14, 17, 21, 24, and 28 post incorporation for A) summer, B) fall, C) winter and D) spring seasonal studies

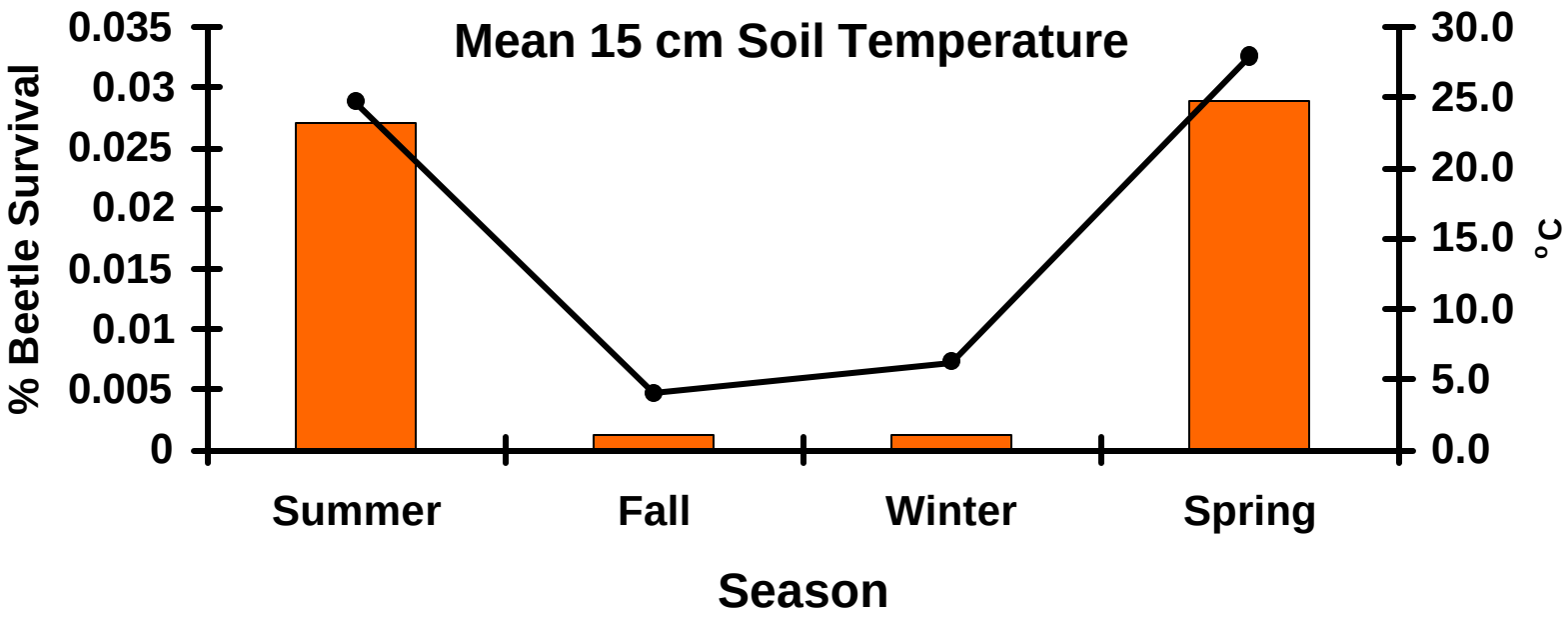


Fig. 3.2. Percent beetle emergence for each season compared to average soil temperature 15 cm below surface.

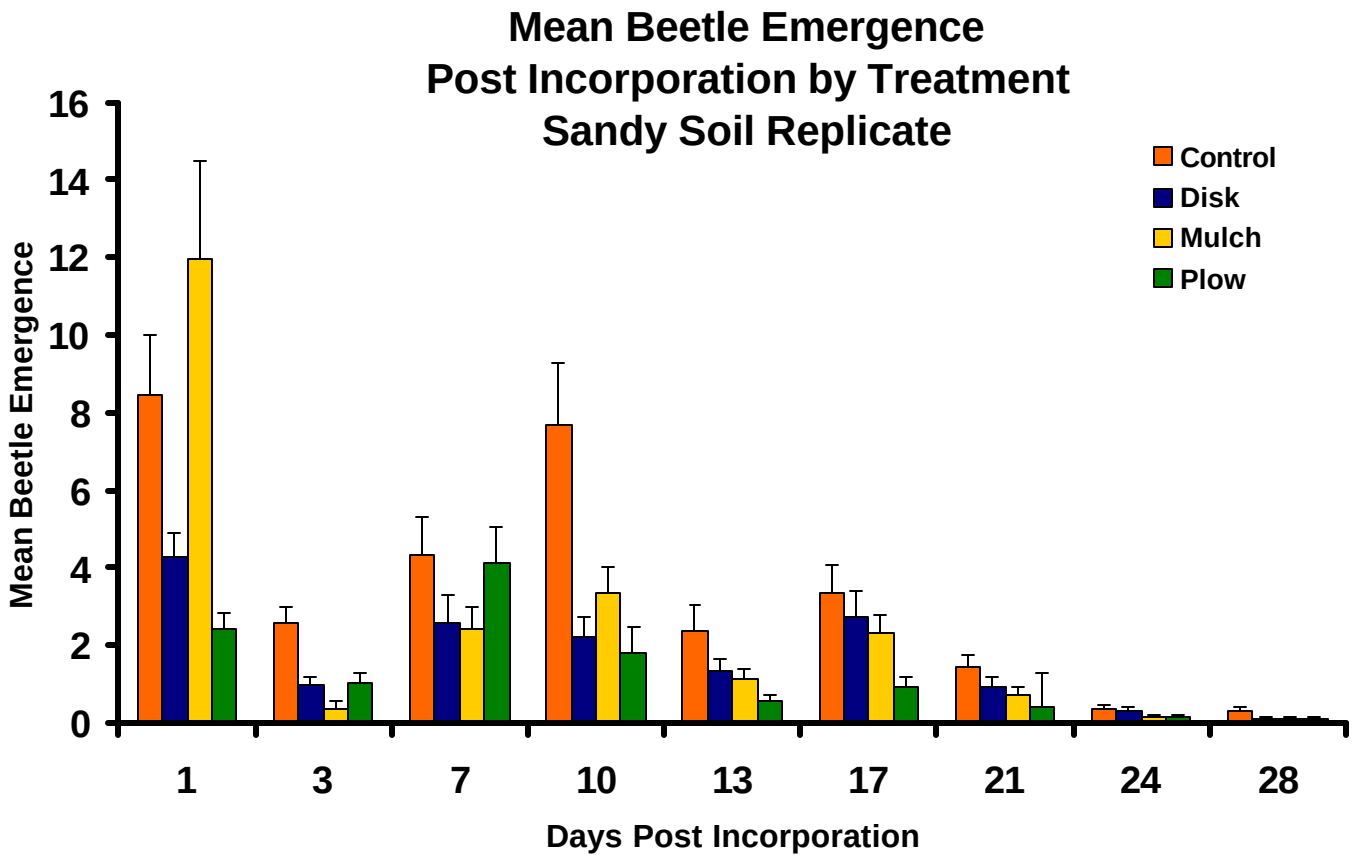


Fig. 3.3. Mean number of beetles that emerged from the soil on days 1, 3, 7, 10, 14, 17, 21, 24, and 28 post incorporation. Graph represents combined data for all four replicates of the sandy soil study.

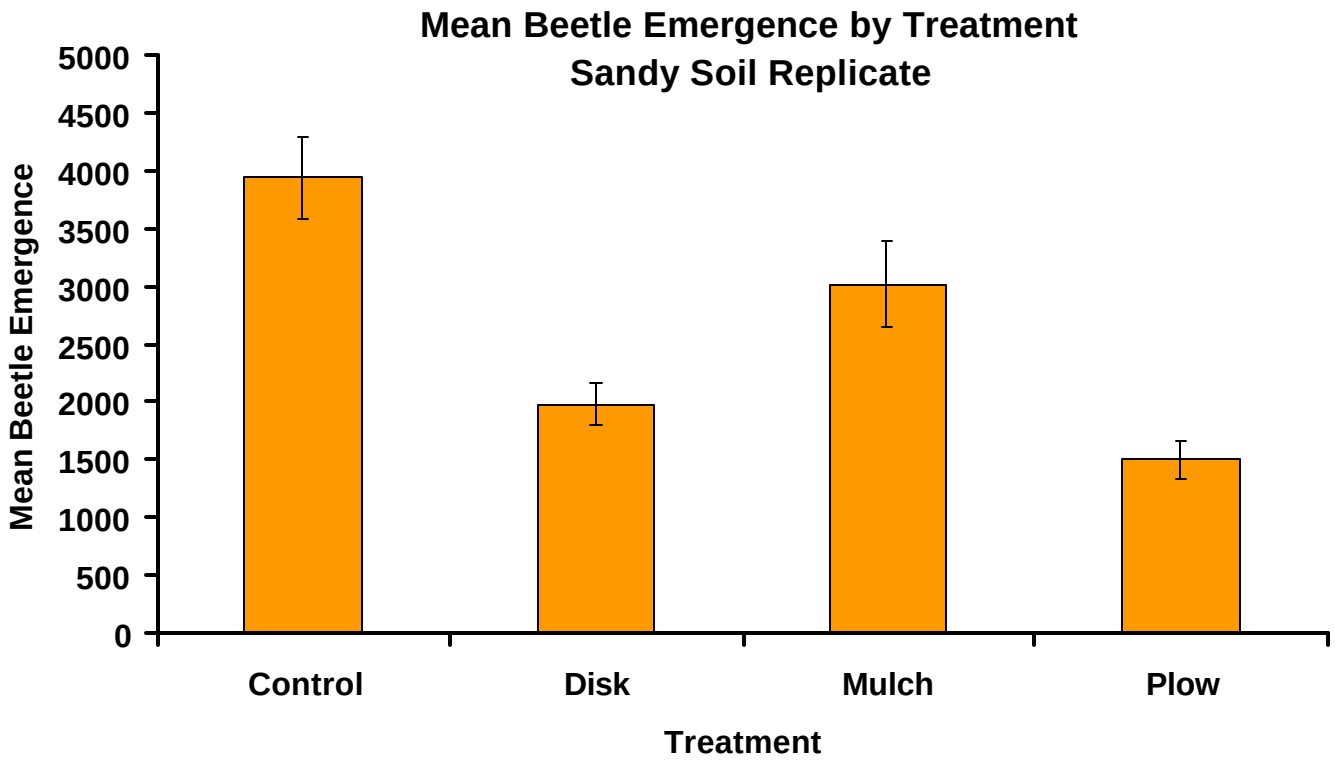


Fig. 3.4. Mean number of beetles that emerged from soil for each treatment; control, disk, mulch, and plow. Graph represents combined data for all four replicates of the sandy soil study. $p \leq 0.0001$.

Chapter Four

Turkey Coronavirus Transmission by House Fly, *Musca Domestica* (L.) (Diptera: Muscidae)

Abstract

Turkey coronavirus (TCV) is a recurrent enteric disease of turkeys in North Carolina characterized by diarrhea, depression, weight loss and increased mortality. This study demonstrated the potential of the house fly to harbor and act as a mechanical vector of TCV. House flies were allowed to feed on liquid medium containing TCV. Crops, mid- and hindgut tissues were dissected from the flies at intervals of 0.5, 1, 3, 6, 9 and 12 hours post-feeding. Turkey embryos were then inoculated with homogenized crops from the infected flies. TCV was detected in house fly crops up to 9 hours post-feeding. No TCV was detected in tissues from house fly mid- or hindguts. The potential of the house fly to directly transmit TCV to live turkey poults was demonstrated by exposing 7 day-old poults to TCV infected house flies at densities of 15, 150 and 1500 flies per treatment. Serology results of poults exposed to fly densities of 150 and 1500 were 75-100% and 87-100%, respectively.

Introduction

Turkey coronavirus (TCV) is a persistent recurrent disease of turkeys in North Carolina (De Las Casas et al., 1973; Carver et al., 2001). Diarrhea, depression, weight loss and increased mortality characterize this acute, highly contagious, enteric disease (Nagaraja and Pomeroy, 1997). Primary means of transmission is oral fecal contact. TCV is often associated with outbreaks of Poult Enteritis and Mortality Syndrome (PEMS). PEMS outbreaks result in mortalities of 80 - 90% of a flock in a few weeks (Barnes and Guy, 1997).

Prevention and control of TCV is difficult. An outbreak of TCV in Minnesota spanned two decades before strictly adhered-to depopulation and decontamination requirements eventually eliminated the disease (Nagaraja and Pomeroy, 1997). The movement of personnel, vehicles and equipment was recognized as a contributing factor in the transmission of TCV (Nagaraja and Pomeroy, 1997). In recent North Carolina outbreaks, TCV was transmitted from infected farms despite strict biosecurity, suggesting a potential vector.

The darkling beetle, *Alphitobius diaperinus* and the house fly, *Musca domestica* (L.) are the predominant pests of poultry (Axtell, 1999). Although, the darkling beetle has been incriminated in the transmission of several viral diseases, its role in TCV transmission is limited (Watson et al., 2000).

The potential role of the house fly in the transmission of TCV is great. House flies reproduce in a wide variety of organic substances including manure, mixtures of manure and bedding, and spoiled feeds by depositing eggs in batches of approximately

200. The growth and development of the fly is temperature dependent, completing their life cycle in a little as 7 to 10 days during the summer.

Adult house flies subsist on semi-solid and liquid diets obtained from the environment. Manure and excreted body fluids provide the house fly with much of the nutrition required to sustain the insect's life. Upon encountering such materials on the poultry farm, the adult fly, using sponging mouthparts, consumes fluids that are stored temporarily in the insect crop. Following 12 hours of starvation and subsequent feeding with 10% sucrose solution, the crop will hold an average of 2.3 ± 0.03 microliters of fluid (Watson, personal communication). Studies of the blow fly, *Phormia regina*, reveal that quantities of crop fluids are mixed with ingested semi-solid foods. This mixture passes into the insect midgut and is digested over the next five hours (Hainesworth et al., 1990). To help liquefy more solid foods before ingestion the fly may regurgitate crop contents onto foods and surfaces (West, 1951). Similarly, flies frequently defecate while feeding and resting on surfaces. As a result of such feeding habits the house fly has been implicated in the transmission of over 30 bacterial, protozoan, and viral diseases including a porcine coronavirus (Greenberg, 1973; Gough and Jorgenson, 1983; Graczyk et al., 1999). This study describes three laboratory experiments designed to evaluate the role of the house fly in the transmission of turkey coronavirus.

Materials and Methods

Virus. TCV (NC95) was isolated from enteritis-affected turkeys and propagated in embryonated turkey eggs as previously described (Guy et al., 1997).

An inoculum containing TCV (NC95) was prepared by amniotic inoculation of 21-day-old embryonated turkey eggs with TCV (NC95) at the 15^{th} embryo passage. At 3 days post-inoculation (PI) embryo intestines were harvested and prepared as a 10% suspension in Dulbecco's minimal essential medium (DMEM) (Sigma Chemical Co., St. Louis, MO). The suspension was homogenized, then clarified by centrifugation for 10 minutes at 1,200 x g. Virus was titered by inoculation of ten-fold dilutions into each of three 22-day-old embryonated turkey eggs with examination of individual embryo intestines on day 3 PI using indirect IFA; virus titer was calculated by the method of Reed and Muench (1938). An inoculum was prepared to contain approximately 5×10^5 50% embryo infectious doses (EID_{50})/0.1 ml and stored at –70°C.

Virus isolation. Intestinal contents were collected using cotton-tipped swabs and immediately immersed in 1 ml DMEM. These were clarified by centrifugation at 1,500 x g for 20 minutes at 4° C, and filtered through a 0.45 µm filter (Gelman Science). Two 21-24 day-old embryonated turkey eggs were each inoculated with 0.2 ml of sample. Three days post-inoculation, embryo intestines were collected for indirect immunofluorescent antibody (IFA) staining.

Serology. TCV-specific antibody was detected in turkey sera using an indirect IFA assay. Antigen for the indirect IFA procedure consisted of epithelial cells exfoliated from the bursae of Fabricius of experimentally infected turkeys (Guy et al., 1997). TCV-infected epithelial cells were spotted onto glass microscope slides, air-dried, and fixed in cold (4°C) absolute acetone for 10 minutes. Sera were diluted 1:20 in phosphate buffered saline (PBS), overlaid onto cells, and incubated at 37° C for 15 minutes. Slides were

washed briefly in two changes of PBS, and cells were overlaid with a 1:40 dilution of fluorescein isothiocyanate-labeled rabbit anti-chicken immunoglobulin G (ICN Biomedicals, Inc., Costa Mesa, CA). Slides were incubated at 37° C for 15 minutes, washed twice with PBS, and examined by epifluorescence.

Insects. House flies used in this study originated from field collected flies held in colony at Cornell University, Ithaca, NY. This colony has been maintained four years at North Carolina State University. All adult flies used in this study were 5 days-old. Target organs for experiments one and two were the crop, where liquids are stored temporarily before passage to the midgut, and mid- and hindgut where foods are digested and formed into fecal droplets.

Experiment one**:** Evaluate the fly crop for the presence of TCV

Adult flies of mixed sex were separated into two groups of 240 flies each and held for 18 hours at 22° C in screened containers without food or water. Flies were anesthetized with cold and 1500 µl of TCV inoculum 1.5 X 10^5 suspended in DMEM was placed in a small reservoir and placed in each cage. The control group was given DMEM without TCV. The flies consumed the TCV inoculated DMEM and uninoculated material within 10 minutes of recovering from the anesthesia. Thirty minutes following the ingestion of the medium, 40 flies were removed from each group (80 total). These flies were placed in sterile petri dishes and held on ice. Whole crops were aseptically removed from the fly (Appendix II, Fig 9) and stored in chilled DMEM plus 1% fetal bovine serum, 0.15 mg/ml gentamycin and 5 µg/ml amphotericin B. Dissection

instruments were sterilized in dilute bleach and ETOH following each operation. Pools of 40 fly crops were then frozen at –70^{o} C. Crop tissues were removed at time intervals 0.5, 1, 3, 6, 9, and 12 hours post-feeding and were evaluated for the presence of TCV by IFA as described above. The experiment was replicated three times.

Experiment two: Evaluate the fly digestive tract, mid- and hindgut, for presence of viable TCV

Crop contents enter the mid- and hindgut of the fly within a few hours of feeding. Flies were fed as described above. Fly mid- and hindguts were aseptically removed from the fly by excising the terminal abdominal plate and gently pulling the digestive tract, midgut and hindgut through the opening. Gut contents and tissues were transferred to chilled DMEM and antibiotic medium and frozen at –70^{o} C. Crop tissues were not used in this experiment. Fly gut tissues were removed at time interval 6, 9, 12 and 24 hr post-feeding and evaluated for the presence of TCV by IFA as described above. The experiment was replicated three times.

Experiment 3: Demonstrate the potential of the house fly to directly transmit TCV to live turkey poults

These experiments were conducted using Horsfal isolation units with negative pressure ventilation, commercial hatchlings and laboratory reared adult flies. One-day-old turkey poults were raised in heated brooders until 7 d of age. Poults were weighed and divided into groups of 15 birds and distributed to each of eight chambers.

Birds in study chambers were exposed to fly densities of zero (control), 15, 150 and 1500 house flies. Each of the four treatments was replicated twice. Flies selected for study were divided into two groups of 15 flies, two groups of 150 and two groups of 1500. Flies were provided with TCV inoculated medium in a reservoir as described above. The large group of 1500 flies consumed 6000 µl of TCV inoculated medium. Three hours post-feeding, the flies were released into the chamber and the bird behavior observed. One day after the flies were released in the brooders, 0.30% pyrethrin insecticide synergized with 2.40% piperonyl butoxide (CB-38 EXTRA™, Waterbury Companies Inc., Waterbury, CT) was sprayed into the fresh air intake to kill the flies. Dead flies were removed from the chambers.

Three days after exposure to the flies, 6 birds were selected randomly from each treatment group. These birds were necropsied and examined for clinical indications of TCV infection. Ilium and bursa of Fabricius tissues were collected and examined for presence of TCV by IFA as described above. On day 21 the remaining birds were tested for TCV-specific antibodies as described above.

Results

After overnight deprivation of food and water, flies readily consumed the liquid medium containing TCV. Preliminary experiments in which dissected crops were weighed immediately after fly feeding on liquid medium containing TCV, demonstrated that flies consumed approximately 2 µl of inoculum under conditions identical to experiments one, two and three. TCV was detected by virus isolation in fly crops up to 9

hours post-feeding liquid medium containing TCV (Table 6.1). No TCV was detected in house flies that were fed control medium. TCV was not detected by virus isolation in mid- and hind gut tissues at any time interval for either TCV fed or control fed flies (Table 6.1).

Seven day-old poults exposed to house flies responded attentively to the presence of the house flies in the chambers. Poults were observed eating flies in all but one chamber one hour after the flies and birds were co-mingled. TCV infection was detected in all treatment groups except in the chamber that no fly feeding was observed (Table 6.2). Rates of TCV infection increased as the number of flies in the chamber increased. Infection rates were 67-83 % and 100% in turkeys exposed to densities of ten and 100 flies per bird, respectively.

Discussion

This study demonstrated the potential of the house fly to harbor and support the transmission of TCV. There is no evidence in our study of cyclodevelopmental or propagative transmission of virus in the house fly. The fly functions strictly as a mechanical vector as TCV remains viable in the crop of the house fly for 9 hours following a single feeding. The relative instability of TCV has been demonstrated elsewhere. Unlike astrovirus, TCV is not environmentally resilient and appears to be inactivated in the absence of the host (Schultz-Cherry, 2001).

Although the fly crop provided time-limited protection, our study suggests TCV is inactivated in the gut of the fly. House flies consume a wide variety of organic

substances including microorganisms for food. Microorganisms play an essential role in the growth and development of house fly larvae, yet there is little evidence of a dietary association in adults (Levinson, 1960; Schmidtmann, 1992; Watson et al., 1993). Digestive enzymes of the adult and larval house fly differ slightly (Terra et al., 1988). Although adult flies consume proteins and lipids, the digestive enzymes of the adult fly are well suited to carbohydrate diets (Sinha, 1976). Adult house flies produce amylase, alpha glucosidases, alpha galactosidase, and beta galactosidase (Sinha, 1976; Terra et al., 1988). Proteases produced in the adult house fly gut include pepsin, trypsin, chymosin, peptidases, and dipeptidases, and (Sinha, 1976; Terra et al., 1988). Presumably these enzymes contributed to the inactivation of the TCV in the gut of the fly.

We successfully demonstrated that flies could harbor TCV in the crop but not the gut. It was important for us to demonstration the actual transmission potential of the fly for TCV at varying fly densities. Turkey poults exposed to house fly densities of 15, 150 and 1500 flies became infected with TCV. A dose dependent effect was seen in the IFA results suggesting as the infected fly density increases, there is a greater likelihood for disease transmission. The dose dependent effect was lost, however, by fourteen days post-exposure when infection was detected by serology. This was expected because of the potential of TCV to spread from bird to bird, independent of the house fly.

The evident consumption of infected flies by the poults suggests a likely method of TCV transmission. In the chambers where no insects were eaten by the poults, results of IFA and serology tests were negative. Contamination of feed and drinkers may have contributed to the transmission of TCV. Further study would confirm this inference.

Results of this study suggest that house flies may act as mechanical vectors of TCV. In addition to strict biosecurity measures, management of fly populations may aid in the prevention and control of TCV outbreaks.

Table 6.1. Expt. 1. Detection of turkey coronavirus (TCV) in fly crops and mid-and hindgut tissues after experimental exposure to virus-containing media. Flies were allowed to consume TCV-containing inoculum (approximately 1.5 x 10^5 50% egg infectious doses TCV in minimal essential media); at selected times post-exposure crops were removed from euthanized flies and examined for presence of TCV by virus isolation. [A]

	Hours post-exposure						
	0.5	**1**	**3**	**6**	**9**	**12**	**24**
TissueType							
Crop							
1	Pos[1]	Pos	Pos	Pos	Neg[1]	Neg	No test[1]
2	Pos	Pos	Pos	Pos	Pos	Neg	No test
3	Pos	Pos	Pos	Pos	Neg	Neg	No test
Gut							
1	No test	No test	No test	Neg	Neg	Neg	Neg
2	No test	No test	No test	Neg	Neg	Neg	Neg
3	No test	No test	No test	Neg	Neg	Neg	Neg

[A] TCV was not detected in crops of sham-exposed flies collected at same times post-exposure. Experiment was replicated three times.
[1] Pos = positive IFA test, Neg = negative IFA test, No test = not tested

Table 6.2. Transmission of turkey coronavirus (TCV) to 7-day old turkey poults by the house fly, *Musca domestica*.

		TCV detection (number positive/number tested)		
Exposure	IFAT[1]	Percent positive	Seroconverted	Percent positive
Control[2]	0/6	0	0/9	0
15 Flies	3/6*	50	7/8	87
	0/6	0	0/8	0
150 Flies	4/6*	67	6/8	75
	5/6*	83	9/9	100
1500 Flies	1/1*	100	7/8	87
	6/6*	100	6/6	100

[1]IFAT= indirect fluorescent antibody test.
[2] No flies were released with the control group.
*Conspicuous consumption of flies by poults observed within one hour of allowing poults and flies to co-mingle.

Conclusions

The relative risk of insects contributing to a TCV epizootic depends on the habits and mobility of the insect. The darkling beetle has been incriminated in the transmission of several avian diseases (De Las Casas et al., 1976; Despins et al., 1994, 1995; McAllister et al., 1994, 1995) and has been shown to harbor and transmit TCV (Watson et al., 2000). The darkling beetle is a common pest of commercial poultry facilities and populations may often reach extremely high densities. Darkling beetle adults inhabit the litter but may often be seen on the walls, drinkers and feeders, and birds will often eat beetles (Despins, 1995). Darkling beetles are also attracted to lights and may take flight, especially during warm summer evenings. However, much of this activity occurs within an individual farm (Geden and Axtell, 1987a). Savage (1992) reports that adult darkling beetles fly as much as one mile per day. This presents the potential for re-infestation and disease transmission if removed litter is applied to land in close proximity of the poultry house from which it was removed or other poultry operations.

The house fly is also a common pest in commercial poultry operations and may be found resting on the walls, drinkers and feeders. In this study we have shown that the house fly is also capable of harboring and transmitting TCV. The potential of the fly to disperse from the poultry facility is great. Bishop and Laake (1921) reported that flies are capable of dispersing 20 km. Greenberg (1973) reported house flies were capable of flights ranging from 2.3 to 11.8 km within 24 hr. Dispersal of house flies from the poultry facility is an important consideration in the transmission of disease.

This study demonstrated that darkling beetle adults are capable of surviving at least 28 days under laboratory conditions simulating land applied litter. Conditions in the field are less than optimum and few beetles emerged from the field after 28 days. Beetles that did not emerge to the surface in the laboratory experiments were later found to be alive under the surface. Used poultry litter was buried along with the beetles in this study and the availability of adequate food and moisture conditions may have allowed the beetle to survive without coming to the surface. Factors that motivated some beetles to move to the surface and others to remain buried are unknown. Similarly, beetles may have remained under the surface the field after each replicate of the incorporation study was completed, particularly in the plow rows of the clay soil study.

In the clay soils experiment, only a handful of beetles were recovered from the sticky traps that surrounded the field over four seasonal replicates (data not shown). In Duplin Co. where the incorporation study was conducted on sandy soils, the sticky traps were literally covered with beetles. Used poultry litter applied at both sites provided the beetles with food source in the field. In addition, the Duplin Co. site was a tilled corn field with broken cobs littered about. Beetles were often found in and on the cobs and corn stubble. Factors that may have contributed to migration of the beetles from the Duplin co. field site are density of applied beetles, proximity to a poultry house and light. The number of beetles estimated to have been applied to the field along with the used litter was much lower at the Wake Co. site. Also, the Wake Co. site is part of an agricultural research station. The field chosen for the incorporation study was a considerable distance from the nearest poultry facility and from any light source. The

Duplin Co. site was situated between the poultry house and the farmer's home approximately 40 m from each. The field was along a road with streetlights and a large floodlight was mounted at the end of the poultry house nearest the field.

It was demonstrated that the darkling beetle may transmit TCV for at least one hour but not more than twelve (Watson et al., 2000). TCV remains viable in the house fly crop for nine hours. In assigning relative risk to transmission of TCV by these two insects the mitigating factor must be mobility. Incidences of beetles actively migrating from a poultry house are rare. Emigration from land applied litter appears to only occur under certain circumstances. The house fly has a much greater range of flight and is more likely to migrate between farms and residential areas. The risk of TCV transmission by the darkling beetle remains a concern especially within an individual poultry house or farm. The house fly, however, is more likely to vector TCV between poultry facilities.

Mechanical incorporation of land applied litter as well as application during the colder months has been demonstrated to reduce beetle emergence. Used poultry litter infested with darkling beetle adults should be applied at least one mile from the nearest poultry facility or residential area to reduce the risk of disease transmission and nuisance complaints.

References

Apuya LC, S.M. Stringham, J.J. Arends, and W.M. Brooks. 1994. Prevalence of protozoan infections in darkling beetles from poultry houses in North Carolina. Journal of Invertebrate Pathology. 63: 255-259.

Armitage DM. 1986. Population changes of four species of insects (Col. & Dipt.) in three deep pit poultry houses. Entomologist's Monthly Magazine. 122: 75-77.

Arends JJ. 1987. Control, management of the litter beetle. Poultry Digest. April. pp. 172-176.

Arnett RH. 1963. The Beetles of the United States. Washington, D.C. The Catholic University Press. 1112 p.

Axtell RC, and J.J. Arends. 1990. Ecology and management of arthropod pests of poultry. Annual Review of Entomology. 35: 101-126.

Axtell RC. 1999. Poultry integrated pest management: status and future. Integrated Pest Management Reviews. 4: 43-73.

Azmi MA, S.N.H. Aqvi, K. Akhtar, K. Yasmin, and M. Jahan. 1993. Comparative toxicity of Solfac (10% Cyfluthrin) and a crude neem extract (RB-A) on *Alphitobius diaperinus*. Geobios. 20: 175-177.

Bala P, D. Kaur, J.J. Lipa, and R.C. Bhagat. 1990. *Gregarina alphitobii* spp. n. and *Mattesia alphitobii* sp. n., parasitizing *Alphitobius diaperinus* Panz. (Tenebrionidae, Coleoptera). Acta Protozoologica. 29 (3): 245-256.

Banjo AD, and O.Y. Soyoye. 1999. The diversity of insect fauna in poultry houses in southwest Nigeria. Discovery and Innovation. 11 (3-4): 181-184.

Barke HE, and R. Davis. 1967. Sexual dimorphism in the lesser mealworm, *Alphitobius diaperinus* (Panz.) (Coleoptera: Tenebrionidae). Journal of the Georgia Entomological Society. 2 (4): 119-121.

Barke HE, and R. Davis. 1969. Notes on the biology of the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Journal of the Georgia Entomological Society. 4 (2): 46-50.

Barnes HJ, and J.S. Guy. Poult enteritis - mortality syndrome ('spiking disease') of turkeys in *Diseases of Poultry*, 10th ed. B.W. Calnek, H.J. Barnes, C.W. Beard, LR. McDougald and Y. M Saif, eds. Iowa State University Press, Ames. pp 1025-1031.

Basak PK, D.K. Choudhuri, and T. Sengupta. 1991. On the beetle Insecta: Coleoptera infestation of milk powder at Calcutta warehouses. Journal of Bengal Natural History Society. 10 (2): 50-55.

Bhattacharyya S. 1995. Coleopteran insects in the nests of birds in West Bengal. Environment and Ecology. 13 (3): 629-632.

Bishopp FC, and E.W. Laake. 1921. Dispersion of flies by flight. Journal of Agricultural Research. 21: 729-766.

Blahutiak A, and V. Barus. 1969. Comparacion de la efectividad de cinco insecticidas para el control de *Alphitobius diaperinus* (Panzer). Academia de Ciencias de Cuba Serie Biologica. 20:1-17.

Breslin JJ, L. G. Smith, F. J. Fuller, and J. S. Guy. 1999. Sequence analysis of the matrix/nucleocapsid gene region of turkey coronavirus. Intervirology. 42: 22-29.

Breslin JJ, L.G. Smith, H.J. Barnes and J.S. Guy. 2000. Comparison of virus isolation, immunohistochemistry, and reverse transcriptase-polymerase chain reaction procedures for detection of turkey coronavirus. Avian Disease. 44: 624-631.

Breslin JJ, L.G. Smith and J.S. Guy. 2001. Baculovirus expression of turkey coronavirus nucleocapsid protein. Avian Disease. 45: 136-143.

Broce AB. 1988. An improved alsynite trap for stable flies, *Stomoxys calcitrans* (Diptera: Muscidae). Journal of Medical Entomology. 25: 406-409.

Buol S. 2001. North Carolina State University. Department of Soil Science. College of Life Sciences. Raleigh, NC.

Burkeholder WE. 1981. Biomonitoring of stored products insects. In: *Management of Insect Pests with Semiochemical: Concepts and Practice*. Mitchell, E.R., ed. Plenum Press. New York. 514 pp.

Carver DK, J. P. Vaillancourt, S. M. Stringham, J. S. Guy, and H. J. Barnes. 2001. Mortality patterns associated with Poult Enteritis Mortality Syndrome (PEMS) and coronaviral enteritis in turkey flocks raised in PEMS-affected regions. Avian Disease. 45: 985-991.

Casteels H, R. Moerman, J.S. Muduturi, and R. De Clercq. 1996. Occurrence of insect pests in imported stored products in Belgium during the period 1991-1995. Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen. 61 (3A): 637-701.

Chatterjee NB, and A. K. Gangopadhyay. 1989. Environmental cues in monthly egg laying and hatching of the lesser mealworm *Alphitobius diaperinus* (Panz)

(Coleoptera: Tenebrionidae). Proceedings of the Zoological Society of Calcutta. 40: 13-18.

Cogan P, D. Webb, and M. Wakefield. 1996. A comparison of four residual insecticides for the control of the lesser mealworm beetle (*Alphitobius diaperinus* Panzer) in turkey broiler houses in UK. International Pest Control. 38 (2): 52-55.

Crawford PJ, W.M. Brooks, and J.J. Arends. 1998. Efficacy of field isolated strains of *Beauveria bassiana* (Monilianes: Moniliacaea) as microbial control agents of the lesser mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Journal of Economic Entomology. 91 (6): 1295-1301.

Crook PG, J.A. Novak, and T.J. Spilman. 1980. The lesser mealworm, *Alphitobius diaperinus*, in the scrotum or *Rattus norvegicus*, with notes on other vertebrate associations (Coleoptera: Tenebrionidae; Rodentia, Muridae). The Coleopterists Bulletin. 34 (4): 393-396.

Dale PS, J.C. Hays, and J. Johannesson. 1976. New plant pests. New Zealand Journal of Agricultural Research. 19: 265-269.

Dass R, A.V Navarajan Paul, and R.A. Aragarwal. 1984. Feeding potential and biology of lesser mealworm, *Alphitobius diaperinus* (Panz) (Col., Tenebrionidae), preying on Corcyra cephalonica St. (Lep., Pyralidae). Journal of Applied Entomology. 98: 444-447.

Davidson S. 1987. Beetles solve a sticky problem. Rural Research. 134 (autumn): 29-30.

De Grisse A, and W. Bohyn. 1985. Rasterelektronenmikroskopische waarnemingen op *Alphitobius diaperinus*, gnathocerus cornutus en tribolium confusum.

Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 50 (1): 173-183.

De Las Casas E, B.S. Pomeroy, and P.K. Harein. 1968. Infection and Quantitative Recovery of *Salmonella typhimurium* and *Escherichia coli* from within the lesser mealworm, *Alphitobius diaperinus* (Panzer). Poultry Science. 47: 1871-1875.

De Las Casas EA. 1970. The Lesser Mealworm, *Alphitobius diaperinus* (Panzer), and its Associations with Certain Bacteria and Fungi in Poultry Brooder Houses. Doctoral Dissertation. University of Minnesota. 78pp.

De Las Casas E. 1972. Bacteria and fungi within the lesser mealworm collected from poultry brooder houses (*Alphitobius diaperinus*). Environmental Entomology. 1 (1) 27-30.

De Las Casas E, P.K. Harein, D.R. Deshmukh, and B.S. Pomeroy. 1973. The relationship between the lesser mealworm and avian viruses 1. Reovirus 24. Environmental Entomology. 2 (6): 1043-1047.

De Las Casas E, P.K. Harein, M.D. York, and B.S. Pomeroy. 1974. *Escherichia coli* serotypes isolated from within the lesser mealworm and evaluated for virulence. Annals of the Entomological Society of America. 67 (6): 967-970.

De Las Casas E, P.K. Harein, D.R. Deshmukh, and B.S. Pomeroy. 1976. Relationship between the lesser mealworm, fowl pox, and Newcastle Disease virus in poultry. Journal of Economic Entomology. 69 (6): 775-779.

DeMilo AB, R. W. Miller, B. Bordas, R.E. Redfern, and G. Mills, Jr. 1995. Larvicidal effects of benzoylphenylureas against the lesser mealworm (Coleoptera:

Tenebrionidae): quantitative structure - activity relationships. Journal of Entomological Science. 30 (3): 324-332.

Despins JL, E.C. Turner Jr., and P.L. Ruszler. 1987. Construction profiles of high rise caged layer houses in association with insulation damage caused by the lesser mealworm, *Alphitobius diaperinus* (Panzer) in Virginia. Poultry Science. 66: 243-250.

Despins JL. 1987. Investigations of the Destructive Behavior and Methods for Control of the Lesser Mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae). Masters Thesis. Virginia Polytechnic Institute and State University. 110 pp.

Despins JL, J.A. Vaughn, and E.C. Turner Jr. 1988. Role of the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) as a predator of the house fly, *Musca domestica* L. (Diptera: Muscidae), in poultry houses. The Coleopterists Bulletin. 42 (3): 211-216.

Despins JL, E.C. Turner Jr., and P.L. Ruszler. 1989. Effects of poultry manure moisture and poultry construction materials on movements of the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), a structural insect pest in high rise caged layer houses. Poultry Science. 68: 1326-1331.

Despins JL, E.C. Turner, Jr., and D.G. Pfeiffer. 1991. Evaluation of methods to protect poultry house insulation from infestations by lesser mealworm (Coleoptera: Tenebrionidae). Journal of Agricultural Entomology. 8 (3): 209-217.

Despins JL, R.C. Axtell, D.V. Rives, J.S. Guy, and M.D. Ficken. 1994. Transmission of enteric pathogens of turkeys by darkling beetle larva (*Alphitobius diaperinus*). Journal of Applied Poultry Research. 3: 61-65.

Despins JL, and R.C. Axtell. 1995. Feeding behavior and growth of broiler chicks fed larvae of the darkling beetle, *Alphitobius diaperinus.* Poultry Science. 74: 331-336.

Edwards JP, and L. Abraham. 1985. Laboratory evaluation of two insect juvenile hormone analogues against *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae). Journal of Stored Products Research. 21 (4): 189-194.

Eidson CS, S.C. Schmittle, R.B. Goode, and J.B. Lal. 1966. Induction of leukosis tumors with the beetle *Alphitobius diaperinus.* American Journal of Veterinary Research. 27 (119): 1053-1057.

Eugenio C, E. De Las Casas, P.K. Harein, and C.J. Marocha. 1970. Detection of the mycotoxin F-2 in the confused flour beetle and the lesser mealworm. Journal of Economic Entomology. 63 (2): 412-415.

Falomo AA. 1986. The Pheromone Biology of the Lesser Mealworm, *Alphitobius diaperinus* (Panzer), (Coleoptera: Tenebrionidae). Doctoral Dissertation. University of Wisconsin. 200 pp.

Fattorini S, and P. Leo. 2000. Darkling beetles from Mediterranean minor islands: new records and biogeographical notes (Coleoptera Tenebrionidae). Bolletino della Societa Entomologica Italiana. 132 (3): 201-217.

Filho EB, V.A. Costa, and T.L. Aageesen. 1989. Occurrence of natural enemies of *Musca domestica* L. (Diptera: Muscidae) in poultry areas of Bastos, State of Sao Paulo, Brazil. Revista de Agricultura. 64.

Fransisco O, and A.P. Prado. 2001. Characterization of larval stages of *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) using head capsule width. Brazilian Journal of Biology. 61 (1): 125-131.

Gangopadhyay AK, A. Bhattacharyya, and N.B. Chatterjee. 1985. Occurrence of neurosecretory cells of the brain during the adult life of female lesser mealworm *Alphitobius diaperinus* (Panz). Indian Journal of Comparative Animal Physiology. 3 (2): 54-60.

Geden CJ, R.C. Axtell, and W.M. Brooks. 1985. Susceptibility of the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) to the entomogenous nematodes *Steinernema feltiae, S glaseri* (Steinernematidae) and *Heterorhabditis heliothidis* (Heterorhabditidae). Journal of Entomological Science. 20 (3): 331-339.

Geden CJ, T.D. Edwards, J..J. Arends, and R.C. Axtell. 1987a. Efficacies of mixtures of disinfectants and insecticides. Poultry Science. 66: 659-665.

Geden CJ, and R.C. Axtell. 1987a. Factors affecting climbing and tunneling behavior of the lesser mealworm (Coleoptera: Tenebrionidae). Journal of Economic Entomology. 80 (6): 1197-1204.

Geden CJ, J.J. Arends, and R.C. Axtell. 1987b. Field trials of *Steinernema feltiae* (Nematoda: Steinernematidae) for the control of *Alphitobius diaperinus*

(Coleoptera: Tenebrionidae) in commercial broiler and turkey houses. Journal of Economic Entomology. 80: 136-141.

Geden CJ, and R.C. Axtell. 1988. Effect of temperature on nematode (*Steinernema feltiae* [Nematoda: Steinernematidae]) treatment of soil for control of lesser mealworm (Coleoptera: Tenebrionidae) in turkey houses. Journal of Economic Entomology. 81 (3): 800-803.

Geden CJ. 1989. Understanding and controlling litter beetles. Poultry Digest. 48 (565): 142-144.

Geden CJ. 1990. Coleopteran and Acarine Predators of House Fly Immatures in Poultry Production Systems. In: *Biocontrol of Arthropods Affecting Livestock and Poultry*. Rutz DA, and R.S. Patterson, eds. San Fransisco: Westview Press. pp 177-200.

Geden CJ, J.J. Arends, D.A. Rutz, and D.C. Steinkraus. 1998. Laboratory evaluation of *Beauveria bassiana* (Moniliales: Moniliaceae) against the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) in poultry litter, soil, and a pupal trap. Biological Control. 13: 71-77.

Geden CJ, and D.A. Carlson. 2001. Mechanical Barrier for preventing climbing by lesser mealworm (Coleoptera: Tenebrionidae) and hide beetle (Coleoptera: Dermestidae) larvae in poultry houses. Journal of Economic Entomology. 94: 1610-1616.

Gogoi AR, and R.P. Chaudhuri. 1982. Contribution to the biology of fowl cestodes *Raillietina tetragon, Raillietina echinobothrida and Raillietina cesticillus*. Indian Journal of Animal Science. 52 (4): 426-253.

Good JA, and D.P. Sleeman. 1988. Extensions in range of two pest species: *Leptocera caenosa* (Rondani) (Diptera: Sphaeroceridae) and *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae). Irish Naturalist's Journal. 22 (11): 501.

Goodwin MA, and W.D. Waltman. 1996. Transmission of Eimeria, viruses, and bacteria to chicks: darkling beetles (*Alphitobius diaperinus*) as vectors of pathogens. Journal of Applied Poultry Research. 5: 51-55.

Gough PM, and R.D. Jorgenson. 1983. Identification of porcine transmissible gasteroenteritis virus in house flies (*Musca domestica*, Linnaeus). American Journal of Veterinary Research. 44: 2078-2082.

Graczyk TK, M.R. Cranfield, F. Fayer, and H. Bixler. 1999. House flies (*Musca domestica*) as transport hosts of *Cryptosporidium parvum*. American Journal of Tropical Medicine. 61: 500-504.

Greenberg B. 1973. Flies and Disease. Volume II, Biology and Disease Transmission. Princeton University Press, Princeton, NJ.

Guatam RD. 1989. Exploration of lesser meal worm for the control of storage insects together with its stages and effect on seed viability. Agricultural Situation in India. 44 (6): 487-489.

Guy JS, H.J. Barnes, L.G. Smith, and J. Breslin. 1997. Antigenic characterization of a turkey coronavirus identified in poult enteritis- and mortality syndrome- affected turkeys. Avian Disease. 41: 583-590.

Hainsworth FR, G. Fisher, and E. Precup. Rates of energy processing by blow flies: The uses for a joule vary with food quality and quantity. Journal of Experimental Biology. 150: 257-268.

Harding WC, and T.L. Bissell. 1958. Lesser mealworms in a brooder house. Journal of Economic Entomology. 51:112.

Harein PK, E. De Las Casas, B.S. Pomeroy, and M.D. York. 1970. *Salmonella* spp. and serotypes of *Escherichia coli* isolated from the lesser mealworm collected in poultry brooder houses. Journal of Economic Entomology. 63 (1): 80-82.

Harris F. 1966. Observations on the lesser mealworm, *Alphitobius diaperinus* (Panz.). Journal of the Georgia Entomological Society. 1: 17-18.

Heimbucher J, and E. Kutzer. 1979. Lesser mealworm (*Alphitobius diaperinus*) in poultry farms (occurrence and control). Aus dem institut fur Parasitologie und Allegemeine Zoologie der Veterinarmedizinischen Universitat Wein. 66 (11): 334-337.

Hewlett PS. 1958. Secondary sexual characters in *Alphitobius laevitigus* (F.) and *A. diaperinus* (Panzer) (Col., Tenebrionidae). Entomologist's Monthly Magazine. 94: 144.

Hinchey F. 1997. Bugged residents sue over beetles. The Columbus Dispatch. Columbus Ohio. Thursday, October 2. 1-2 A.

Hinkle NC, and L.A. Hickle. 1999. California caged layer pest management evaluation. Journal of Applied Poultry Research. 8: 327-338.

Hopkins JD, C.D. Steelman, and C.E. Carlton. 1992. Anatomy of the adult female lesser mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) reproductive system. Journal of the Kansas Entomological Society. 65 (3): 299-307.

Hopkins JD, C.D. Steelman, and C.E. Carlton. 1994. Internal reproductive system of the adult male lesser mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Journal of the Kansas Entomological Society. 66 (4): 446-450.

Hopkins JW. 1990. A Study of the Reproductive Anatomy and Distribution of the Adult Male and Female Lesser Mealworm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) Masters Thesis. University of Arkansas. 92 pp.

Hulley P, and M. Pfleiderer. 1988. The coleoptera in poultry manure - potential predators of house flies, *Musca domestica* Linnaeus (Diptera: Muscidae). Journal of the Entomological Society of South Africa. 51 (1): 17-29.

Ichinose T, S. Shibazaki, and M. Ohta. 1980. Studies on the biology and mode of infestation of the tenebrionid beetle, *Alphitobius diaperinus* Panzer, harmful to broiler-chicken houses. Japanese Journal of Applied Entomology and Zoology. 24 (3): 167-174.

Jerrard PC, and K.B. Wildey. 1980. Beetle plague from deep-pit muck spreading. Poultry World. 131 (8): 20-21.

Kahn BA, P.A. Day, L.A. Goonewardene, M.J. Zuidhof, and G. Hawkins. 1998. Efficacy of tetrachlorvinphos insecticide dust against darkling beetles in commercial broiler chicken barns. Canadian Journal of Animal Science. 78: 723-725.

Karlson P, and M. Luscher. 1959. Pheromones: a new term for a class of biologically active substances. Nature. 183: 55-56.

Keville R, and P.B. Kannowski. 1975. Sexual excitation by pheromones of the confused flour beetle. Journal of Insect Physiology. 21: 81-84.

Kumar P. 1983. Flesh eating behaviour of *Alphitobius diaperinus* Panz. (Tenebrionidae: Coleoptera). Indian Journal of Entomology. 48: 113-115.

Lancaster JL, and J.S. Simco. 1967. Biology of the Lesser Mealworm. Arkansas Experiment Station Report Series. 159: 1-12.

Lancaster JL, and J.S. Simco. 1969. Pre-treated rice hull litter for the control of the lesser mealworm. Arkansas Experiment Station Report Series. 174: 1-13.

Le Torc'h JM. 1979. A new pest of livestock buildings (*Alphitobius diaperinus* in pigstyes, Brittany). Phytoma. 308: 31-33.

Le Torc'h JM, and R. Letenneur. 1983. Essais en laboratoire de resistance de differents isolants thermiques aux perforations de tenebrion, *Alphitobius diaperinus* Panzer, (Col. Tenebrionidae). Academie D'agriculture de France Comptes Rendus de Seances. 69: 188-200.

Legner EF, G.S. Olton, R.E. Eastwood, and E.J. Dietrick. 1975. Seasonal density, distribution, and interactions of predatory and scavenger arthropods in

accumulating poultry wastes in coastal and internal Southern California. Entomophaga. 20: 269-283.

Levinson ZH. 1960. Food of housefly. Nature. 188: 427.

Lonc E, M. Mazurkiewicz, W. Doroszkiewicz, A. Kolpa, and M. Manka. 2001. Microbial control of coleopteran larvae of *Alphitobius diaperinus* and *Tenbrio molitor* - grain pests. Medycyna Weterynaryjna. 57 (4): 258-262.

Lorenzo P. 1990. Aplicacion de las radiaciones gamma de 60 Co en la control de insectos que afectan el arroz durante el almacenamiento. Ciencia y Tecnica en la Agricultura. 13 (1-2): 67-76.

Majchrowicz I. 1985. Occurrence of *Alphitobius diaperinus* in poultry farms. Wiadomosci Entomologiczne. 6 (1-2): 77-78.

McAllister JC, C.D. Steelman, and J.K., Skeeles. 1994. Reservoir competence of the lesser mealworm (Coleoptera: Tenebrionidae) for *Salmonella typhymurium* (Eubacteriales: Enterebacteriaceae. Journal of Medical Entomology. 31 (3): 369-372.

McAllister JC, C.D. Steelman, and C.E. Carlton. 1995a. Histomorphology of the larval and digestive systems of *Alphitobius diaperinus* (Coleoptera; Tenebrionidae). Journal of the Kansas Entomological Society. 66 (2): 195-205.

McAllister JC, C.D. Steelman, L.A. Newberry, and J.K. Skeeles, 1995b. Isolation of infectious bursal disease virus from the lesser mealworm, *Alphitobius diaperinus* (Panzer). Poultry Science. 74: 45-49.

McAllister JC, C.D. Steelman, J.K. Skeeles, L.A. Newberry, and E.E. Gber. 1996. Reservoir competence of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) for *Escherichia coli* (Eubacteriales: Enterobacteriacaea). Journal of Medical Entomology. 33 (6): 983-987.

Mekada H, N. Imaeda, S. Shigezumi, S. Ebisawa. 1983. Control of *Alphitobius diaperinus* by polyurethane boards impregnated with some insecticides. Japanese Poultry Science. 20 (3): 149-157.

Mekada H, N. Imaeda, and K. Umeda. 1984. Effect of insecticide-impregnated insulation board on growth of chickens and vaporizing rate of the insecticide. Japanese Poultry Science. 21 (6): 330-332.

Merkl O. 1998. Corrections and new records of Tenebrionidae (Coleoptera) from Nicaragua. Revista Nicaraguense de Entomologica. 43 (4): 1-6.

Miller J.P. 1997. That crunchy stuff in your cereal bowl may not be granola: Beetles invade an Ohio town when chicken farmer's plan for fly control goes awry. Wall Street Journal. New York City, New York. November 3.

Miller RW, and R.E. Redfern. 1988. Feed additives for control of lesser mealworm (Coleoptera: Tenebrionidae). Journal of Economic Entomology. 81 (4): 1137-1139.

Miller RW. 1990. Use of ivermectin to control the lesser mealworm (Coleoptera: Tenebrionidae) in a simulated poultry broiler house. Poultry Science. 69: 1281-1284.

Minitab User's Guide. 1996. Release 11 for Windows.

Mishchenko AA, and I.A. Mashkei. 1987. Harmful beetles (Coleoptera) - inhabitants of livestock premises. Veterynariia-KYIV. 62: 68-71.

Mohammad A, G.A. Jan, and K. Asmatullah. 1985. Insect fauna of birds nests in N.W.F.P. Bulletin of Zoology, Pakistan. 2: 1-7.

Nagaraja KV, and B.S. Pomeroy. 1997. Coronaviral enteritis of turkey (bluecomb disease). In: *Diseases of Poultry*, 10th ed . B.W. Calnek, H.J. Barnes, C.W. Beard, LR. McDougald and Y. M Saif, eds. Iowa State University Press, Ames. pp. 686-692.

Nemeseri L, and T. Gesztessy. 1973. Mass incidence of *Alphitobius diaperinus* on poultry farms. Magyar Aallatorvosok Lapja. 28 (6): 335-338.

Nolan Jr. M. 1982. Beetle control in broiler houses. Poultry Digest. 41 (487): 458-459.

O'Connor JP. 1987. *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) damaging polystyrene insulation on an Irish piggery. Entomologist's Monthly Magazine. 123: 1472-1475.

Olsen AR, and T. S. Hammack. 2000. Isolation of Salmonella spp. from the housefly, *Musca domestica* L., and the dump fly, *Hydrotaea aenescens* (Wiedemann) (Diptera: Muscidae), at caged layer houses. Journal of Food Protection. 63 (7): 958-960.

Pajni HR, I. Jit, and P. Lata. 1976. A report on tenebrionidae (Coleoptera) of Chandigarh. Research Bulletin of the Panjab University. 27: 123-124.

Pena G. LE. 1973. Nuevos insectos introducidos accidentalmente en Chile. Revista Chilena de Entomologia. 7: 251.

Pfeiffer DG, and R.C. Axtell. 1980. Coleoptera of poultry manure in caged-layer houses in North Carolina. Environmental Entomology. 9: 21-28.

Pospischil R. 1996. The litter beetle. Der Praktische Schladlingsbekampfer. 48: 14-15.

Preiss FJ, and J.A. Davidson. 1968. The effect of temperature and humidity on egg hatch of the lesser mealworm. University of Maryland Agricultural Experiment Station Miscellaneous Publication Number 660.

Preiss FJ, and J.A. Davidson. 1970. Characters for separating late-stage larvae, pupae, and adults of *Alphitobius diaperinus* and *A. laevigatus* (Coleoptera: Tenebrionidae). Annals of the Entomological Society of America. 63 (3): 807-809.

Preiss FJ, and J.A. Davidson. 1971. Adult longevity, pre-oviposition period and fecundity of *Alphitobius diaperinus* in the laboratory (Coleoptera: Tenebrionidae). Journal of the Georgia Entomological Society. 6 (2): 105-109.

Preiss FJ. 1971. Bionomics of the Lesser Mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Doctoral Dissertation. University of Maryland. 131 pp.

Propp GD, and P.B. Morgan. 1985. Mortality of eggs and first-stage larvae of the house fly, *Musca domestica* L. (Diptera: Muscidae), in poultry manure. Journal of the Kansas Entomological Society. 58 (3): 442-447.

Prudnikova MA. 1991. On impossibility of the use of the Tenebrionid beetles *Alphitobius diaperinus* Pz. (Coleoptera, Tenebrionidae) for biological control of the chicken mite *Dermanyssus gallinae*. Entomologicheskoe Obozrenie. 70 (1): 53-56.

Rahman MF, S. Hussain, and R. Rahman. 1991. Alimentary canal and malpighian tubules of the adult *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae). Bangladesh Journal of Zoology. 19 (1): 21-28.

Ratti E. 1986. Faunistic researches by the Civic Natural History Museum of Venice on the island of Patelleria I. Introductory notes; Coleoptera Tenebrionidae. Bollettino del Museo civico di storia naturale di Venezia. 35: 7-41.

Reed LJ, and H. Meunch. 1938. A simple method of estimating fifty percent endpoints. American Journal of Hygiene. 27: 493-497.

Renault D, C. Salin, G. Vannier, and P. Vernon. 1999. Survival and chill-coma in the adult lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), exposed to low temperatures. Journal of Thermal Biology. 24: 229-236.

Reyna PS, L.R. McDougal, and G.F. Mathis. 1982. Survival of Coccidia in poultry litter and reservoirs of infection. Avian Disease. 27 (2): 464-473.

Roelofs WL, and R.T. Carde. 1971. Hydrocarbon sex pheromone in tiger moths (Arctiidae). Science. 171: 684-686.

Rueda LM, and R.C. Axtell. 1996. Temperature dependent development and survival of the lesser mealworm, *Alphitobius diaperinus*. Medical and Veterinary Entomology. 10: 80-86.

Rueda LM, and R.C. Axtell. 1997. Arthropods in litter of poultry (broiler chicken and turkey) houses. Journal of Agricultural Entomology. 14 (1): 81-91.

Safrit RD, and R.C. Axtell. 1984. Evaluations of sampling methods for darkling beetles (*Alphitobius diaperinus*) in the litter of turkey and broiler houses. Poultry Science. 63: 2368-2375.

Salin C, P. Vernon, and G. Vannier. 1999. Effects of temperature and humidity on transpiration in adults of the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Journal of Insect Physiology. 45: 907-914.

Salin C, D. Renault, G. Vannier, and P. Vernon. 2000a. A sexually dimorphic response in supercooling temperature, enhanced by starvation, in the lesser mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Journal of Thermal Biology. 25: 411-418.

Salin C, Y.R. Delettre, M. Cannavacciuolo, and P. Vernon. 2000b. Spatial distribution of *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) in the soil of a poultry house along a breeding cycle. European Journal of Soil Biology. 36: 107-115.

Sarin K. 1972. On food utilization and nutrients by *Alphitobius diaperinus* (PANZ). Zool. Jahrbucher. Abt. Allg. Zool. Phys. Tiere. 76 (3): 408-415.

Sarin K. 1973. Digestive enzymes in immature and adult stages of *Alphitobius diaperinus* (Panz.). Kontyu. 41 (1): 10-17.

Sarin K, and S.C. Saxena. 1975. Food preference and site of damage to preferred products by *Alphitobius diaperinus* Panz. (Pests of stored cereals). Bulletin of Grain Technology. 13 (1): 50-51.

Sarin K. 1978. Population variation of *Alphitobius diaperinus* (Panz) during a year. Indian Journal of Entomology. 40: 358-359.

Sarin K. 1980a. On population variation of a stored product pest *Alphitobius diaperinus* (Panz) during a year. Pestology. 4 (9): 30-31.

Sarin K. 1980b. On the morphology of insects of economic importance. III. Neck and thoracic region on *Alphitobius diaperinus* (Panz) (Tenebrionidae--Coleoptera). The Journal of Animal Morphology and Physiology. 27: 279-289.

Savage S. 1992. Reducing darkling beetles. Poultry Digest. 51 (10): 34-36.

SAS Institute, 1987. SAS System for Elementary Statistical Analysis. SAS Institute Inc., Cary, NC.

Saxena SC, and K. Sarin. 1972. Relative toxicity of some common insecticides to the adults of *Alphitobius diaperinus* (Panz). Indian Journal of Entomology. 34: 349-351.

Schmidtmann ET, and P. A. W. Martin. 1992. Relationship between selected bacteria and the growth of mature house flies, *Musca domestica*, in an axenic test system. Journal of Medical Entomology. 29: 232-235.

Schmitz VM, and R. Wohlgemuth. 1988. Untersuchungen zum massenauftreten und verhalten von *Alphitobius diaperinus* Panz. (Coleoptera: Tenebrionidae) in Huhnermastbetrieben als Grundlage zur praxiisgerechten bekampfung. Anzeiger Fuer Schaedlingskunde Pflanzenschutz Umweltschutz. 61: 108-114.

Schultz-Cherry S, D. J. King, and M. D. Koci. 2001. Inactivation of an astrovirus associated with poultry enteritis mortality syndrome. Avian Disease. 45: 76-82.

Sharma GP, S.M. Handa, and S. Sharma. 1977. Karyometrical analysis of three species of tenebrionid beetles (Coleoptera). Research Bulletin of the Panjab University. 28: 197-199.

Sinha M. 1976. Digestive enzymes in the gut and salivary glands *of Sarcophaga ruficornis* Fab. and *Musca domestica* L. (Diptera: Insecta). Applied Entomology and Zoology. 11: 260-262.

Silfverberg H. 1979. *Alphitobius diaperinus* (Panz) (Tenebrionidae) etablerad i Finland. Notulae Entomologicae. 59 (4): 171.

Skewes PA, and J.L. Monroe. 1991. The effects of darkling beetles on broiler performance. Poultry Science. 70:1034-1036.

Spilman TJ. 1966. On the generic names *Alphitobius, Phthora, Clamoris,* and *Cataphronetis.* Proceedings Entomological Society of Washington. 68: 6-10.

Stafford III KC, and C.H. Collison. 1987. Manure pit temperatures and relative humidity of Pennsylvania high-rise poultry houses and their relationship to arthropod population development. Poultry Science. 66: 1603-1611.

Stafford III KC, C.H. Collison, J.G. Burg, and J.A. Cloud. 1988. Distribution and monitoring lesser mealworms, hide beetles, and other fauna in high-rise caged-layer poultry houses. Journal of Agricultural Entomology. 5 (2): 89-101.

Steenberg T, and J.B. Jesperson. 1998. Microbial pest control in animal husbandry. Insect Pathogens and Insect Parasitic Nematodes: Proceedings of the 6th European Meeting "Microbial Control in Insect Pests in Sustainable Agriculture". Copenhagen Denmark. 10-15 August 1997. p 25-29.

Steenburg T, and R.A. Humbert. 1999. Entomopathogenic potential of *Verticillium* and *Acremonium* species (Deuteromycotina: Hyphomycetes). Journal of Invertebrate Pathology. 73: 309-314.

Steinkraus DC, C.J. Geden, and D.A. Rutz. 1991. Susceptibility of lesser mealworm (Coleoptera: Tenebrionidae) of *Beauveria bassiana* (Moniliales: Moniliaceae): Effects of host stage, substrate, formulation, and host passage. Journal of Medical Entomology. 23 (3): 314-321.

Steinkraus DC, W.M. Brooks, and C.G. Geden. 1992. Discovery of the neogregarine *Farinocystis tribolii* and an eugregarine in the lesser mealworm, *Alphitobius diaperinus.* Journal of Invertebrate Pathology. 59: 203-205.

Steinkraus DC, and E.A. Cross. 1993. Description and life history of *Acarophenax mahunkai*, n. sp. (Acari, Tarsonemina: Acarophenacidae), an egg parasite of the lesser mealworm (Coleoptera: Tenebrionidae). Annals of the Entomological Society of America. 86 (3): 239-249.

Strother KO, and C.D. Steelman. 2001. Spatial analysis of *Alphitobius diaperinus* (Coleoptera:Tenebrionidae) in broiler production facilities. Environmental Entomology. 30 (3): 556-561.

Swatonek VF. 1970. The biology of the shining black wheat beetle (*Alphitobius diaperinus* Panz. = A. piceus Oliv.). Anz. Schaedlingsk. Pflanzenschutz. 43 (7): 101-104.

Tabassum R, S.M. Hassan, M. Jahan, S.M. Nurulain, M.F. Khan, and M.A. Azmi. 1998. Determination of the toxicities of Fenpropathrin (Pyrethroid) and Neem

Formulation (RB -a+PBO+TX-100) against *Alphitobius diaperinus* adults and their effects on transaminases. Turkish Journal of Zoology. 22 (4): 319-322.

Terra WR, F.P. Espinosa-Fuentes and C. Ferreira. 1988. Midgut amylase, lysozyme, aminopeptidase, and trehalase from larvae and adults of *Musca domestica*. Archives of Insect Biochemistry and Physiology. 9: 283-297.

Toyoshima K, H. Banba, and Kamiya. 1996. Control of *Alphitobius diaperinus* (Panzer) on chicken house I: sensitivity to insecticides on imago. Research Bulletin of the Aichi Ken Agricultural Research Center. 28: 363-367.

Tseng YL, J.A. Davidson, and R.E. Menzer. 1971. Morphology and chemistry of the odiferous gland of the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Annals of the Entomology Society of America. 64 (2): 425-430.

Turner Jr. EC. 1986. Structural and litter pests. Poultry Science. 65: 644-648.

Vannier G. 1987. Mesure de la thermotorpeurchez les insectes. Bulletin de Societe d'Ecophysiologie. 12: 165-186.

Vaughan JA. 1982. Biology and control of the lesser mealworm: *Alphitobius diaperinus* a structural pest in poultry houses. Masters Thesis. Virginia Polytechnic Institute and State University.

Vaughan JA, E.C. Turner Jr., and P.L. Ruszler. 1984. Infestation and damage of poultry house insulation by the lesser mealworm, *Alphitobius diaperinus* (Panzer). Poultry Science. 63: 1094-1100.

Vaughan JA, and C.E. Turner Jr. 1984. Residual and topical toxicity of various insecticides to the lesser mealworm (Coleoptera: Tenebrionidae). Journal of Economic Entomology. 77 (1): 216-220.

Victor JR, and P.S. Ogonor. 1987. Humidity reactions in two species of tenebrionid beetles infesting poultry and food storage houses in Nigeria. Revue de Zoologie Africaine. 101: 423-430.

Voris JC, J.A. Meyer, R. Pfost, and R. Woodbury. 1994. Temperature affects lesser mealworm populations in turkey brooder houses. California Agriculture. 48 (2): 18-21.

Wallace MMH, R. G. Winks, and J. Voestermans. 1985. The use of a beetle, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) for the biological control of poultry dung in high-rise layer houses. The Journal of the Australian Institute of Agricultural Science. 51 (3): 214-219.

Watson, DW, P. A. W. Martin, and E. T. Schmidtmann. 1993. Egg yolk and bacterial growth medium for the house fly, *Musca domestica* L. (Diptera: Muscidae) Journal of Medical Entomology. 30: 820-823.

Watson DW, D.A. Rutz, K. Keshavarz, and J.K. Waldron, 1998. House fly (*Musca domestica* L.) survival after mechanical incorporation of poultry manure into field soil. Journal of Applied Poultry Research. 7: 302-308

Watson DW, J.S. Guy, and S.M. Stringham, 2000. Limited transmission of turkey coronavirus in young turkeys by adult *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Journal of Medical Entomology. 37: 480-483.

Watson DW, P.E. Kaufman, D.A. Rutz, and C.S Glenister. 2001. Impact of darkling beetle *Alphitobius diaperinus* (Panzer) on establishment of the predaceous beetle *Carcinops pumilio* (Erichson) for *Musca domestica* control in caged layer - poultry houses. Biological Control. 20: 8-15.

Watson DW. 2002. North Carolina State University. Department of Entomology. College of Life Sciences. Raleigh, NC.

Weaver DK. 1989. The Role of Selected Frass Chemicals and Cuticular Lipid Components in the Orientation of Certain Larval Tenebrionidae. Doctoral Dissertation. McGill University, Montreal, Quebec.

Weaver JE, and V.A. Kondo. 1987. Laboratory evaluation of insect growth regulators in producing lesser mealworm mortality and egg infertility. Journal of Agricultural Entomology. 4 (3): 233-245.

Weaver JE. 1996. The lesser mealworm, *Alphitobius diaperinus*: field trials for control in a broiler house with insect growth regulators and pyrethroids. Journal of Agricultural Entomology. 13 (2): 93-97.

West LS. 1951. The House Fly: Its natural history, medical importance, and control. Comstock Publishing, Cornell University Press, Ithaca, NY.

White NDG, P.S. Berker, and C. J. Demianyk. 1995. Beetles associated with stored grain captured in flight by suction traps in southern Manitoba. Journal of the Entomological Society of Manitoba. 51: 1-5.

Wildey KB, and P.L.G. Bateman. 1983. Insect pests in animal houses - current control developments. September 7-10. Robinson College, Cambridge. p 1-11.

Wills LE, and B.A. Mullens. 1991. Vertical distribution of dipterous larvae and predatory arthropods in accumulated caged layer poultry manure in southern California. Journal of Agricultural Entomology. 8 (1): 59-66.

Wilson DD, E.T. Schmidtmann, R.D. Richard, and R.D. Lehman. 1986. Isolation of avian influenza from insects. In: *Arbovirus Research in Australia - Proceedings 4th Symposium*. St. George, T.D., B.H. Kay, and J. Blok, eds. Brisbane Australia.

Wilson TH, and F.D. Miner. 1969. Influence of temperature on development of the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Journal of the Kansas Entomological Society. 42: 294-303.

Wohlgemuth R. 1989. Control of mealworm (*Alphitobius diaperinus* Panz.) in poultry housing. Healthy animals, safe foods, healthy man; Proceedings of the Xth jubilee international symposium world association of veterinary food hygienists. pp 18-20.

Worley JR, M.L. Cabrera, and L.M. Risse. 2000. Reduced levels of alum to amend broiler litter. Applied Engineering in Agriculture. 16 (4): 441-444.

Worley JW, L.M. Risse, M.L. Cabrera, and M.P. Nolan, Jr. 1999. Bedding for broiler chickens: two alternative systems. Applied Engineering in Agriculture. 15 (6): 687-693.

Yagi AI, and M.T. Abdel Razig. 1972. *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) in pigeon houses in the Sudan. Current Science. 41 (17): 642-643.

Yoshida T. 1983. Historical change in the status of stored product insect pests especially in Japan. Proceedings of the Third International Working Conference on Stored Product Entomology. pp 655-668.

Zaedee IHM, and S. Ignatowicz. 1993. Mortality of the larvae and adults of the lesser mealworm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), caused by gamma radiation. Annals of Warsaw Agricultural University-SGGW Veterinary Medicine. 18: 131-140.

Zublena, J. P., J. C. Barker and T. A. Carter. 2002. Soil Facts: Poultry manure as a fertilizer source. Extension on-line Fact sheet: Department of Soil Science, North Carolina State University, Raleigh, NC. http://www.soil.ncsu.edu/publications/Soilfacts/AG-439-05/

Appendix I

Preliminary Studies on Odoriferous Gland Extract as a Potential Aggregation Pheromone in the Darkling Beetle, *Alphitobius diaperinus*.

Abstract

The darkling beetle, *Alphitobius diaperinus*, is a cosmopolitan pest of stored products and commercial poultry facilities. Darkling beetles damage poultry house insulation, affect bird performance when consumed by poultry and have been implicated in the transmission of over thirty avian diseases including turkey coronavirus (TCV). Common litter management practices include the removal of used poultry litter for use as organic fertilizer. Land applied litter may serve as a source of re-infestation or dispersal of darkling beetles to nearby farms or residential areas. Darkling beetles are commonly found in aggregations poultry litter. Male and female darkling beetles possess an odoriferous gland that has been shown to produce quinones and hydrocarbons. To evaluate the potential aggregation pheromone activity of these hydrocarbons, odoriferous glands were dissected from unmated male and female adult darkling beetles 1, 3, 5, 7, 9, 12, and 15 days-old. Hydrocarbon components of the odoriferous glands of were extracted with hexane, isolated by column chromatography and subjected to analysis by gas chromatography. Comparisons of gas chromatograms of odoriferous gland extracts revealed several peaks shared by all samples (14.99, 12.40 and 17.23 minutes retention time) although intensity varied with age and sex. A peak at approximately 19.20 minutes decreases in intensity in males from 1 day-old (90, 000 mV) to 3 days-old (23, 000 mV) and was only present in gland extracts of females at 5 days-old (17, 750 mV) and 9 days-old (18, 000 mV). Chromatograms of gland extracts of females 3 days-old and 7 days-old have a group of several peaks between 22 and 24 minutes retention time. Female adult darkling beetles become sexually receptive between 3 and 6 days-old. These peaks are

not present in chromatograms of gland extracts of males of any age. These preliminary data suggest the presence of an aggregation and/or sex pheromone. Hydrocarbon identification and confirmation of biological activity via olfactometer assays are underway.

Introduction

The darkling beetle, *Alphitobius diaperinus* is often found in great numbers in poultry litter (Pfeiffer and Axtell, 1980; Rueda and Axtell, 1997). High populations are a concern to producers due to the potential of the beetle to transmit pathogens including bacteria in the genera *Escherichia*, *Salmonella*, *Bacillus*, and *Streptococcus* and viruses that cause leukosis and infectious bursal disease in poultry (Despins et al., 1994; McAllister et al., 1994, 1995b, 1996; Goodwin and Waltman, 1996). Broiler chicks and poults readily consume darkling beetle adults and larvae aiding in the transmission of pathogenic organisms. Feeding on darkling beetles by birds also has negative effects on weight gain and feed consumption (Despins and Axtell, 1995).

Modern poultry houses are insulated to maintain an optimal temperature range in the house. Tunneling by beetle larvae in search of pupation sites reduces the insulating value of the material and inability to maintain temperature in an optimal range results in higher costs associated with reduced feeding efficiency, replacement of the insulating material and the time the house is out of production (Vaughn et al., 1984; Geden, 1995).

Current litter management practices include the periodic clean out of the poultry litter and its use as an organic fertilizer. Emigration of beetles from land applied litter to nearby homes and businesses may result in litigation and become a public relations problem (Miller, 1997; Hinchey, 1997).

Infestations of lesser mealworm can be severe. Summer populations of beetles in poultry farms in North Carolina can reach densities of 10,000 to 20,000 individuals per square meter (Arends, 1987). Adults and larval darkling beetles are found in the pit of

high rise poultry houses at the lower portion of the manure cone (Stafford III and Collison, 1988; Wills and Mullins, 1991; Geden,) and in broiler houses and turkey grow-out houses in and under the litter and any objects that might be lying on the surface of the litter (feeders, dead birds, nest boxes, etc.) (Lancaster and Simco, 1967). Darkling beetle larvae and adults are not evenly distributed in these microhabitats in a poultry house but rather are found in clumps or aggregations. Darkling beetle adults placed in a petri dish with wood shavings will, after only a few minutes, form a tight aggregation (Appendix II, Fig. 10).

Pheromones are secreted chemical substances that release specific behaviors or responses in the recipient and serve as a form of communication between insects (Karlson and Luscher, 1959). Pheromones may be used to monitor or control insects (Burkeholder, 1981). Studies on potential aggregation pheromones of the darkling beetle have been few. Weaver (1989) studied the attractiveness of frass to larval darkling beetles. Falomo (1986) observed that while calling, male and female adult beetles will rub the seventh and eighth abdominal tergites with the hind tarsi in a wiping motion as if to disperse pheromone into the air. Scanning electron microscopy revealed the presence of setiferous patches on the dorsal abdomen. Adult darkling beetles of both sexes were attracted to solvent extracts of patch secretions, suggesting a possible pheromone source.

Adult darkling beetles possess a pair of eversible posterior abdominal glands (Wilson and Miner, 1969) described as follows: "Adults of both sexes possess a pair of large, fleshy scent glands that protruded when pressure was applied to the posterior region of the abdomen. The fluid obtained by puncturing the extruded gland produced a

musky odor and caused sexual excitement in both sexes." These observations suggested the presence of a sex and/or aggregation pheromone. In experiments using live beetles and a convection current olfactometer, no evidence of a sex pheromone was found (Preiss, 1971). Tseng et al. (1971) described the morphology of the paired glands and found the glands to be equally well developed and morphologically identical in both sexes. Each gland consists of two structures: a large bifurcating sac that serves as a reservoir for the secretory fluid and the glandular secretory cells. Infrared analysis of the gland secretion resulted in identical spectra for males and females. Thin layer chromatography, gas chromatography and mass spectrophotography revealed the major components of the gland secretion to be quinones and hydrocarbons. Quinones are known to be defensive compounds and are common defensive secretions in tenebrionid beetles (Tseng et al., 1971). Hydrocarbons have been found to function as pheromones in the stored grain beetle *Tribolium confusum* (Keville and Kannowski, 1975), several species of arctiid moths (Roelofs and Carde, 1971) and some diptera (Howard and Blomquist, 1982).

The objective of this study was to examine the role of the hydrocarbon constituents of the odoriferous gland of the darkling beetle adult as potential aggregation pheromones.

Materials and Methods

Insects

Darkling beetles collected from a poultry house were maintained in a colony at North Carolina State University. Adult male and female darkling beetles were placed inside a plastic container with a handful of chicken starter feed. The center of the container lid was cut out and replaced with fabric mesh. Water was added though the mesh as needed. Stacks of 10 pieces of 5 cm x 5 cm of black construction paper secured with a staple through the center of the stack were placed inside the container. Female adult beetles oviposited in the spaces between the individual pieces of paper. After ovoposition, the papers were removed and placed into a new container with fresh feed. Darkling beetle larvae were allowed to progress to the pupal stage at which time the pupae were removed from the colony, sexed and placed into individual cells of a plastic microcentrifuge tube rack. Males and females were placed in separate racks. Racks were placed in an incubator at 30° C, 75% RH, and an 18/6 L/D photoperiod. Upon eclosion beetles were removed from the individual cells and placed in petri dishes with a small amount of starter feed and a crumpled kim-wipe moistened with water. Petri dishes were returned to the incubater until insects were needed.

Dissections

Beetles were washed three times in distilled water to remove frass and food residue. A single beetle was placed on a silicone dissecting dish that had previously been washed with dilute bleach solution, distilled water, 75% ethyl alcohol, distilled water and then air dried. The beetle was pinned through the thorax, ventral side down and the elytra removed with scissors. The soft tissues of the abdomen were reflected back exposing the paired eversible glands at the extreme posterior of the abdomen attached to the abdominal wall (Appendix II, Fig. 11). The glands were grasped gently with fine forceps and cut away from the abdominal wall with a small scalpel. The glands were immediately placed in three mls hexane. Glands were removed from unmated male and female beetles at ages 1, 3, 5, 7, 9, 11, 13, and 15 days. Five pairs of glands were pooled for each sample. In addition, five beetles from each sample group were placed in three mls of hexane and shaken gently for five minutes. Gland extracts and cuticular extracts were stored at -70° C.

Column Chromatography

To separate hydrocarbon components from the hexane samples, columns were prepared by plugging the tapered end of pasture pipettes with glass wool and packing the tube with 500 mg silica gel. Columns were conditioned in an oven for one hour at 150° C. Once at room temperature, columns were placed in a rack over a metal pan and washed with one ml of hexane. Gland extracts were evaporated under nitrogen gas to a volume of one ml, added to the column and eluted with hexane until 7 ml of eluant was

collected. Eluant was evaporated under nitrogen gas to a volume of one ml and stored at -70° C.

Gas Chromatography

Gland extracts of unmated males 1, 3, 9 and 15 days-old and unmated females 3, 5, 7, and 9 days-old were analyzed with a Hewlett-Packard GCD (HP GCD) Gas Chromatograph-Mass Spectrometer equipped with a 30 m x 0.25 mm HP-5 0.25 µm film thickness column. The carrier gas was hydrogen at a flow rate of 1 ml/min. The samples were temperature programmed from 50 - 200° C at 5°/min with five minute initial hold and a five minute final hold. Inlet and detector temperature were both 280° C. All other samples were analyzed with a Hewlett-Packard (HP) Model 5890 Gas Chromatograph (GC) with a 15m x 0.25mm HP-5 0.5 µm film thickness column. The carrier gas was hydrogen at a flow rate of 1 ml-min. The samples were temperature programmed from 50 - 200° C at 5°/min with one minute initial hold and a five minute final hold. Inlet temperature was 225° C and detector temperature was 220° C.

Preliminary Results and Discussion

Whole gland extracts from unmated male darkling beetles 1, 3, 7, 9, and 15 days old and unmated female beetles 3, 5, 7, 9, and 12 days old were analyzed. Chromatograms from the HP GCD better illustrated peaks from the gland extract samples. Presumably, this is due to greater sensitivity of the detector in this instrument. Chromatograms of gland extracts of unmated males 7 and 15 days-old and unmated

females 12 days old illustrated only a single peak at approximately 14.99 minutes retention time (Figs. A1.5, 8 and 10).

Although preliminary, some trends in the data were apparent. Several compounds were present in all samples as demonstrated by similar peaks in gas chromatograms. A peak at approximately 14.99 minutes retention time was shared by all samples (Figs. A1.1-10), although intensity varied with age and sex. In males, the intensity was highest at 1 day-old (270, 000 mV) (Fig. A1.6) and declined through day 15 (7700 mV) (Fig. A1.10). In females, the intensity of this peak increased from 2700 mV at 3 days-old (Fig.A1. 1) to 340, 000 mV at day 9 (Fig. A1. 4) then declined to 4000 mV at day 12 (Fig. A1.5). Similar patterns were apparent for peaks at approximately 12.40 and 17.23 minutes retention time (Figs. A1.1-10). The peak at approximately 19.20 minutes decreased in intensity in males from 1 day-old (90, 000 mV) (Fig A1. 6) to 3 days-old (23, 000 mV) (Fig. A1. 7). However, this same peak was only present in gland extracts of females at 5 days-old (17, 750 mV) (Fig. A1.2) and 9 days-old (18, 000 mV) (Fig. A1. 4). Chromatograms of gland extracts of females 3 days-old and 7 days-old have a group of several peaks between 22 and 24 minutes retention time (Figs. A1. 1 and 3). These peaks are not present in chromatograms of gland extracts of males of any age. Hopkins et al., (1992) states that female darkling beetles become receptive to mating at 7 days, although Gangopadhyay et al., (1985) claims females are able to mate at 3 days-old in the presence of males 7+ days old.

Using gas chromatography and mass spectrometry, Tseng et al., (1971) identified the quinone components of the odoriferous gland but not the hydrocarbons.

Hydrocarbons in the odoriferous glands may act as a solvent for the quinones. Darkling beetles have been observed to emit a brownish fluid from the odoriferous glands when handled roughly. This liquid dries immediately upon contact with air to form a crusty solid. Some insects, including the stored grain beetle, *Tribolium castaneum*, use hydrocarbons as pheromones (Roelofs and Carde, 1971; Keville and Kannowski, 1975; Howard and Blomquist, 1982). Hydrocarbon based pheromones provide a close range attractant. The relatively short residues of a hydrocarbon pheromone are advantageous to a stored grain, or in the case of the darkling beetle, litter inhabiting, insect as a pheromone with a lingering residue would shortly saturate the environment. The peak at 14.99 minutes retention time is common to all samples analyzed by the GC and is possibly an aggregation pheromone. Other peaks that vary in intensity with age and sex suggest the presence of an aggregation and/or sex pheromone. Further study is needed to determine the exact compounds present in the odoriferous gland and to what extent these compounds contribute to aggregation and sexual behaviors.

Future Research

These preliminary data suggest the presence of an aggregation and/or sex pheromone. The following is a list of work in progress and that to be completed:

1. Improve CG Method. Chromatograms produced by the HP 5890 are not as detailed as those produced by the HP GCD. Experimentation with various method parameters is needed to yield better quality chromatograms.

2. Replication. Each odoriferous gland extract sample is a pool of five individual glands. These samples must be replicated by rearing and dissecting additional darkling beetles as described above.

3. Bioassays. Compounds that may be potential compounds must be fractionated by High Pressure Liquid Chromatography (HPLC) or column chromatography and subjected to olfactometer bioassays to determine activity as pheromones.

4. Identification. Candidate compounds must be identified by comparison to known databases or known standard compounds.

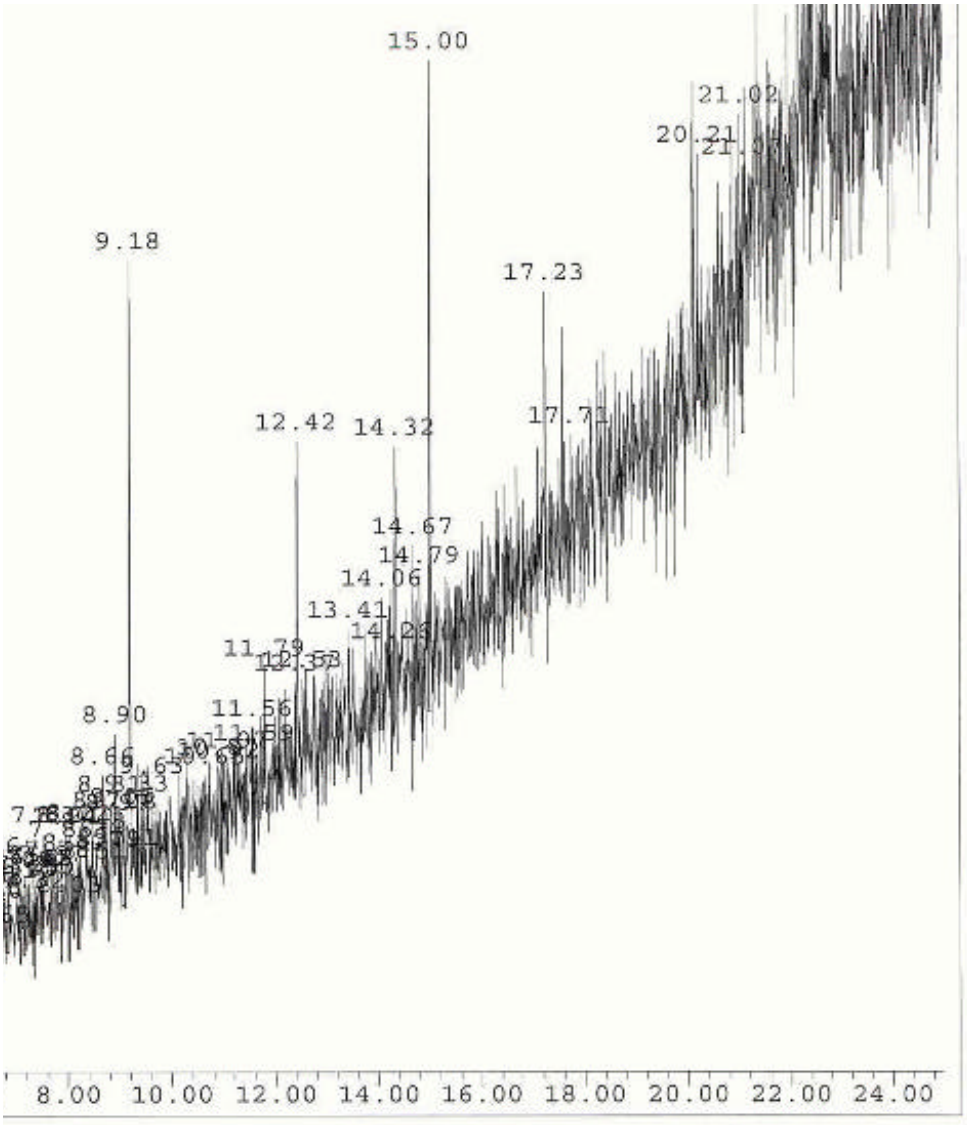


Fig. A1.1. Gas chromatogram of odoriferous gland extract from three-day-old unmated female darkling beetle adult.

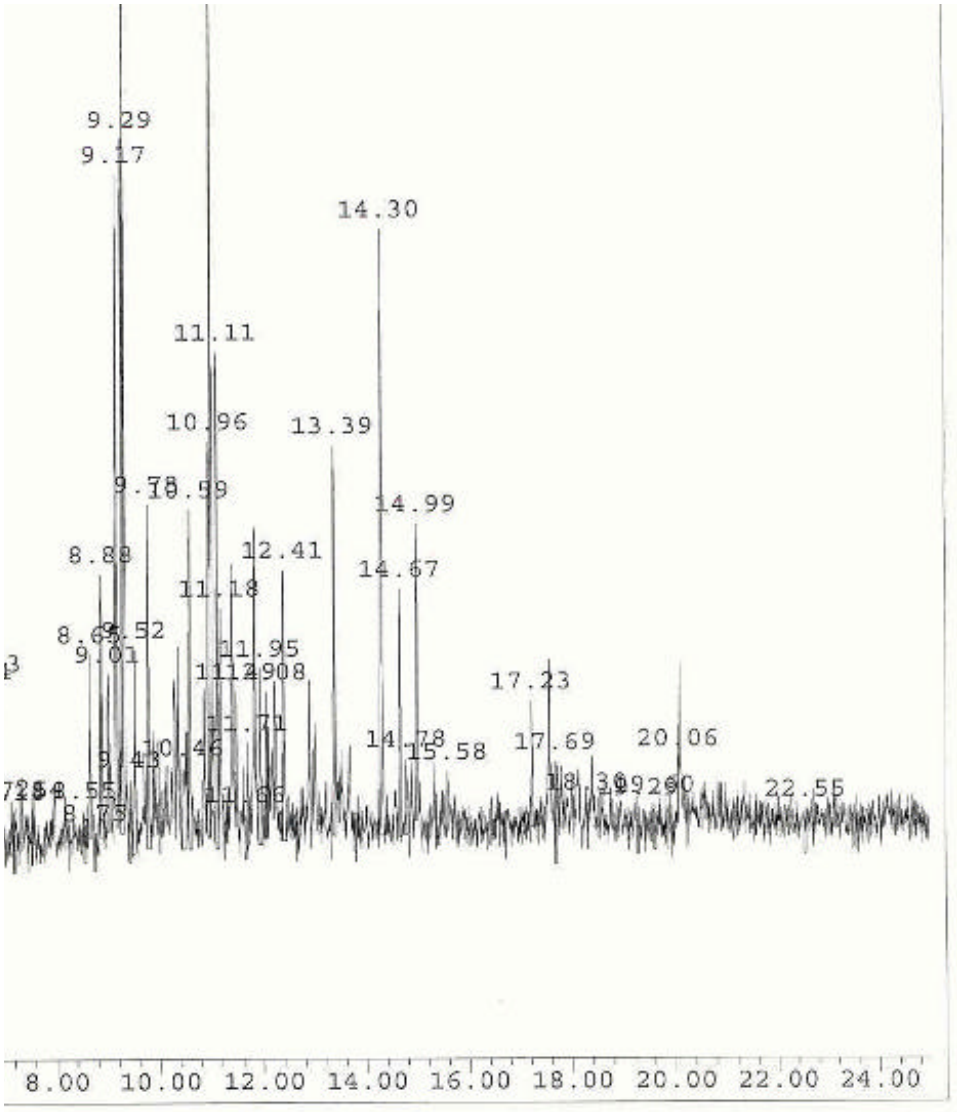


Fig. A1.2. Gas chromatogram of odoriferous gland extract from five-day-old unmated female darkling beetle adult.

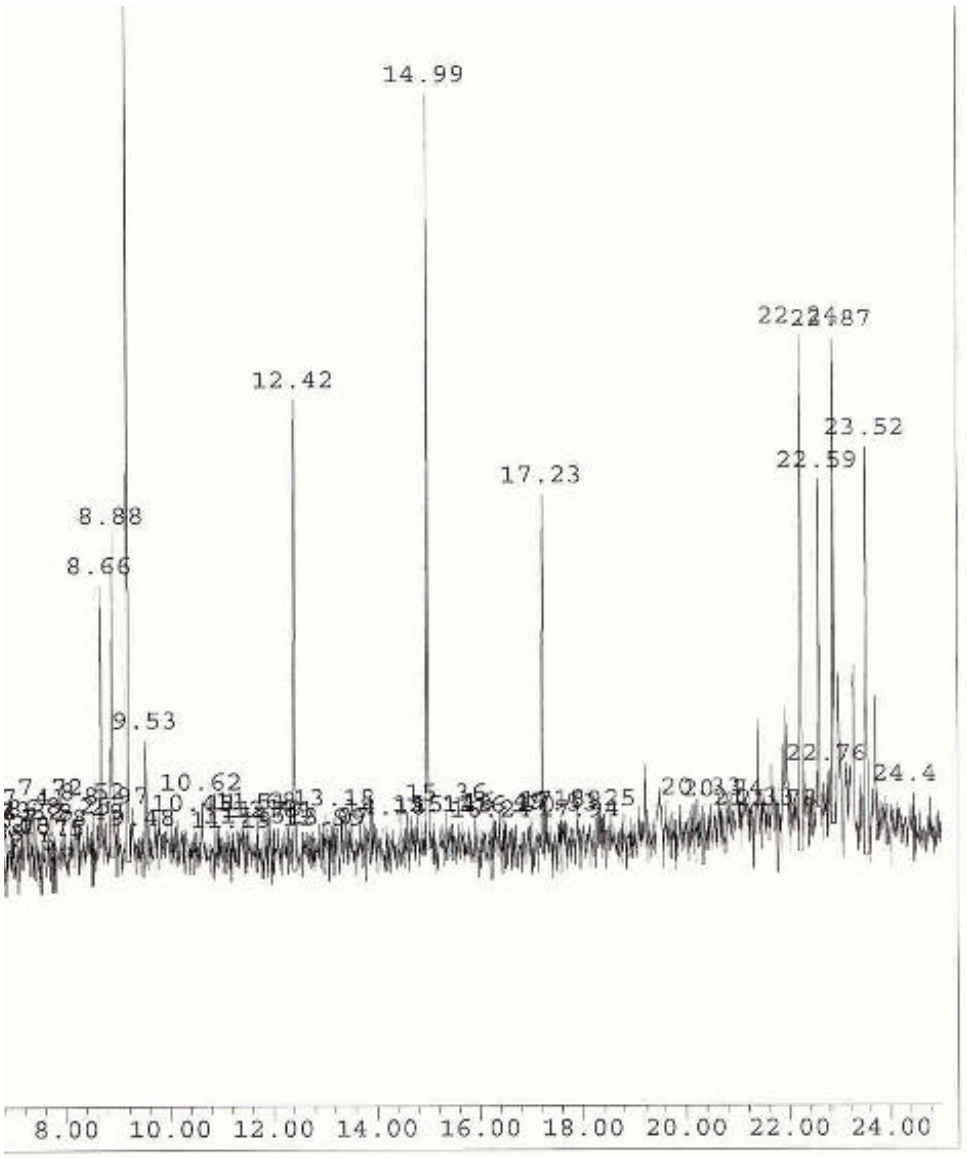


Fig. A1.3. Gas chromatogram of odoriferous gland extract from seven-day-old unmated female darkling beetle adult.

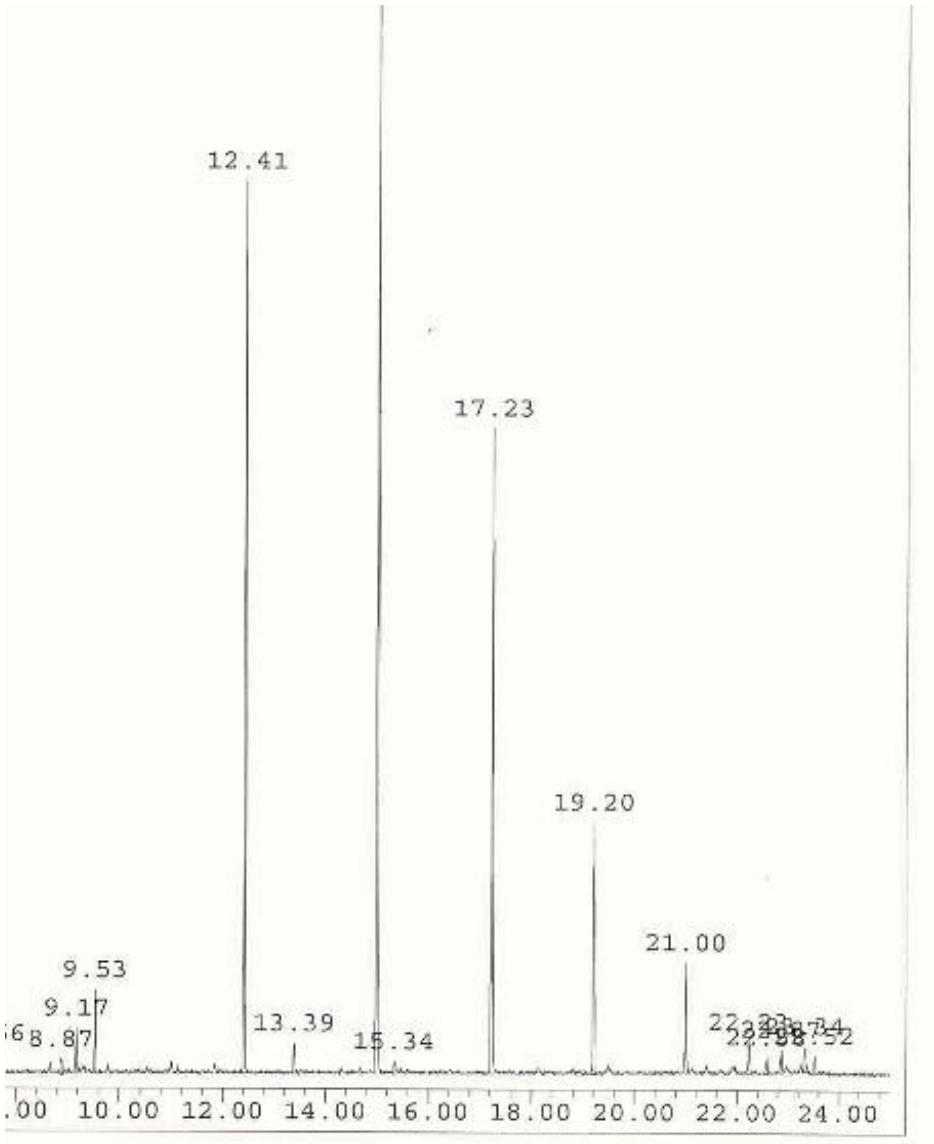


Fig. A1.4. Gas chromatogram of odoriferous gland extract from nine-day-old unmated female darkling beetle adult.

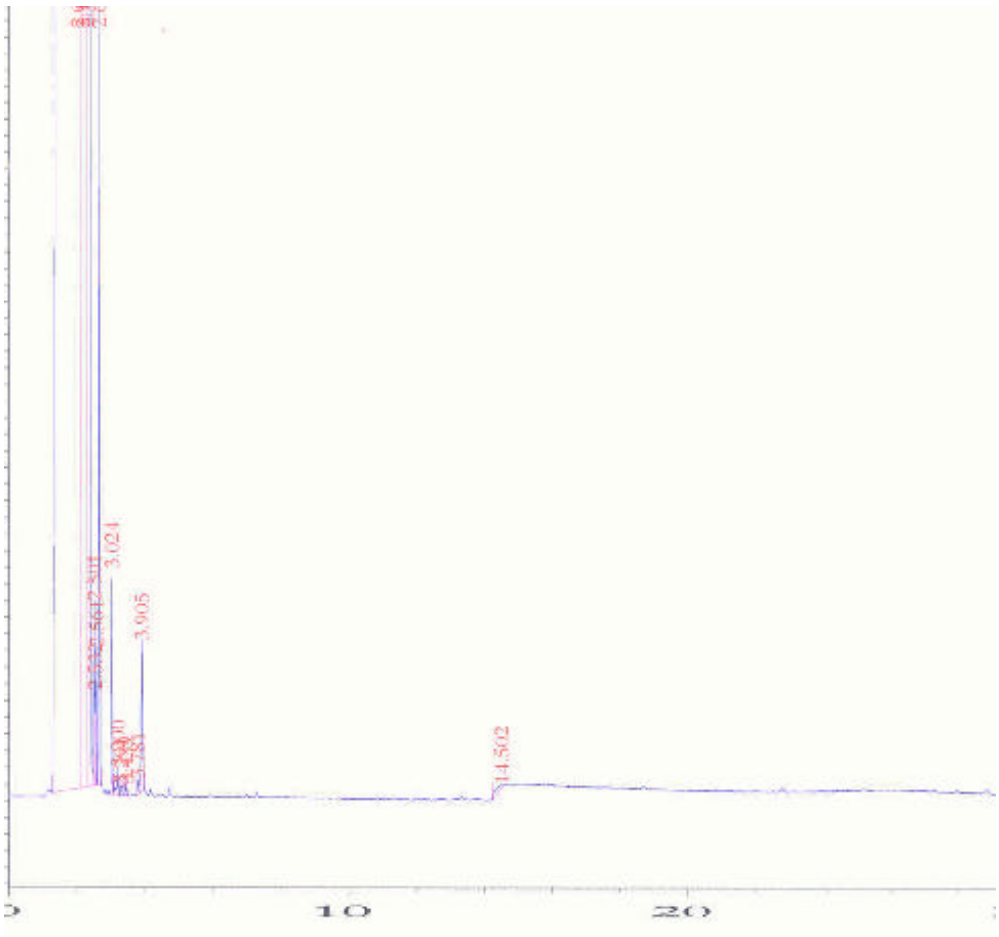


Fig. A1.5. Gas chromatogram of odoriferous gland extract from twelve-day-old unmated female darkling beetle adult.

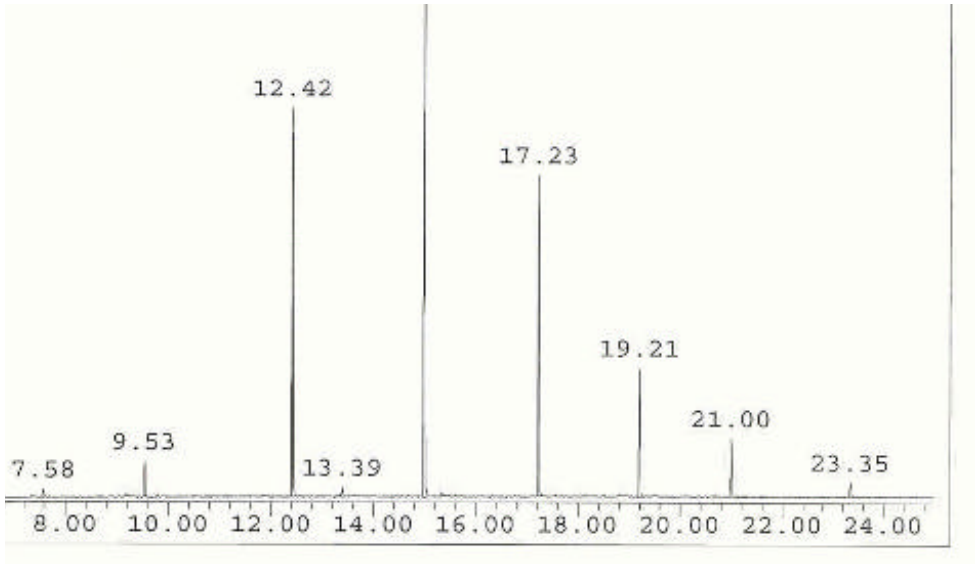


Fig. A1.6. Gas chromatogram of odoriferous gland extract from one-day-old unmated male darkling beetle adult.

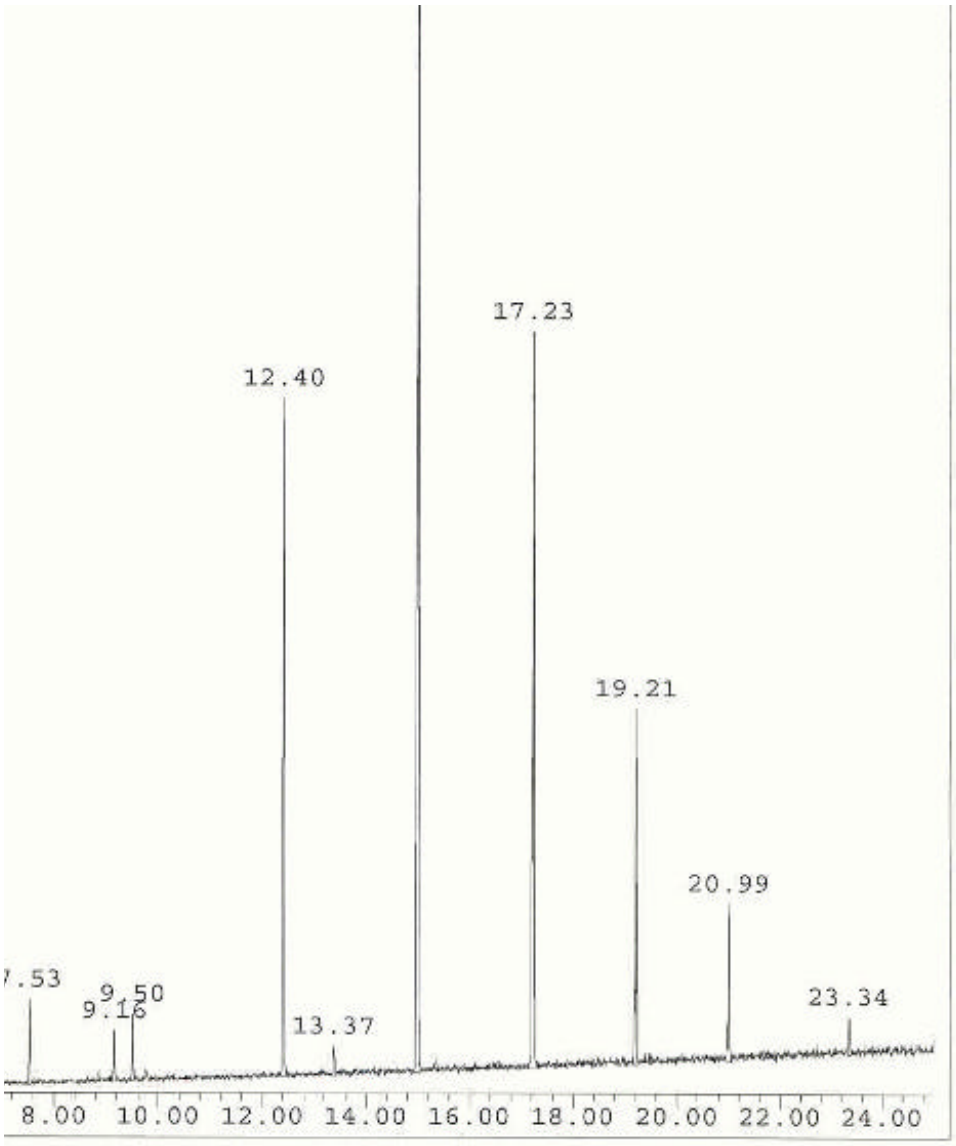


Fig. A1.7. Gas chromatogram of odoriferous gland extract from three-day-old unmated male darkling beetle adult.

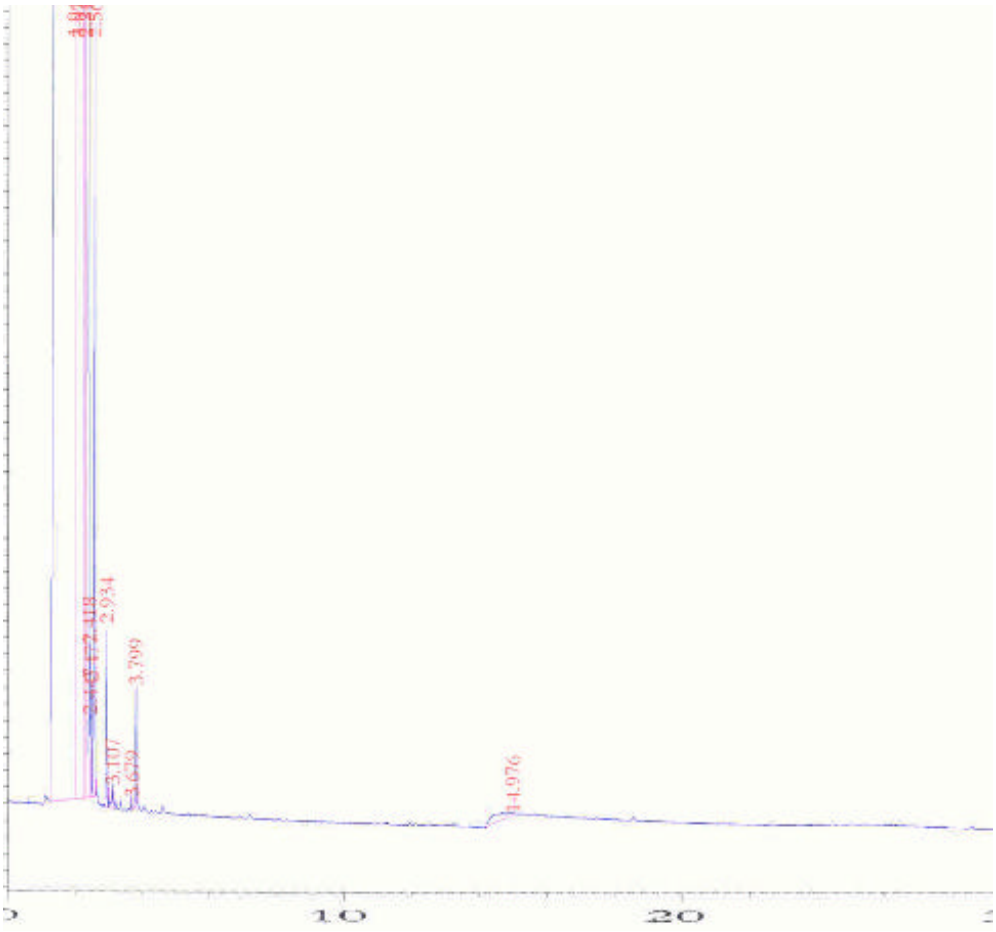


Fig. A1.8. Gas chromatogram of odoriferous gland extract from seven-day-old unmated male darkling beetle adult.

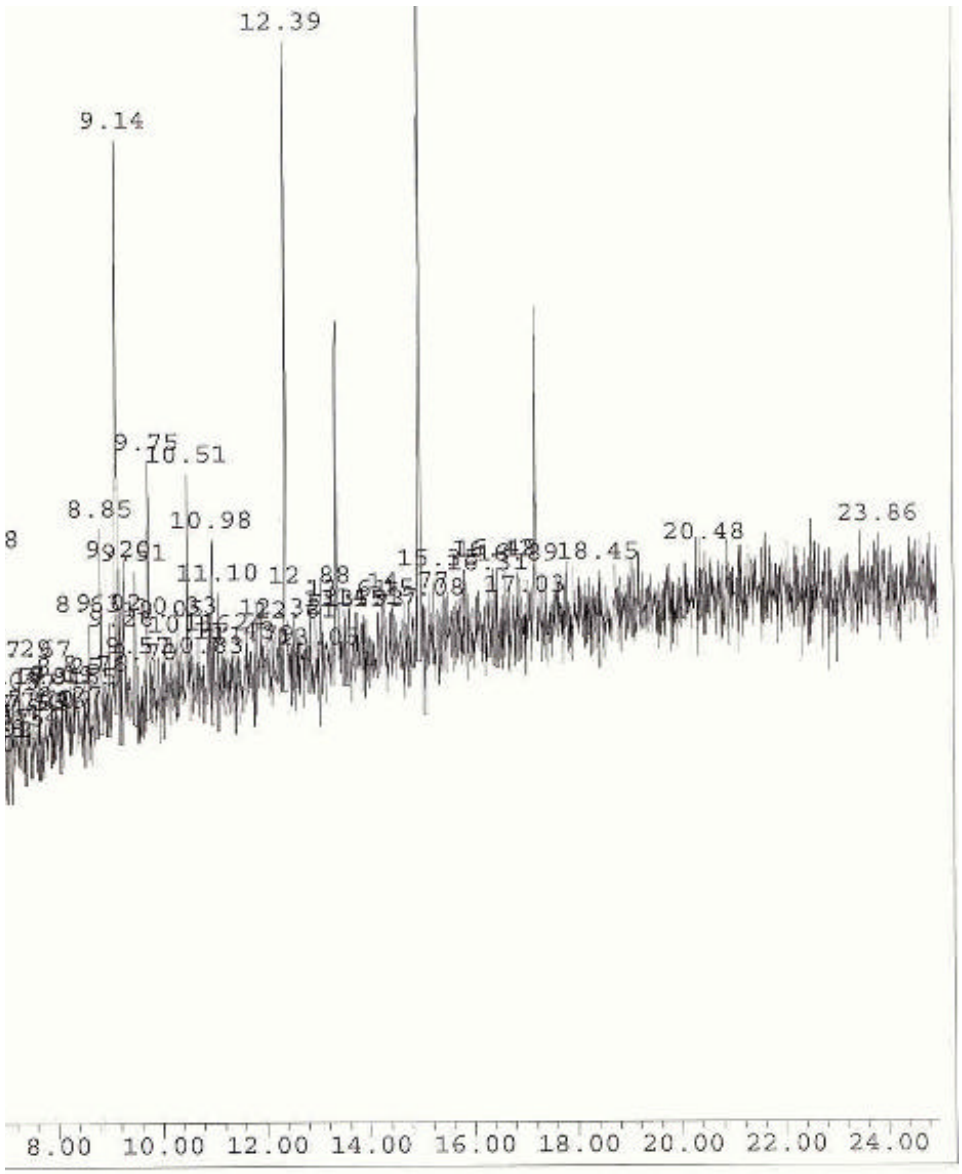


Fig. A1.9. Gas chromatogram of odoriferous gland extract from nine-day-old unmated male darkling beetle adult.

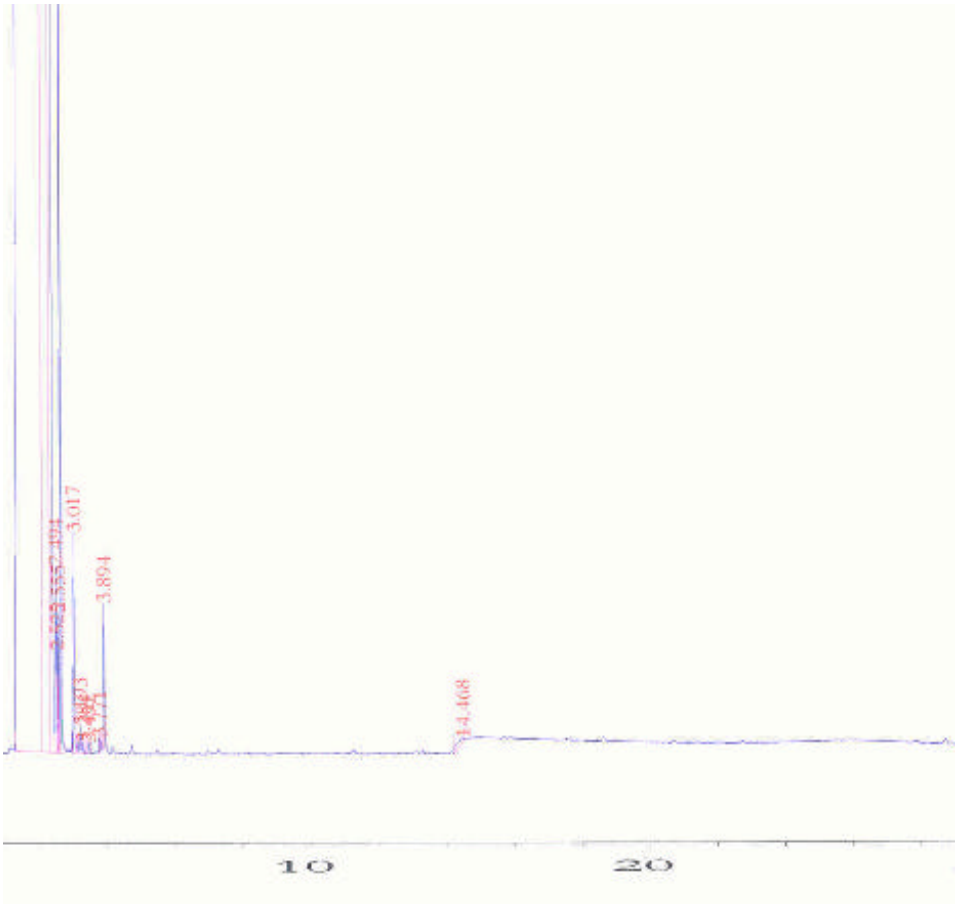


Fig. A1.10. Gas chromatogram of odoriferous gland extract from fifteen-day-old unmated male darkling beetle adult.

Appendix II

Diagrams and Photographs

Photo by Wes Watson

Fig. 1 Adults and larvae of the darkling beetle, *Alphitobius diaperinus*.

Fig. 2. Damage to insulation caused by tunneling darkling beetle larvae.

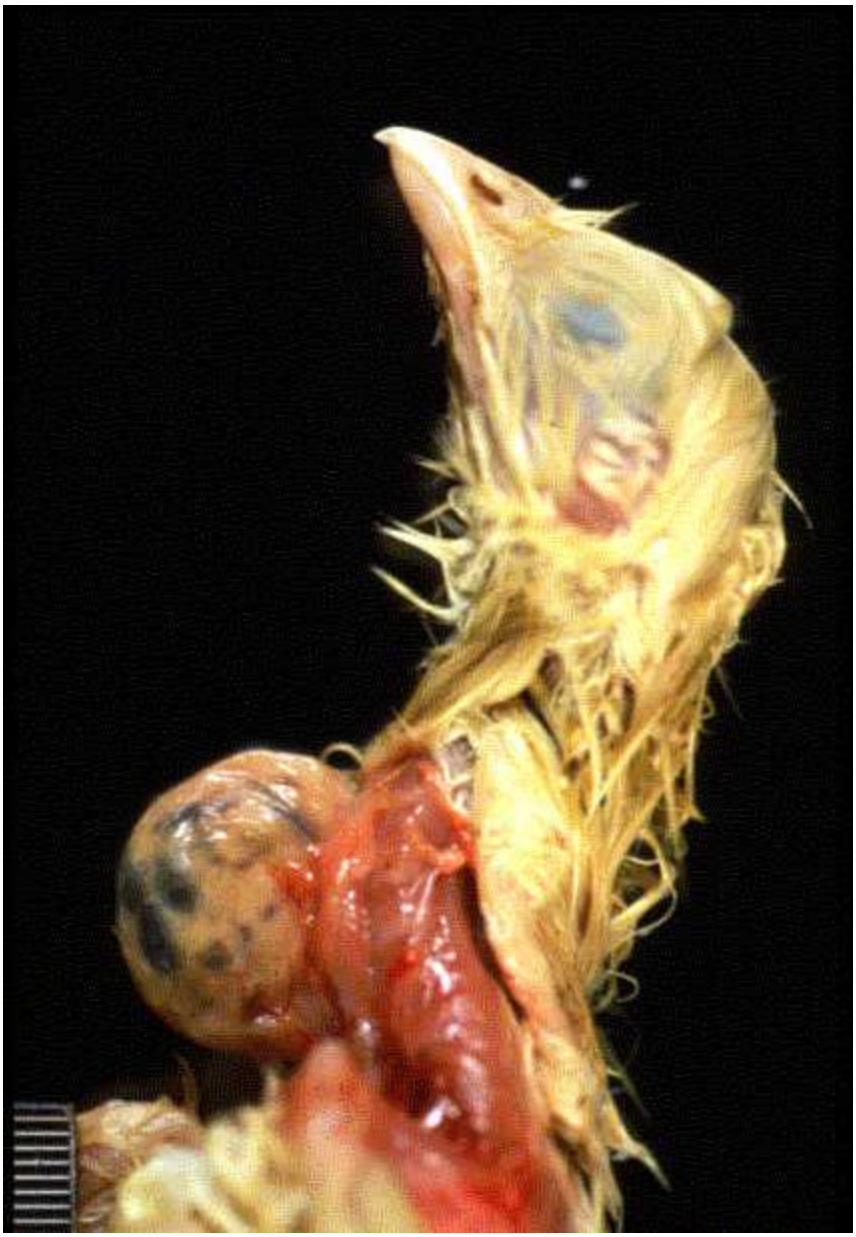


Photo by J. Barnes

Fig. 3. Distended crop due to chick feeding on darkling beetle adults and larvae.

Fig. 4. Lengths of PVC pipe used to simulate land application of poultry litter. Beetles were buried at depths of 0, 8, 15, 23, and 30 centimeters.

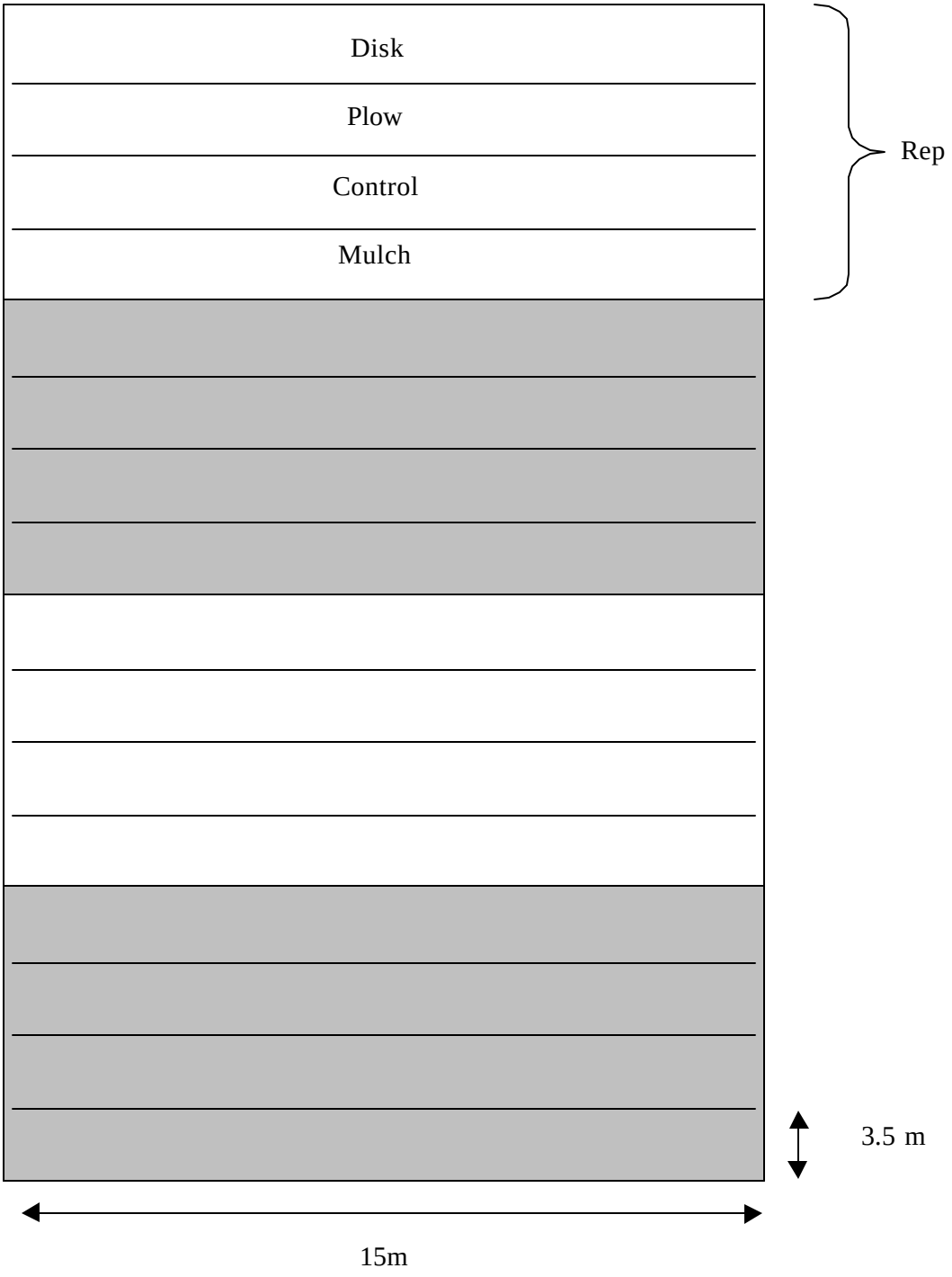


Fig. 5. Diagram of randomized treatments.

A

B

C

Fig. 6. Implements used in mechanical incorporation of poultry manure: A) disk, B) mulch, and C) plow.

A

B

C

Fig. 7. Methods used to estimate number of beetles applied to field prior to mechanical incorporation of poultry manure: A) spreading manure, B) placing trays in each treatment plot to capture beetles from each pass of the manure spreader and C) tray with captured poultry litter and beetles.

A

B

C

D

Fig. 8. Traps used to monitor darkling beetle emergence from field soil following mechanical incorporation of poultry litter: A) cylinder trap, B) tile trap, C) pitfall trap and D) sticky trap.

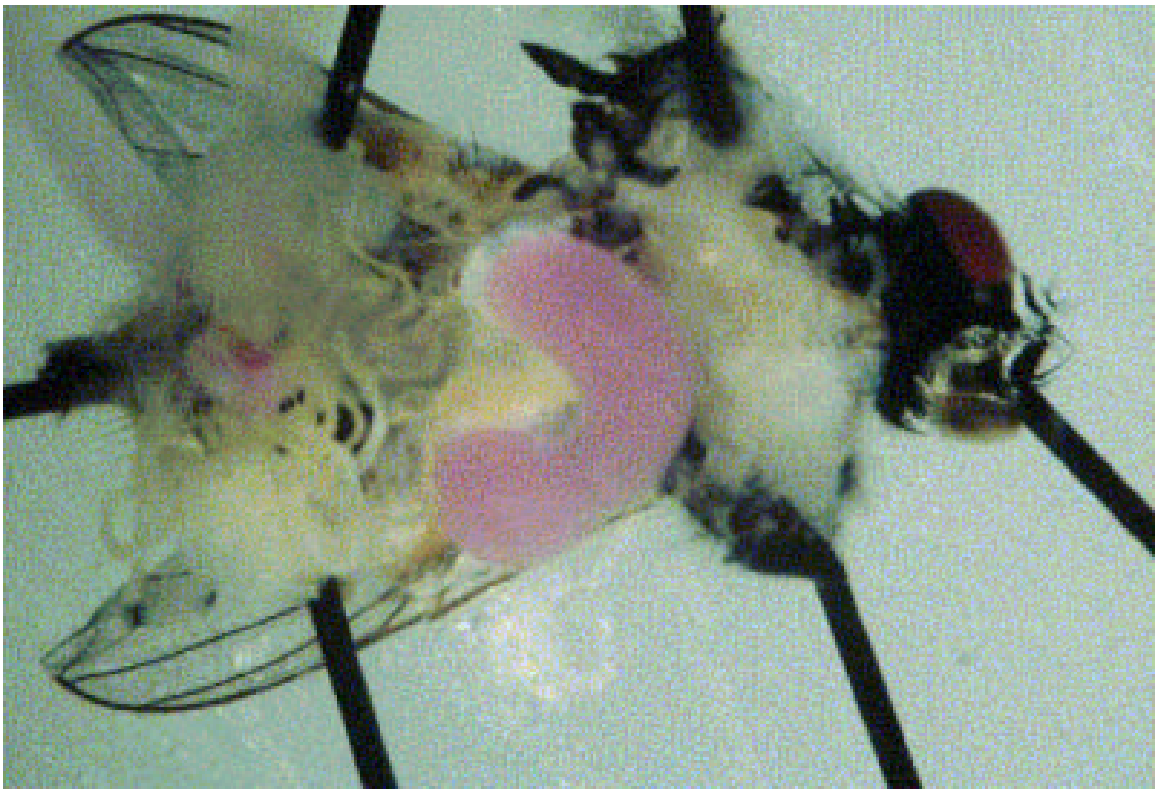


Fig. 9. Dissection of house fly, *Musca domestica*, showing fully distended crop (stained pink).

Fig. 10. Beetle aggregation in petri dish.

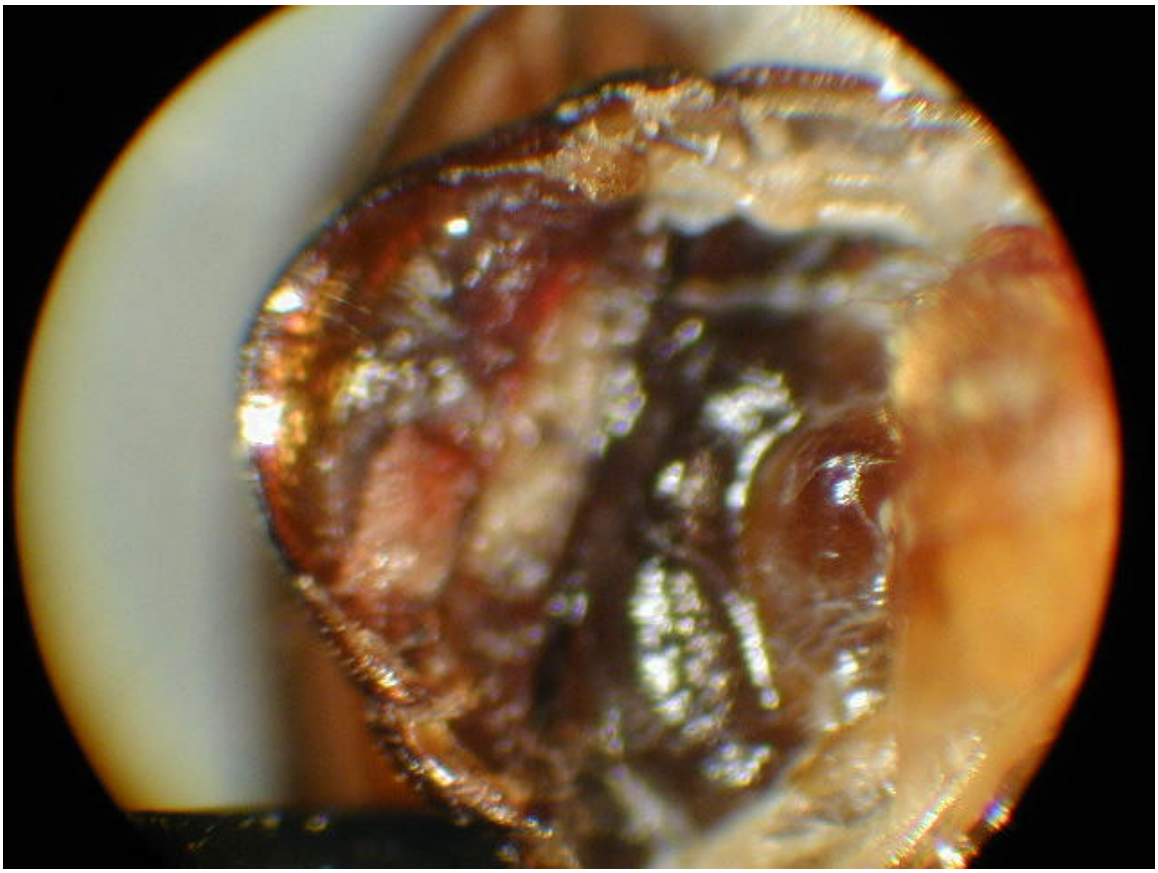


Fig. 11. Dissected darkling beetle adult showing paired eversible glands.