Manure characteristics affecting the management of house fly (Musca domestica L.) populations in duck production facilities

by

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MANURE CHARACTERISTICS AFFECTING THE MANAGEMENT OF HOUSE FLY
(*MUSCA DOMESTICA* L.) POPULATIONS IN DUCK PRODUCTION FACILITIES

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Management techniques rendering poultry manure less suitable for fly reproduction creates unfavourable conditions for larval growth, egg-laying and adult feeding and may reduce fly infestations. Four types of poultry manure (duck, turkey, broiler & layer) were compared. House fly landing preference was highest on duck manure. Laboratory tests investigated house fly preference to duck manure of different moisture levels (40-90%). Adult emergence numbers were highest when moisture content was 65–85%. Adult fly emergence and landing preference from manures treated with 7 concentrations of acetic acid (0.5, 1.0, 2.0, 3.25, 4.5, 5.75 and 7.0 %) and boric acid (0.25, 0.5, 1.0, 1.5, 2.0, 3.25, and 4.5%) was also assessed. All concentrations of acetic and boric acid tested significantly reduced adult emergence, but only the highest dose of acetic acid repelled adults. Practicality and cost of applying the acids to duck manure in production facilities needs to be further investigated.
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<tr>
<td>AA</td>
<td>acetic acid</td>
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<tr>
<td>BA</td>
<td>boric acid</td>
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<td>BCA</td>
<td>biological control agent</td>
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<td>ca.</td>
<td>approximately</td>
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<td>CA</td>
<td>citric acid</td>
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<td>Canadian dollar</td>
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<td>d</td>
<td>day</td>
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<td>DE</td>
<td>diatomaceous earth</td>
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<tr>
<td>h</td>
<td>hours</td>
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<tr>
<td>IPM</td>
<td>integrated pest management</td>
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<tr>
<td>HB</td>
<td>hister beetle</td>
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<td>HL</td>
<td>hydrated lime</td>
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<td>NOS</td>
<td>naturally-occurring substances</td>
</tr>
<tr>
<td>OMAFRA</td>
<td>Ontario Ministry of Agriculture, Food and Rural Affairs</td>
</tr>
<tr>
<td>PMRA</td>
<td>Pest Management Regulatory Agency</td>
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<td>RM</td>
<td>rearing media</td>
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CHAPTER 1: INTRODUCTION

1.1 POULTRY PRODUCTION IN ONTARIO

The poultry industry is an important, expanding sector in Canadian agriculture. In 2012, $3.8 billion of poultry products were sold in Canada, contributing 7.1% of cash receipts to all farming operations (Figure 1.1) (Agriculture and Agri-Food Canada, 2013). Poultry are typically raised for meat and egg consumption, and Canada’s poultry industry is comprised of chicken, turkey, ducks, geese, hatching eggs, and table eggs (Agriculture and Agri-Food Canada, 2013). Commercial poultry production occurs primarily in enclosed buildings; birds are housed indoors to provide protection against extreme weather conditions, predators, and the spread of diseases from wild birds (United States Environmental Protection Agency, 2012). In 2006, 2,706 duck farms existed in Canada; 34% of these were located in Ontario (Figure 1.2), making it the largest duck-producing province (Statistics Canada, 2006).

Poultry species raised for meat, such as broiler chickens, turkeys, ducks, and geese, are generally housed freely in a barn (i.e., floor-raised). Birds are placed in the barn as one-day-old chicks on bedding material (chopped straw or wood shavings). Manure accumulates in the barn for the duration of the grow-out period (period in which birds are housed before they are shipped to market), which can last from 5 weeks for broiler chickens, to 12 weeks for ducks, to 17 weeks for turkey toms (adult males). Normally, the soiled litter is removed prior to placement with the next flock, although some turkey growers re-use the litter for one or more flocks, such that manure remains in the barn for up to a year. Feed is delivered as a complete balanced ration in a pelleted or mash
form via an automatic auger system to open feed pans or troughs suspended from the ceiling that the birds can access at all times. The type of watering equipment varies with bird species and has an important role in the moisture management of litter because water leaks or spillage can create wet spots in the barn. Broiler chickens and ducks are provided a nipple type drinker on a suspended water line that is raised as the birds grow. Turkeys are provided a bell or special inverted nipple type drinker that is also suspended from the ceiling and raised to accommodate growth.

Some poultry species, such as breeders (i.e., parent flocks that produce fertilized eggs for the next generation) and laying hens (chickens that produce eggs for human consumption), have a long production cycle that can last for up to 52 weeks. Broiler breeders, and laying hens in single-tier free-run barns, are normally housed on a partial litter system with a raised slatted area where the watering equipment, hen feeding equipment, and nest boxes are located. Manure accumulates under the slats and on the litter. High-rise layer barns are two-story buildings in which laying hens are housed in wire cages on the top floor, with manure falling through the cage floor to the lower level where it is stored for the life of the flock.

Duck meat is not sold on a quota basis (like chicken meat is), but is instead sold as a niche commodity. Although niche poultry markets are smaller than the commercial poultry industry, there is a large demand for duck meat in Asian and European countries; so much of this specialty product is exported.
An increased frequency of calls to the Ontario Ministry of Agriculture and Food (OMAFRA) regarding nuisance flies from citizens residing near poultry facilities have been reported in Ontario since 2012 (D. Ward, pers. comm.). Neighbours believe the expanding fly populations observed in their homes originated from the nearby litter-based poultry farms (ducks, turkeys, broiler chickens). Poultry barns provide ideal fly breeding grounds for flies, and due to increasing urban pressures, often settle at neighboring residences or businesses once they find their way out of these animal housing facilities. *Musca domestica* L. (Diptera: Muscidae) has been identified as the most abundant fly species found within poultry facilities, creating both nuisance and health concerns for the poultry, barn workers and neighbouring residents (Axtell, 1990).

### 1.2 INSECT PESTS OF POULTRY PRODUCTION FACILITIES

Insect pests in poultry production have become a growing concern within the industry in recent years. The management requirements of poultry housing facilities often create favourable conditions for the development of *Alphitobius* darkling beetles, *Dermestes* hide beetles, and manure-breeding flies (*M. domestica* - house fly, *Fannia canicularis* L. - little house fly, *Hydrotaea aenescens* L. - black garbage fly) (Axtell, 1999; Kaufman et al., 2000; Kaufman et al., 2010). Abiotic factors (i.e., temperature or physical and chemical traits of the habitat) and biotic factors (i.e., the presence of natural enemies or competition among species) determine the type and size of the pest populations in these production facilities (Axtell, 1999).
Poultry manure provides an ideal substrate for manure-breeding flies; when the grow-out period or the production cycle is long, or when litter is re-used, several fly breeding cycles can occur. Coupled with feed and water spillage, accumulated manure can create ideal breeding conditions for manure-breeding flies, making poultry barns very attractive for fly growth and reproduction.

The house fly is considered the most abundant and pestiferous fly species in poultry production facilities globally (Axtell, 1990), so the focus of this research will be aimed at primarily controlling this manure-breeding fly. Closely resembling the house fly, the little house fly is ca. 3/16 inch smaller, and are typically found in poultry housing with open-window ventilation (Kaufman et al., 2000). Less annoyance issues are reported with little house flies, as they do not readily settle on people or food when they invade nearby residential areas (Kaufman et al., 2000). Although least commonly observed in poultry facilities, *Hydrotaea aenescens* (Wiedemann) - shiny black garbage flies have been identified as facultative larval predators of *M. domestica* (Hogsette and Washington, 1995). Although this appears beneficial in terms of house fly control, large numbers of black garbage flies both on-farm and in nearby communities can create nuisance issues (Kaufman et al., 2000).

1.2.1 *House fly*

*Musca domestica* L. (Diptera: Muscidae) (Figure 1.3) rapidly infest accumulating poultry manure and increase its population size very quickly (Axtell, 1990; Mwamburi et al., 2011). Increased fly populations in poultry production can cause a reduction in feed
efficiency and an increase in production costs, and have a significant impact on the
growth and welfare of the poultry being housed (Moon and Meyer, 1985; Mwamburi et
al., 2011). In addition, house flies can also act as disease vectors and transmit intestinal
pathogens to both humans and poultry. This results in health concerns for poultry
production workers and neighbours who reside in homes near barns (Malik et al., 2007).

Male house flies average 7-9 mm in length, and have larger compound eyes than
females. Females are typically larger in size, and the space between their eyes is
almost twice as wide as in males (Keilding, 1986). Musca domestica is often confused
with the stable fly - Stomoxys calcitrans (Linnaeus), the false stable fly - Muscina
stabulans (Germar), and the face fly Musca autumnalis (DeGeer). House flies can be
distinguished from S. calcitrans and M. stabulans by the four black stripes on their
greyish coloured thorax and the lack of piercing mouthparts. Their abdomen is a grey-
brown colour; males have yellow-coloured markings and thinner narrower space
between the eyes, characteristics that can differentiate it from closely related M.

The life cycle of the house fly in poultry manure can be completed in only 7-10 days in
warm housing, and as many as 10-12 generations may proliferate in one summer
(Axtell and Arrends, 1990; Sanchez-Arroyo, 1998). Ideal growth conditions occur when
the temperature is 25-30°C and relative humidity is 70% (Axtell 1970). If conditions are
less favourable, the life cycle can take up to 60 days to complete (Sacca, 1964).
Although M. domestica are most active at ca. 33°C, 7°C to 43°C are suitable conditions
for proliferation (Schoof, 1964). In poultry operations, house flies develop best in fairly wet, but not liquefied, manure (Mullens et al., 1996). The average life span of an adult house fly is typically 2-3 weeks, however in cooler conditions flies can live up to three months (WHO, 1991). Female flies typically have a longer lifespan than males; at ca. 26°C females can survive for 30 d, whereas males will only survive 17 d (Schoof, 1964; Spiller, 1966). *Musca domestica* undergo 4 distinct stages to complete metamorphosis: egg, larva or maggot (3 instars), pupa and adult (Figure 1.4).

Female house flies prefer to mate three days post emergence (Sacca, 1964). Male house flies will reach sexual maturity 24 h post emergence, but can begin mating as early as 10-12 h post emergence (Sacca, 1964; Chang, 1965). Females only mate once (Zingrone et al., 1959), however males are capable of mating with 4-8 females in a 24-h period (Chang, 1965). After mating, eggs are laid in areas with the most attractive odour and moisture content for *M. domestica* (i.e., manure) (Axtell, 1999). House fly eggs are white, and approximately 1.2 mm in length. Each female fly can lay up to 500 eggs (deposited in batches of 75-150) over a 3-4 day period (Moon and Meyer, 1985). Fly preferences due to physical factors (i.e., temperature and moisture content of breeding material) or chemical factors (i.e., decomposition and fermentation products responsible for changing the nutritive quality of products) are not fully understood (Malik et al., 2007).

The cylindrical, creamy-white coloured, larvae undergo 3 instars and develop rapidly. At the end of the 3rd instar, larvae are fully grown and ca. 7-12 mm long (Keilding, 1986).
Larvae will crawl up to 15 m to a drier, cooler place near breeding material and transform into a pupae (Keiding, 1986). Pupations occur in the drier portions of the breeding material (e.g. near the surface and edges of manure) (Axtell and Arrends, 1990). Pupae are ca. 8 mm long, and as the pupae ages, the colour changes from yellow, red, brown, and finally black. Using the ptilinum, a vesicular organ on the front of the head that ruptures the puparium, adults are able to emerge from the puparium usually in 4-7 days (Keiding, 1986; Moon and Meyer, 1985).

Adult flies feed primarily on feces and other organic matter and due to their mobility, often act as mechanical disease vectors by transporting harmful organisms to both humans and poultry (Levine and Levine, 1991; Chakrabarti et al., 2010). House flies landing on feces or organic matter use regurgitated saliva (containing digestive juices and enzymes) to moisten and partially digest the food. The liquefied food is then taken up with the proboscis, at which point microorganisms from the fly’s body or mouthparts can be transferred to human food or areas where humans come in contact (Malik et al., 2007).

*Musca domestica* is the fly species most frequently associated with disease transmission worldwide (Wales et al., 2010). The correlation between endemic disease outbreaks and the seasonal abundance of house flies has been examined; a reduction in disease prevalence in both urban and rural areas is observed when house fly control is implemented (West, 1951; Graczyk et al., 2001). House flies can carry more than 65 vertebrate intestinal pathogens and are responsible for numerous protozoan, bacterial,
helminthic and viral infections (Malik et al., 2007). Numerous studies have outlined the role house flies play in the transmission of pathogens, such as: 

- Campylobacter spp. (Rosef and Kapperud, 1982),
- Shigella (Levine and Levine, 1991),
- Vibrio cholera (Fotedar et al., 1992),
- Escherichia coli strain O157:H7 (Szalanski et al. 2004),
- Salmonella Enteritidis (Mian et al., 2002), and
- Bacillus anthracis (Fasanella et al., 2010).

Pathogens can be transmitted by house flies using their mouthparts, hair, vomit or feces (Malik et al., 2007).

Flies that disperse away from farms may come in contact with locations where food is prepared, transferring harmful microorganisms (Chakrabarti et al., 2010). As urbanization continues to expand into rural areas, public health concerns arise; the risk of disease transmission increases as residential areas move closer to poultry operations (Kaufman et al., 2010). Interrupting the house fly life cycle by decreasing reproductive conditions within the barns will help reduce fly dispersal to neighbouring houses and businesses, limit potential litigations and conflicts faced by producers, as well as decrease the risk of transmitting harmful pathogens to barn workers and neighbouring residents (Gerry et al., 2011; Heinrich et al., 2010).

The frequent use of antibiotics in commercial livestock operations has enabled house flies to acquire antibiotic-resistant bacterial strains (Wales et al., 2010). Graham et al (2009) isolated drug resistant Enterococci and Staphylococci bacteria from flies captured near poultry feeding operations. Flies that come in contact with antibiotic
residues in manure/feed may harbor antibiotic resistant bacteria, causing further public health concerns for citizens residing near these productions (Chakrabarti et al. 2010).

1.3 Poultry Manure – Significance to House Fly Reproduction

The modern poultry production facility is an ideal breeding environment for fly populations. Suitable fly-breeding conditions are present year-round due to manure accumulation and controlled temperatures (Kaufman et al., 2000). As the poultry industry expands to meet the needs of world-wide consumers, higher bird densities are observed in production facilities. In high density production systems, more manure and used litter is generated, increasing the material available for fly reproduction and consequently the expansion of fly populations (Axtell, 1999).

While poultry production facilities or barns provide a suitable breeding or reproductive environment for house flies, poultry manure itself may be particularly suitable for fly reproduction over other types of manure. Khan et al. (2012) conducted a study comparing the effects of various livestock manures on M. domestica fitness and relative population growth rate. House fly larvae reared on poultry manure developed faster than on any of the other host manures (buffalo, cow, nursing calf, dog, horse, sheep, and goat). The adult flies produced had high fecundity and longevity, partially related to the nutrient composition in the manure (Khan et al. 2012). Awmack and Leather (2002) suggested that a plant host which enables insects to have rapid development times and high reproductive rates, must provide a more suitable feeding environment than other hosts. The principles may also apply to house flies feeding on organic matter.
Poultry manure is often warm and moist while fermenting, providing an ideal habitat for the development of large populations of the \textit{M. domestica} (Axtell 1986). Stafford and Bay (1987) found house fly population numbers to be highest when the poultry manure moisture content was between 70 and 79%; a significant decrease in population numbers was observed when moisture levels were below 60% (Mullens et al., 1996). Higher levels of moisture and more litter compaction are observed in operations where producers keep birds longer, in order to reach higher market weights. These factors contribute to further optimizing conditions for house fly reproduction (Axtell, 1999).

Good management practices are necessary to reduce manure suitability for house fly development. Leaky water nipples, condensation from non-insulated overhead pipes, improper ventilation and seepage from the exterior can all contribute to moist, humid areas in the barns, resulting in a further increase in fly numbers. Sufficient ventilation throughout the housing facilities aids in the drying of manure by maximizing airflow and reducing humidity in the barns (Axtell, 1990). In addition to moist manure, house flies will lay their eggs in spilled feed or other moist, decaying organic material (Kaufman et al., 2000). The monitoring of “wet spots” and feed spills, as well as the removal of deadstock decreases the favourable development conditions for \textit{M. domestica}.

Numerous fly control techniques have been developed for poultry facilities (Kaufman et al., 2001b), but producers must ensure implementation strategies are compatible with management requirements for the birds that are being housed (Axtell and Arrends, 1990). Temperature, humidity, ventilation, type of housing system and feed, light/dark schedules and management of manure will vary between production facilities (Axtell
and Arrends, 1990). Therefore, a thorough understanding of *M. domestica* ecology in various management settings is necessary to develop an effective pest control system that targets all life stages of the house fly.

Salivary secretions released by the flies during feeding and regurgitation may contain enzymes and microorganisms that initiate the breakdown process of the carcass or the organic matter, making a specific area more attractive to other flies (Dethier, 1955; Telford et al., 2012). Brodie et al. (2014) confirm that blow flies appear to co-opt semiochemicals associated with feeding flies when selecting a site to oviposit. Rather than oviposition pheromones, it is likely that semiochemicals (located in the alimentary canal) attract flies to a particular area for oviposition. Such knowledge could be used to manipulate oviposition patterns and potentially limit egg deposition in manure or other organic material.

### 1.4 MANAGEMENT STRATEGIES FOR HOUSE FLIES

The management of house fly populations in poultry operations deserves a serious effort from poultry producers. Historically, pest control measures have relied primarily on insecticides to reduce economic losses and nuisance concerns within the barns (Kaufman et al., 2000). The overuse and/or improper use of insecticides have enabled house flies to develop resistance to many active ingredients (Geden et al., 1995), as well as put producers at risk of contaminating eggs or meat with chemical residues (Kaufman et al., 2010). To reduce the dependence on insecticides, physical, cultural, biological and chemical control tactics can be combined in an overall integrated pest
management (IPM) program (Axtell, 1986). Integrated pest management programs implemented for house fly control can be very effective in decreasing house fly numbers in poultry operations, as well as reduce the development of insecticide resistance in *M. domestica* populations (Malik et al., 2007).

Various sampling methods should be used to monitor house fly populations both inside and outside poultry production facilities to evaluate the effectiveness of IPM programs. Surveillance of fly populations is also needed to identify increases in fly densities and to determine when introduction of control measures is necessary (Birkemoe and Sverdrup-Thygeson, 2011; Kaufman et al, 2001a). There is variability between studies in estimating fly populations due to the inconsistency of monitoring methods (Kaufman et al, 2001a), mostly related to the types of traps, trap positioning (i.e., if placed in areas of high air exchange) and duration of monitoring (i.e., too short of monitoring period). As a result, it is often difficult to draw conclusions on the effectiveness of fly monitoring methods. However, most of the methods have relied on sticky cards to evaluate populations (Gerry et al., 2011). Birkemoe and Sverdrup-Thygeson (2011) outline the need for a standardized fly surveillance/monitoring method that could be used across the industry. A consistent monitoring method would more accurately depict differences between test sites and assess the efficacy of the control measures implemented. The absence of an established surveillance program in poultry production facilities can cause producers to identify house flies as a problem after populations have already exceeded a manageable level, thus limiting their options of control (Geden and Hogsette 1994).
1.4.1 Physical Control

Physical control methods will trap house flies and can be used to monitor pest populations. Sticky tapes, cards and ribbons suspended inside poultry barns will capture adult house flies. These controls also give a relative measure of house fly abundance and activity (Gerry et al., 2011; Kaufman et al., 2005). Sticky traps can be used to manage small to moderate house fly populations, as they are coated with a sticky adhesive that entraps flies once they land on its surface (Kaufman et al., 2005). Several commercial sticky traps contain (Z)-9-tricosene (Muscalure®), a sex pheromone released by female house flies, to enhance house fly capture (Butler et al., 2010; Darbro et al., 2005). The effectiveness of sticky traps decreases with dust and fly accumulation on the trap surface, and they should be changed once every 1-2 weeks (Kaufman et al., 2005; WHO 1991). Trapping devices are sometimes combined with insecticides and baits.

1.4.2 Cultural Control

Cultural control methods modify the favourable reproductive environment by manipulating abiotic factors (i.e., temperature, moisture of breeding habitat, and relative humidity) to reduce the prevalence of house flies in poultry operations (Walker and Stachecki 1996; WHO, 1991). Management techniques that render the manure less suitable for fly reproduction are important in reducing existing and future fly infestations. The approaches must be compatible with the poultry management and production procedures in a specific facility (Axtell and Arrends, 1990).
Inspection of leaky water nipples and other sources of moisture should be conducted routinely to identify the potential for “wet spots” in the production facility (Kaufman et al., 2010), and apply corrective measures. Appropriate ventilation throughout facilities also will help reduce moisture in the barns, decreasing fly reproduction (Axtell, 1990). The type of fan utilized in a poultry production facility depends on the management practices for the birds being housed there (i.e., circulating fans for poultry housed in accumulated manure or exhaust fans located in manure pits or poultry houses on slates).

Manure removal is another key component in a successful fly control program. However, poultry manure generally cannot be removed while birds are inside the barns, so removal can only be performed after the birds have reached market weight and have been shipped out. Due to the production requirements of poultry, manure may sometimes only be removed from the housing facilities once or twice per year. It is often suggested that producers should remove manure prior to the earliest date of fly activity (i.e., during the cooler months in temperate climates when flies are less active) to reduce the rapid proliferation of house flies in accumulating manure piles when favourable conditions occur (Axtell, 1970; Kaufman et al., 2000).

1.4.3 Biological Control

Biological control uses live organisms, such as parasitoids, predators and entomopathogens to suppress house fly populations in poultry production facilities. Various biological control agents (BCAs) have been introduced to livestock manures to help control pest flies before they reach adulthood (Lysyk, 1995; Dry et al. 2007). The release of BCAs has the advantage of controlling flies before they reach the adult stage
and disperse from the farms, reducing the risk of nuisance and disease transmission to neighbouring residents (Mwamburi et al., 2011). When coupled with physical, cultural or even chemical control tactics, biological control can help to significantly reduce house flies in poultry operations (Crespo et al., 1998; Axtell and Arrends, 1990; Axtell, 1999; Kaufman et al., 2000).

**Parasitoids**

Parasitoids are defined as natural enemies that attack a single host organism, ultimately killing it upon emergence. House flies have many parasitoid natural enemies including several species of pteromalid wasps - *Muscidifurax* sp. and *Spalangia* sp. (Hymenoptera: Pteromalidae) (Sanchez-Arroyo, 1998). Numerous studies have outlined the potential of these parasitic wasps in controlling house fly populations within poultry facilities (Lysyk, 1995; Dry et al., 2007; Geden and Hogsette, 2006).

Female parasitoid wasps will locate fly pupae and deposit their eggs inside the puparium (Figure 1.5). Parasitoid larvae will feed on the fly larvae, kill the host, and emerge as an adult (Axtell, 1986; Kaufman et al., 2010). The implementation of parasitic wasps into poultry production facilities is possible because the parasitoids are host-specific and will not sting poultry, production facility workers or neighbouring residents (Tomberlin and Whitney, 2003). Geden and Hogsette (2006) observed a significant reduction in house flies and a high parasitism rate in poultry manure when *Muscidifurax raptor* and *Spalangia cameroni* were released. *Muscidifurax raptor* and *S. cameroni* can co-exist and complement each
other due to their similar microhabitat selection and searching behaviours (Skovard and Jesperson, 1999). *Muscidifurax raptorellus* are able to rapidly establish populations in poultry facilities, as they can produce more than four offspring per parasitized pupa (as a gregarious species) (Geden and Hogsette 2006; Kaufman et al., 2012). Highly variable results have been reported after the mass release of parasitoids in poultry facilities. McKay et al. (2007) placed 10 bags of 30 house fly sentinel pupae weekly in broiler-breeder manure, to determine the efficacy of biweekly releases of *Muscidifurax zaraptor* Kogan and Legner, *M. raptorellus* Kogan and Legner, and *Spalangia cameroni* Perkins. Only 18.8% of the bags retrieved were parasitized during the duration of study.

Varying results can be attributed to the species of the parasitoid released and the environmental conditions at the farm (Mwamburi et al., 2011). Surveys of natural parasitism within the barn will help determine which species of parasitoids should be deployed (Geden and Hogsette, 2006). Parasitoid wasps occur naturally in low numbers in poultry manure, so mass augmentative releases of commercially reared parasitic wasps can increase parasitism rates within poultry production facilities (Kaufman et al., 2010).

Research outlining the manure characteristics favoured by parasitoid wasps should be conducted to maximize their efficiency. Producers could then use this information to manipulate the manure in their barns to not only decrease fly breeding suitability, but hopefully increase the developmental time for flies, allowing BCAs a longer opportunity to search and parasitize their house fly host (Khan et al., 2012).
**Predators**

Predators of *M. domestica* will actively search for house fly eggs, larvae, pupae and adults in poultry production facilities, contributing to the overall control of all life stages. Although some predators occur naturally in poultry manure, commercially-reared beetles and mites can be added to manure by producers for additional control. Muscovy ducks have also been used to control house flies in poultry facilities.

The hister beetle (HB), *Carcinops pumilio* (Erichson), has been identified as an important predator of house flies in poultry production facilities (Stoffolano and Geden, 1987). Larval and adult stages of the HB feed on house fly eggs and larvae (Kaufman et al., 2010) but if prey is scarce, they will disperse away from the release site, decreasing their effectiveness of controlling *M. domestica* populations (Kaufman et al., 2010). The HB can survive in both wet (Stoffolano and Geden, 1987) and dry (Peck and Anderson, 1969) manure, so this BCA can be utilized in various types of poultry operations.

Common predatory mites found in poultry manure include the macrochelid mite (*Macrocheles muscae domesticae*) and the uropodid mite (*Fuscuropoda vegetans*) (Kaufman et al. 2010). The mites can be used in conjunction with a number of BCAs in a poultry production system. *Macrocheles muscae domesticae* feed on house fly eggs on the surface of manure, and *F. vegetans* feed on fly larvae located deeper in the manure (Kaufman et al., 2010).
In tandem with good sanitary practices (e.g., regular removal of manure), Muscovy ducks (*Coirina moschata* L.) are effective predators of house flies by actively seeking and consuming flies (Glofcheskie and Surgeoner, 1993). In a study outlining the use of Muscovy ducks as BCAs in dairy operations, Glofcheskie and Surgeoner (1990) observed adult ducks remove flies from enclosed 0.24 m$^3$ spaces 30 times faster than coiled fly paper rolls, fly traps or bait cards. However, in situations where the Muscovy ducks are in production, it was observed that fly populations were often reaching high densities causing complaints by neighbouring residents.

**Nematodes**

Laboratory studies on the utilization of entomopathogenic nematodes to manage house flies in poultry manure looks promising, but field applications have been relatively unsuccessful (Geden et al., 1986). Georgis et al. (1987) conducted a production facility study in which three nematodes - *Steinernema feltiae* Filipjev All Strain, *Steinernema bibionis* Steiner SN strain, and *Heterorhabditis heliothidis* NC strain were used as BCAs to control house flies in poultry manure. Seventy to 100 % of the nematodes applied to the manure remained on the surface and died within 18 h of release. The nematodes poor survival rate and limited movement in a realistic production system environment makes them a less than optimal candidate for inclusion in a biological control program for house flies at this point.
1.4.4 Chemical Control

The current control of house flies in poultry operations relies primarily on synthetic insecticides (Meyer et al., 1987; Scott et al., 2000). The overuse of insecticides has resulted in resistance development in *M. domestica* populations world-wide (Kaufman et al., 2001a). The continual application of insecticides from similar chemical classes has accelerated resistance issues (Geden, 2012), and enabled house flies to develop resistance to virtually every insecticide used for their control (Scott et al., 2000). More stringent regulations for new control products, as well as the loss of others from the market continue to limit producers’ choice of insecticides, contributing further to resistance concerns (Kaufman et al., 2001a).

*Musca domestica* is a highly adaptable species that utilizes both physiological and behavioural defense mechanisms to develop resistance to a wide range of insecticides (Bull and Pryor, 1990; Learmount et al., 1996; Learmount et al., 2002). Development of resistance can be attributed to the house fly’s cosmopolitan distribution, high populations in livestock facilities, rapid developmental time and cross-resistance among insecticide classes (Kaufman et al., 2010). Insecticide resistance in pest populations can unfortunately lead producers to increase application rates and frequency of application consequently resulting in increasing the rate at which resistance develops (Scott et al., 2000).

Numerous studies on insecticide resistance in house fly populations have been conducted world-wide. House flies have shown resistance to organophosphorus
(Keiding, 1965), organochlorine (Busvine 1963), carbamate (Harris et al., 1982) and pyrethroid insecticides (Scott et al., 2000). Resistance to newer insecticides like abamectins (Scott et al., 1991), spinosyns (i.e., spinosad) (Shono and Scott, 2003) and neonicotinoids (i.e., imidacloprid and thiamethoxam) (Kaufman et al. 2010) have already been observed in house fly populations as well.

The implementation of cultural, biological and biopesticide control measures can be used as part of an integrated pest management (IPM) program, to control house fly populations and reduce the heavy dependence on synthetic insecticides (Axtell 1986; Axtell, 1999). IPM programs that target various life stages of *M. domestica* can be effective in reducing population numbers and decreasing insecticide resistance development (Malik et al., 2007; Scott et al., 2000).

**Conventional Insecticides**

Historically, organophosphorus insecticides (OPs) have been used for fly control in the poultry industry, as they are readily available to producers and relatively inexpensive (Axtell, 1970). In addition to the use of OPs (e.g., diazinon, dichlorvos and fenthion), organochlorine insecticides (e.g., dieldrin), carbamates (e.g., dioxacarb and propoxur), and pyrethroids (e.g., cypermethrin, fenvalerate and permethrin) can be used for chemical control of house flies as well (Keiding 1986).

Since insecticides are often used as the primary source of fly control in poultry productions, house fly resistance concerns have been observed throughout the industry. Frequent reapplication of insecticides is required, as many of the insecticides
have short residual times. Improper timing of insecticide applications (i.e., when fly populations have already exceeded manageable thresholds) may lead farmers to apply more of the product. Both these factors will end up contributing to an increased rate of insecticide resistance development in house fly populations (Axtell, 1970). Many insecticides are not compatible with introduced BCAs associated with poultry production systems, so it is important to identify conventional insecticides and/or biopesticides that can be used in these agricultural systems without fear of negatively impacting established biological control programs (Kaufman and Rutz, 2010).

**Botanical Insecticides**

Pyrethrins are natural compounds that are derived from the flowers of the *Chrysanthemum* genus of plants (Casida, 1980). Space sprays (liquid insecticide applied as fog) comprised of pyrethrins enable producers to perform a quick knock down of adult flies in their production facilities. Pyrethrins become unstable when exposed to light, therefore a synthetic form, pyrethroid, was created for agricultural use. Pyrethroids are synthetically produced pyrethrins that contain similar insecticidal and toxicologic properties, but are more stable compounds (Valentine, 1990). Unlike pyrethrins, pyrethroids cannot be used in organic operations. Traps with pyrethroid baits are effective at suppressing adult *M. domestica* populations when fly numbers in poultry facilities are low (Kaufman et al., 2010). Larvicides including pyrethroids should be used selectively and applied only as spot treatments on poultry manure, to minimize any potential negative impact on BCAs (Keilding, 1986; Kaufman et al., 2000).
**Biopesticides**

Biopesticides are derived from natural materials such as animals, plants, bacteria, and certain minerals and are typically less toxic than conventional pesticides; they are grouped with “reduced-risk chemicals” for registration in Canada (EPA, 2008; PMRA, 2002). Biopesticides are categorized into three major classes: (1) microbial pesticides - active ingredient is a microorganism (e.g., bacterium, fungus, virus or protozoan), (2) plant-incorporated-protectants (PIPs) - pesticidal substances are produced by plants containing added genetic materials (e.g., *Bacillus thuringiensis* in corn), and (3) biochemical pesticides - naturally occurring substances that control pests using non-toxic mechanisms (e.g., pheromones, semiochemicals, insect growth regulators) (EPA, 2008).

**1. Microbial Biopesticides**

**Fungi**

*Beauveria bassiana* (Balsamo) Vuillemin is an entomopathogenic fungus that is a naturally occurring pathogen of house flies (Mwamburi et al., 2009). *Beauveria bassiana* is prevalent in low levels under natural conditions, however when used as a biopesticide, the control of house fly populations was highly successful (Mwamburi et al., 2009; Steinkraus et al., 1990). Field studies revealed that *B. bassiana* is comparable to other insecticides, however more frequent applications were required (Geden, 2012). Kaufman et al. (2005) compared poultry facilities sprayed with *B. bassiana* or pyrethrin applications for house fly control. After spraying, adult populations were not only lower in the *B. bassiana*-treated facilities, but the number of larvae...
recovered from the manure was less than half of what was recovered in the pyrethrin-treated facilities (Kaufman et al., 2005). This fungi has been registered in Canada for the control of pest flies in poultry production since 2010, and can be applied as a spray or bait (PMRA, 2010; Kaufman et al., 2012). **Beauvaria bassiana** is often combined with other entomopathogens (i.e., *Entomophora muscae* (Chon) Fresenius, *Metarhizium anisopliae*) to more effectively control fly larvae densities (Malik et al., 2007). The exact mechanisms are not understood, however multiple studies observed enhanced fly control when entomopathogens were combined (Mwamburi et al. 2009; Wraight and Ramos, 2002). **Beauveria bassiana** alone can take 4-6 days to kill its host (Geden, 2012). Seasonal variation may be the cause of variation in effectiveness of entomopathogenic fungi; temperature and relative humidity appear to be the two main abiotic factors (Malik et al., 2007).

*Entomophthora muscae* (Cohn) Fresenius is another fungal pathogen that has been studied to determine whether it can reduce house fly populations. However, its intolerance to high temperatures and short-lived spores make it a less optimal choice compared to *B. bassiana* (Geden et al., 1995).

**(2) Plant-incorporated-protectants**

**Bacteria**

*Bacillus thuringiensis* (*Bt*) is a bacterium that can be effectively incorporated into a biopesticide. Although *Bt* has not been approved for use in animal production systems in Canada (Mwamburi et al., 2011), it has been used widely for agricultural pest control
since 1960 (Johnson et al., 1998). Several *Bt* isolates (strains HD-1, HD-73, and HD-11) have been found to be effective against house fly larvae (Johnson et al., 1998; Mwamburi et al., 2009). Indrasith et al. (1992) observed 50-80% mortality in house flies exposed to *B. thuringiensis* var. *kurstaki*. Larval mortalities for fly populations treated with *B. thuringiensis* var. *israelensis* alone and *B. thuringiensis* var. *israelensis* coupled with *B. bassiana* were 41% and 45-52%, respectively. These results suggest that the larval effects of *Bti* were improved when *B. bassiana* was added. Limited information is available on the uses of other bacteria species to control fly populations, however Padmanabhan et al. (2005) identified *Pseudomonas fluorescens* Migula as being less effective than *Bt*.

**Viruses**

In laboratory studies, both sexes of house flies infected with salivary gland hypertrophy virus (SGHV) develop hyperplastic salivary glands and the ovarian development in infected females was completely inhibited (Lietze et al., 2007; Lietze et al., 2009; Geden et al., 2011). The sterilization of female house flies will rapidly decrease populations, as reproduction cannot occur. The replication of SGHV occurs in the salivary glands of adult house flies, and can be easily transferred to healthy flies who feed on a substrate that has been infected with SGHV viral particles (Prompiboon et al., 2010). An infected house fly can deposit an average of 10^6* viral genome copies onto a solid food substrate; Leitze et al. (2007) reported an infection rate of 66% in newly emerged flies. No field applications of SGHV or other viruses for the control of house fly populations in poultry productions have been reported.
(3) **Biochemical Pesticides**

**Naturally-occurring substances (NOS) with insecticidal properties**

Naturally-occurring substances are naturally present or produced in the environment and are not chemically modified before their potential usage as an insecticide. Many of the NOS have low mammalian toxicity and could potentially be used as a safer, low-risk method of pest control in poultry housing facilities. Some of the products listed may qualify as “reduced-risk products” by PMRA (PMRA 2002), but none of them are registered for house fly control in Canada at the moment.

**Boric Acid**

There are three compounds containing boron that are typically used as insecticides: boric acid (H$_3$BO$_3$; hydrogen borate, boracic acid, orthoboric acid), borax (Na$_2$B$_4$O$_7$·10H$_2$O; sodium borate, sodium tetraborate, or disodium tetraborate) and disodium octaborate tetrahydrate (Na$_2$B$_8$O$_{13}$·4H$_2$O; boron sodium oxide, sodium octaborate, Polybor$^\text{®}$ or Timbor$^\text{®}$). Borax is a mineral salt of boric acid, whereas disodium octaborate tetrahydrate is an alkaline salt (often confused with boric acid).

Boric acid (H$_2$BO$_3$) can be used as an environmentally-safe, alternative method of insect control, as it has a relatively high acute oral LD$_{50}$ ($\geq$ 2000 mg/kg in rats), and is not classified as a skin/eye irritant or carcinogen (Balme et al., 2013; Hogsette et al., 2002). Numerous studies have used boric acid (BA) baits or solutions for the control of: cockroaches (Gore and Schal, 2004; Zurek et al., 2003), worker ants (Sumida et al., 2010), adult mosquitoes (Xue and Barnard, 2003) and house flies (Hogsette and...
Koehler, 1994; Hogsette et al., 2002). Hogsette and Koehler (1992) had great success controlling adult house flies when a liquid formulation of BA was combined with 10% sucrose. Flies that fed on 2.25% BA concentrations died within a few hours; repellency effects were observed when higher concentrations of BA were used (Hogsette and Koehler, 1994). Due to dust accumulation in barns, liquid formulations of BA may not be practical in agricultural settings, as dust may settle on the applicators, preventing house flies from consuming the necessary dose needed for death (Hogsette et al., 2002). Hogsette et al. (2002) developed granular baits made by freeze-drying liquid formulations of boric acid and sugar. Although the granular baits appeared to have a slower rate of kill than the liquid boric acid formulations, the potential for the development of resistance was decreased, as flies are more likely to consume the necessary dose for mortality. Boric acid must be ingested to cause death, as it acts a stomach poison and disrupt water balance of house flies (Hogsette et al., 2002). More research on dosage and repellency is needed to determine the effectiveness of boric acid as a method for house fly control, and the direct application of boric acid to manure has yet to be tested.

**Diatomaceous Earth**

Diatomaceous Earth (DE) is a naturally-occurring siliceous ($\text{SiO}_2$) mineral, composed of fossilized remains of marine diatoms that can easily crumble into a white powder. DE is recognized as a safe, non-toxic substance that has low mammalian toxicity, and is considered one of the most effective mineral dusts for the protection of stored products (i.e., grain) from insect pest infestations (Shafighi et al., 2014). Laboratory studies
treated hard red spring wheat (*Triticum aestivum* (L.)) with 0.5 and 0.75 g of diatomaceous earth (Protect-It®) per kg of wheat, and populations of the rice weevil (*Sitophilus oryzae* (L.)), red flour beetle (*Tribolium castaneum* (Herbst)) and the lesser grain borer (*Rhizopertha dominica* (Fabricius)) were reduced by 98 to 100% (Korunić and Mackay, 2000). Researchers also identified the enhanced efficacy of *B. bassiana* against larval *T. castaneum* after the addition of diatomaceous earth (Akbar et al., 2004). Future studies should be conducted on DE as a potential control agent for *M. domestica* as a standalone control method, or in tandem with *B. bassiana*.

**Hydrated Lime**

Hydrated lime (Ca(OH)$_2$) has historically been used as a sanitizing agent, but recent studies have revealed other benefits it may possess when used as a poultry litter additive (Bennet et al, 2003). In addition to the reduction of in vitro *Salmonella enteritidis* survival, hydrated lime also reduced the overall low level aerobic forming units of bacteria by changing the pH of the litter (Bennet et al, 2003; Bennet et al, 2005). The alkalization of litter may reduce other pathogens, possibly explaining the significant poult weight gains observed in lime-treated pens (Bennet et al, 2005). The use of hydrated lime may also be beneficial as the potential of flies transferring pathogens from the manure to food sources may be decreased due to reduction in pathogenic bacteria in the manure. In another study, researchers applied hydrated lime to calf bedding at a rate of 17.7 and 26.5 g/0.05 m$^2$, and house fly larval survival was reduced by 99 and 100% respectively (Calvo et al., 2010). Continued research needs to assess the
potential efficacy of hydrated lime and the benefits of alkalizing poultry litter for house fly control and pathogen reduction.

**Citric Acid**

Citric acid (C₆H₈O₇) is commonly used as an acidifier or flavouring agent in the food and beverage industry, and is therefore categorized as a relatively safe NOS. Although limited research has been conducted on its entomological uses, Abd El-Baset El-kady et al. (2010) prepared soluble citric acid powder using sodium dodecyl sulphate (SDS) as a wetting agent to decrease cow pea aphid (*Aphis craccivora*) populations. Citric acid showed effectiveness against all tested life stages for this major pest of leguminous crops; at all exposure periods, citric acid was more effective against nymphs, followed by apterous adult and then winged stages. Future research should assess the application of citric acid to poultry litter and its impact on controlling different *M. domestica* life stages.

**Acetic Acid**

Acetic acid (CH₃COOH), also known as ethanoic acid, is a weak acid that is most commonly known as the main ingredient in household vinegar. Vinegar is comprised of 4-8 % acetic acid and water, and plays two important roles in the food industry; it is typically used directly as a condiment or used to pickle vegetables and meats. Similar to citric acid, it is safe to use for human consumption as it has a relatively low toxicity to mammals. Qain et al. (2013) examined a commercial vinegar product (ChinKiang®) that was found to be highly attractive to adult house flies. Seven volatile compounds (i.e.,
acetic acid, furfural, butanoic acid, isovaleric acid, hexanoic acid, 2-phenylethanol, and p-cresol) elicited significant responses from antennae of female and male house flies. The incorporation of acetic acid into house fly traps, or the application of acetic acid to a substrate to attract *M. domestica* to a particular area should be further investigated to contribute to the control of pest fly populations. Acetic acid may also have an impact as an insecticide, as it may significantly lower the pH of the substrate it is added to.

### 1.5 RESEARCH OBJECTIVES

Chicken and turkey manure has been shown to be suitable rearing substrates for house flies; however, limited research has been conducted on duck manure. Previous research (unpublished) identified duck manure as having higher moisture content than turkey or chicken manure, suggesting that duck manure might provide better reproductive conditions for *M. domestica*.

The objectives of this research were to:

1. Evaluate duck manure as a reproductive medium for the house fly;
2. Evaluate the impact of moisture content of duck manure on oviposition and adult house fly reproduction; and
3. Evaluate the effectiveness of naturally-occurring substances added to duck manure (acetic acid, boric acid, citric acid, hydrated lime, diatomaceous earth) at reducing house fly reproduction.
Three two-floored duck barns (in close proximity to nearby wineries, restaurants, and residential areas) in Beamsville, ON were selected for manure collection (laboratory studies) and the field research.

This research will contribute to our understanding of effective fly control measures by investigating the reproductive success of all life stages of the house fly in duck manure, with the goal of diminishing fly populations before they reach adulthood (the nuisance stage). The implementation of reduced-risk strategies to decrease dependence on chemical insecticides will be assessed; the knowledge gained from this research can potentially be applied to other poultry sites experiencing fly problems. Producers will have the ability to manage fly populations before these pests disperse from the barns, thereby decreasing neighbour tensions. The co-existence of poultry operations and neighbouring residents is essential for continued poultry production in Ontario and many other jurisdictions.
CHAPTER 2: Duck manure as a suitable medium for house fly oviposition and development

2.1 INTRODUCTION

The house fly, *Musca domestica*, has been identified as a major pest in commercial poultry production globally (Axtell, 1990). Modern poultry housing facilities often provide *M. domestica* with an ideal breeding ground, due to the manure and bedding accumulation, as well as the controlled year-round temperatures in these facilities (Kaufman et al., 2000). Poultry manure provides an ideal growth substrate for the house fly, as it is typically warm and moist making it very attractive to adult flies (Axtell 1986; Kaufman et al., 2000). As the poultry industry continues to rapidly expand, larger quantities of manure and used litter are being generated in the high-density confined housing facilities, creating model conditions for fly development (Axtell, 1999).

House flies will lay their eggs in areas with the most attractive odour and moisture content, such as manure (Axtell, 1999). Stafford and Bay (1987) found house fly larvae numbers to be highest when the poultry manure moisture content was between 70% and 79%. The location where female adults choose to oviposit is not fully understood, but appropriate substrate conditions for egg hatching and larval growth (e.g. moisture content, temperature, available nutrients) likely play a role in oviposition site selection. When fully grown, larvae will usually crawl to drier portions of the breeding substrate (e.g., near the surface and edges of manure) to begin pupation (Axtell and Arrends, 1990). Manure quality, moisture and temperature significantly impact the establishment
and expansion of fly populations (Barnard and Geden, 1993; Barnard et al., 1995; Fatchurochim et al., 1989).

Khan et al. (2012) conducted a study comparing the effects of various livestock manures on *M. domestica* fitness and relative population growth rate. House fly larvae reared on poultry manure developed faster than on any of the other host manures (buffalo, cow, nursing calf, dog, horse, sheep, and goat). The adult flies produced had high fecundity and longevity, partially related to the nutrient composition in the manure (Khan et al. 2012). Patricia and Claudio (2008) conducted a similar study comparing livestock manures (poultry, cow, milking calf, goat, swine, composted swine) and dog feces. Thirty neonatal house fly larvae (< 12 h old) were reared in each substrate at 28 ± 2°C, and a reduction in the length of the larval period and the production of larger progeny from house flies reared in poultry manure was found. On day 15 of the study, the percent emergence of adult *M. domestica* was 100% for chicken manure (Patricia and Claudio, 2008). Although house flies can feed on a wide variety of organic substrates, some may possess certain nutritional factors, such as an adequate source of protein (used for egg maturation), that make it more favourable than others (Beard et al., 1973; Malik et al. 2007).

Complaints of fly nuisance to the Ontario Ministry of Agriculture and Food (OMAFRA) by residents and businesses surrounding duck farms have been recently reported (D. Ward, pers. comm.). Studies have shown turkey (Axtell, 1999), caged-layer (Hinton and Moon, 2003), and broiler-breeder (Dry et al., 2007) manure to be suitable rearing
substrates for house flies; however, limited research has been conducted on the suitability of duck manure for fly reproductive activities. An understanding of the typical characteristics of duck manure and its effects on fly development were conducted to help understand its suitability compared to other poultry manure types, as a growth medium for house flies.

2.2 MATERIALS AND METHODS

House fly colony

A house fly (*M. domestica*) colony was established at the University of Guelph – Alfred Campus in January 2013, from pupae obtained from Dr. Kevin Floate at Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada. Pupae were then shipped to the University of Guelph – Main Campus and reared at the School of Environmental Sciences (SES). Adult flies were placed in Plexiglas® cages (26.5 cm³). Each cage had a 15 cm diameter circular opening at the front side covered with a long mesh sleeve for free access to the inside of the cage. A binder clip was used to seal each sleeve to prevent flies from escaping. The adult flies were provided with sugar water (5 mL of sugar in 50 mL water) using a brown recycled paper towel soaked in the sugary solution in a white 500 mL plastic container. Every two to three days, the sugar water was changed. The oviposition media consisted of a brown recycled paper towel soaked in powdered milk (20 mL) and water (50 mL) and placed in white 500 mL plastic containers. Two oviposition containers were placed in the middle of each cage, every two to three days. New flies were added to the cages every week by placing three plastic containers (500 mL) full of rearing media (described below) containing pupae.
Approximately 300 to 400 flies were kept in each cage. Every two to three days, eggs were collected in the adult cages by gently washing the oviposition media with water into a fine screen filtration device. Between 0.75 and 1.0 mL of fresh eggs (about 600 eggs), and sometimes young larvae, was collected and placed into the larval rearing media (RM), which consisted of 850 mL of water, 150 mL of blood meal, 250 mL of powdered milk, 14.8 mL of brewer’s yeast and 1,500 mL of wheat bran. The RM was placed into an open-faced plastic container (10.8 L) and stirred at least twice daily (except weekends) until pupation; water and wheat bran was added as necessary to keep the humidity in the range of 65-75% for proper larval development. However, the moisture content of the substrate was allowed to decrease to approximately 35-45% to improve pupation conditions. Temperature of the colony growth room was 22 ± 3°C and photoperiod at 14:10 L:D. Both larvae and adult house flies were used for the experiments.

**Poultry manure tested**

Five to seven litre samples of poultry manure from a Muscovy duck farm (Beamsville, ON), turkey farm (Guelph ON), broiler chicken farm (Guelph), and a conventional-caged layer chicken farm (Guelph) were collected < 72 h prior to the experiments from 5-10 “wet manure spots” in the poultry barns. Manure was collected when the birds were 6–8 weeks into the grow-out period (duck, turkey, and broiler) or production cycle (layer), and frozen for > 24 h to kill any house fly eggs, larvae, pupae, or other arthropods. The frozen manure was thawed and allowed to reach room temperature before further use,
and was well mixed. The dry content of the manure was assessed by drying three 15 g aliquots of each manure type, ≤ 6 hours before the beginning of the experiment.

**Adult house fly emergence of larvae reared in different types of poultry manure (laboratory)**

Two hundred grams of the four different types of poultry manure (the treatments) were placed into 1.9 L plastic containers. The water content of the manure was adjusted to 75% by adding the appropriate amount of distilled water to the manure. For each treatment, 25 neonate *M. domestica* larvae (1-2 d) were placed in contact with the manure and reared until adult fly emergence. Five grams of manure was collected on Day 1, 5, and 10 to assess the pH; samples collected for pH testing had no larvae in them. The manure was mixed every 48 h and emergence of adult flies recorded every 48 h for 21 d. Observations on the manure characteristics (consistency, presence of molds) and larval behaviour (location in the manure and inhabitual movement) were recorded. Containers were placed at room temperature (22 ± 2°C) and four replicates of each treatment were performed.

**Adult house fly landing preference and egg-laying in different types of poultry manure (laboratory)**

Fifteen grams of each type of poultry manure was placed in individual petri dishes (i.e., one dish per type of manure for a total of 4 dishes) 15 min before the beginning of the experiment. A 3 cm² piece of brown recycled paper towel was placed in the centre of each petri dish to encourage oviposition; 3-4 drops of distilled water were applied to the
paper towel to moisten it before placement. The petri dishes were then placed in a fly cage containing 50 *M. domestica* females (7-10 d old). Females used for testing were previously in contact with males to ensure the possibility of copulation. Sugar water was provided to the adult flies before the beginning of the experiment then removed when the petri dishes were placed in the cage. The experiment was conducted at room temperature (22 ± 2°C). Observations on landing and oviposition were recorded visually to assess house fly preference. Landing preference was observed by glancing at each petri dish at T = 0, 30, 60, and 120 min and recording the number of flies landing on the manure at that time interval. Four replicates of this experiment was performed. Counts for landing on the manure and landing on the paper towel were grouped together and averaged to reduce the variability of punctual observation points. Egg-laying was assessed by counting eggs on manure and paper towel after T = 120 min using a microscope at 10 X magnification.

**Adult house fly landing preference and egg-laying in different substrates (barn)**

Fifteen grams of dry rearing media, wet rearing media, commercial duck feed, white granulated table sugar, Muscovy duck manure, layer chicken manure, cow manure, and molasses were prepared in individual petri dishes and placed randomly on a 50 cm x 30 cm plastic tray. The tray was placed inside the duck barn near the entrance door and observations on landing and egg-laying were recorded to assess house fly preference. The experiment was performed 12 weeks into the ducks’ grow-out period at 24.5 ± 0.5°C. Landing preference was observed at T = 0, 30, and 60 min. The number of flies landing on each substrate at each time was recorded for each petri dish and averaged.
to reduce the variability of punctual observation points. Egg-laying was assessed after T = 60 min using a microscope at 10 X magnification. Four replicates were performed for each substrate, and the number of flies landing on each substrate, at each of the three time intervals (T= 0, 30, and 60 min) were averaged.

**Adult house fly landing preference and egg-laying in fed-on versus non fed-on duck manure (laboratory)**

Fifteen grams of Muscovy duck manure was placed in two petri dishes 15 min before the beginning of the experiment. A 3 cm² piece of brown paper towel was placed in the centre of each petri dish to encourage oviposition; 3-4 drops of distilled water were applied to the paper towel to moisten it before placement. One petri dish was placed into a fly cage containing 100 house flies (♂ and ♀, 10 d old), and the other dish was placed into an empty fly cage for two hours. The number of flies landing on the petri dish in the cage with house flies (fed-on) was counted five times (at T = 0, 30, 60, 90, and 120 min) and averaged over the five observations. Egg-laying was assessed after T = 120 min using a microscope at 10 X magnification.

Following the 2 h period, the petri dish from the empty cage (non fed-on) was placed into the cage containing the flies, beside the fed-on petri dish. Observations on landing and egg-laying were recorded on both petri dishes to assess house fly preference. Landing preference was observed at T = 0, 30, 60, 90, and 120 min. The number of flies landing on the manure at each time was recorded for each petri dish. Egg-laying was
assessed after $T = 120$ min using a microscope at 10 X magnification. The experiment was conducted at room temperature ($22 \pm 2^\circ C$) and 4 replicates were performed.

**Nutrient analysis of poultry manure**

Five hundred gram samples of manure from a Muscovy duck farm (Beamsville), Mule duck farm (Beamsville), turkey farm (Guelph), broiler chicken farm (Guelph), and conventional-caged layer chicken farm (Guelph) were collected and submitted to the University of Guelph’s Agriculture and Food Laboratory (AFL). A standard manure test kit was used on all samples to determine the dry matter content (% DM), potassium ((K) % wet), phosphorous ((P) % wet), total Kjeldahl nitrogen ((N) % wet), and ammonium nitrate ((N) mg/kg wet) levels.

### 2.2.1 DATA ANALYSIS

**Adult Emergence/ Landing Preference**

The PROC UNIVARIATE procedure was run for all experiments and the results of the Shapiro-Wilk and Kolmogorov-Smirnov D tests were used to verify the assumptions of normality (SAS Institute, 2010). The square root transformation was carried out to percentage data by computing $\sqrt{\text{%emergence/100}}$. The log transformation was used if necessary for counts (other than %) to meet the normality assumptions. Differences among treatments for each poultry manure were analyzed using the Tukey’s multiple means comparison in PROC GLM (SAS Institute, 2010). Differences among treatments were considered significant at $\alpha = 0.05$. The untransformed means are presented in graphs.
Fed-on vs Non fed-on manure

Landing preference data from the fed-on duck manure versus non fed-on duck manure experiment at each period was analyzed using a PROC GLM and repeated measures analysis (SAS Institute, 2010). The assumption of sphericity was verified for repeated measures analysis. If the data do not satisfy the assumption of sphericity, an adjustment was performed in PROC GLM and appropriate significance levels associated with the $F$ test (SAS Institute, 2010). Means for each treatment were separated using Tukey's multiple means comparison, and differences among treatments were considered significant at $\alpha = 0.05$.

2.3 RESULTS

Adult house fly emergence of larvae reared in different types of poultry manure (laboratory)

No significant differences for adult emergence were found between the four different types of poultry manure tested ($P = 0.2790$) (Figure 2.1). Duck manure had an average emergence of 98%, while turkey, broiler, and layer manure had an average emergence of 90, 92, and 96%, respectively.

Adult house fly landing preference and egg-laying in different types of poultry manure (laboratory)

Although the average number of flies landing on the different types of poultry manure was low (Figure 2.2), significantly more adult flies landed on duck manure than either
type of chicken manure ($P < 0.0001$). No eggs were found on any of the manure samples after 120 min.

**Adult house fly landing preference and egg-laying in different substrates (barn)**

The wet rearing media had the highest average number of flies landing on it (Figure 2.3), followed closely by molasses (14.3 and 10.6 flies, respectively); no statistical differences were identified between these substrates ($P < 0.0001$). Although the average number of flies landing on Muscovy duck manure (4.5 flies) was higher than chicken or cow manure (2.5 and 3.2 flies, respectively), no statistical differences were identified between the three types of livestock manure. No eggs were found on any of the substrates after 120 min.

**Adult house fly landing preference and egg-laying in fed-on versus non fed-on duck manure (laboratory)**

No statistical differences were identified between the average number of flies landing on non-fed-on and fed-on manure after $T = 120$ min (2.5 and 3.3 flies, respectively) ($P = 0.1481$) (Table 2.2). Significantly more flies landed on the fed-on manure at $T = 60$ min ($P = 0.0193$).

**Nutrient analysis of poultry manure**

Turkey manure had the highest dry matter (55.7%), potassium (1.35% wet), total Kjeldahl N (4.69% wet), and Ammonium-N (16,300 mg/kg wet) content compared to the other types of poultry manure, yet had a lower pH (8.00) (Table 2.1). Muscovy duck manure had the highest pH (9.15) followed closely by Mule duck and broiler manure.
Layer manure had the lowest phosphorous content (0.49% wet). Broiler manure had the lowest dry matter content (29.5%) making it the wettest poultry manure tested.

2.4 DISCUSSION

Poultry manures appear to be excellent rearing substrates for *M. domestica* and results from this study are consistent with previous research (Khan et al., 2012; Patricia and Claudio, 2003). No significant differences for adult emergence were identified between the different types of poultry manure, and adult emergence rates were found to be very high for all of the manure tested (between 90-98%). Manure accumulation within poultry facilities enables house fly populations to readily establish; fresh manure is deposited daily by the birds, supplying this pest with a constant supply of new substrate (Bailey et al., 1968). Because producers can implement different management practices (e.g. type and amount of bedding, stocking rates, ventilation) at their facilities, it is difficult to define what would be the typical bedding characteristics specific for each type of poultry production. A collection of manure at multiple barn locations for each poultry type would permit the variability of the litter and the impact it might have on nutrient and moisture content and fly development.

Duck manure had high adult emergence (98%). The number of adults landing on the different types of manure was low in the preference study conducted in the laboratory, and no eggs were found on the tested manure. This can be explained, at least in part, by the short period of contact the adult females had with the manure. It is possible that the small mass of manure used in the petri dishes (15 g) did not elicit oviposition or that...
the surface dried rapidly. House flies feed on a wide variety of organic substrates, including feces and other decaying matter (Malik et al., 2007). In addition to feeding, house flies will lay their eggs in organic substrates that provide a suitable environment for their offspring; suitable locations include areas that provide larvae with sufficient nutrients for development (West, 1951; Sanchez-Arroyo, 1998). Upon hatching, larvae will begin to feed on organic material for several days to weeks, in order to store enough nutrients for the pupal stage. A larger female population size, a longer observation period, and/or conditions more conducive to oviposition, such as larger substrate volumes, might improve egg-laying and allow for differences between treatments to be determined. Due to practicality and biosecurity reasons, samples were collected from one barn for each type of poultry manure. As such, the samples used for each test (replicate) were not truly independent. Further studies should use numerous duck, turkey, broiler and layer farms to depict a more accurate representation of typical manure characteristics of a poultry farm and its impact on growth and egg-laying.

Adult landing preference on a number of organic substrates in a field setting identified wet RM as being the most attractive to adult house flies. The RM is composed of all organic substances (water, blood meal, powdered milk, brewer’s yeast, and wheat bran), making it very attractive for house fly reproduction as it provides a good source of nutrients (proteins, carbohydrates, and fats) for developing larvae, likely due to the fermentation. Molasses was also highly attractive for adult landing. Both RM and molasses have the potential to be incorporated into physical (e.g. a lure-kill trapping mechanism) or chemical (e.g. combined with a spinosyn or neonicotinoid) control
methods, to aid fly control in poultry facilities. Unfortunately, the RM does not store well, and will dry out inside poultry facilities due to the barn conditions, making it less attractive to house flies and limiting its commercial use. Molasses has a longer shelf-life and is readily available; it could potentially be used indoors and at residential areas, as it has a much more pleasant odour than many of the commercially available baits (Quinn et al., 2007).

The development and reproductive success of an insect is dependent upon the chemical and biological characteristics of the substrate on which it grows/feeds (Farkas et al., 1998). Nutrient analyses were conducted on five different types of poultry manure. Mule duck manure had the highest level of phosphorus (0.90% wet). Barnard et al. (1995) identified the phosphorus content of chicken manure as being the key element responsible for the development of house fly larvae into larger pupae; pupal size was not assessed in this research and therefore no conclusions on the phosphorous level in manure can be drawn. Khan et al. (2012) identified poultry manure as having higher house fly pupal weights (16.89 mg) than any of the other livestock manures (nursing calf - 16.56 mg; cow - 15.16 mg; buffalo - 15.26 mg; goat - 12.97 mg; sheep - 12.68 mg; horse - 11.58 mg) or dog feces (16.09 mg) tested; further research should explore the role phosphorous plays in pupal development.

According to West (1951) and Sacca (1964), female house flies seek protein-containing substrates to feed on or oviposit. Muscovy and mule duck manure had the lowest levels of total Kjeldahl N (1.05 and 1.16% wet, respectively) and ammonium-N (2,760 and
1,910 mg/kg wet, respectively). These manure types had high pH (9.15 and 9.06, respectively). Khan et al. (2012) identified poultry manure as a preferred substrate for *M. domestica*, resulting in short developmental time. Although our study showed females landing on duck manure (which had the lowest amount of nitrogen), in general poultry manure has higher levels of nitrogen than other types of livestock manure (Hogsette, 1996; Patricia and Claudio, 2008). Patricia and Claudio (2008) suggested that high nitrogen levels might be explained by the birds’ diet, as typical poultry feed contains protein as the major component; horse, goat, and sheep (manure that had the longest developmental time) diets are primarily comprised of crop residues, fodders, and grasses, all substrates that are low in nitrogen. Further studies on the chemical composition of poultry manure should be conducted to identify specific compounds responsible for house fly attraction. More manure samples from various locations within poultry facilities should be collected and analyzed to consider the variability in manure characteristics. Diet modification could potentially play a role in decreasing fly populations within poultry facilities, as nutrients in manure could be manipulated and may not be as suitable for house flies to use as a reproductive medium; however, the health impact of the diet alterations on birds may be a limiting factor of the method.

Dethier (1955) reported that fed-on food was more attractive to blow flies and house flies than non-fed on food. Brodie (2014) suggested that the attraction to a particular area to feed and/or oviposit may be associated with semiochemicals released by flies that previously fed at the location. Our landing preference trial did not identify any significant differences between duck manure that had been in contact with house flies
(fed-on), and fresh, no contact duck manure (non-fed-on). The duration of the experiment was short (2 hours) and results might have been different if flies were given more time to deposit semiochemicals on the fed-on manure. Cosse and Baker (1996) suggested that resource-derived semiochemicals are what stimulate females to oviposit near other house fly eggs. The researchers identified nine volatiles from pig manure that generated a response on female house fly antennae. These chemicals are involved in female attraction to a certain area for oviposition, as flies flew upwind (an uncommon behaviour) in wind-tunnel behavioral assays, to land on the pig manure containing these volatiles (Cosse and Baker, 1996). The role semiochemicals play in house fly attraction and oviposition site-selection should be further investigated.

A thorough understanding of oviposition preferences and larval development of house flies in poultry manure can contribute to the development of cultural control methods within poultry facilities. Duck manure, although highly suitable for house fly maggot development, does not likely contribute more than other types of poultry manure to large fly populations in barns. Management techniques that render the manure less suitable for fly reproduction are important for reducing existing infestations and preventing future ones. The identification and removal of fly breeding “hot spots” in manure, the elimination of leaky water nipples, the modification of manure moisture content, and the addition of drying agents to poultry manure have the potential to make this substrate less suitable for house fly reproduction and contribute to a successful fly control program within poultry production facilities.
CHAPTER 3: Impact of moisture content of duck manure on house fly development and reproduction

3.1 INTRODUCTION

The control of pest flies in poultry operations has become a growing concern within the industry in recent years. The management of poultry housing facilities often create favourable conditions for the development of manure-breeding flies (predominately Musca domestica); house flies rapidly infest poultry manure and can reach large population sizes very quickly (Mwamburi et al., 2011). House flies can be a nuisance to barn workers, reduce bird feed efficiency, and transmit pathogens to both humans and the poultry being housed (Moon and Meyer, 1985; Mwamburi et al., 2011).

Moist poultry manure provides a highly attractive oviposition medium for adult flies, as it provides ideal conditions for their offspring’s development (Axtell, 1986; Kaufman et al., 2000). House flies will lay their eggs in areas with appropriate moisture content; larval numbers were observed to be highest when the poultry manure moisture content was 70-79% (Stafford and Bay, 1987; Axtell, 1999). Mullens et al. (1996) reported a significant decrease in fly populations when moisture levels were below 60%. In poultry housing facilities, manure with a moisture content of 40-50% is comprised of easily separated clumps, whereas manure with a moisture content of 55-70% preserves some structure, and manure with a moisture content of 80-90% is nearly liquid (Mullens et al., 1996). Manure can accumulate differently in various areas of the barn (e.g. under feed lines, under water lines, near entrances or air inlets), creating locations of varying moisture content and consequently, of varying suitability for fly development. Removing
or modifying the conditions of the areas of the manure where flies tend to congregate and develop could help reduce fly populations by discouraging oviposition and decreasing its suitability for larval development.

Moisture management is a key component of cultural control; keeping manure as dry as possible is essential for reducing fly infestations in poultry facilities (Axtell, 1999; Kaufman et al., 2000). In addition to reducing the suitability of poultry manure for larval development and oviposition, a more desirable habitat for fly predators and parasites is also created when manure moisture is reduced (Axtell, 1981). A less favourable environment lengthens the developmental time of *M. domestica*, allowing beneficial predators and parasites a longer opportunity to search for and parasitize their host (Khan et al., 2012). Geden (1999) evaluated the searching ability of five species of parasitoids and found that *Muscidifurax raptor* preferred manure with ≤ 75% moisture content, whereas the three *Spalangia* spp. preferred 45-65% moisture, and *Dirhinus himalayanus* preferred drier manure (although host attack rates were low). Drier manure not only decreases the suitability of the manure for house fly development, it can also give producers a longer time frame to implement biological and/or cultural control measures, thus reducing the number of fly generations per bird grow-out period or production cycle.

Proper ventilation in poultry barns can help reduce the moisture content of the manure (Axtell, 1999; Kaufman et al., 2000). In addition to exhaust fans (found in standard poultry barns), supplemental fans can also be installed to help keep the manure dry,
thereby reducing its suitability for house fly reproduction (Kaufman et al., 2000). Sufficient ventilation can also improve air quality by removing gases, such as ammonia, from the barn (Kaufman, 2000). High levels of ammonia have been shown to significantly compromise poultry welfare (Dawkins et al., 2004).

Routine inspection and maintenance of equipment is necessary to prevent excess water from contributing further to the wet manure conditions. Leaky water nipples can be a major contributor to increased moisture levels in a poultry barn, as can condensation from non-insulated overhead water lines and seepage from the exterior (Kaufman et al., 2000). Eliminating these problems is crucial in a fly management program.

A thorough understanding of the impact manure moisture content has on house fly development is necessary to maximize the effectiveness of cultural control methods in poultry facilities. There is a lack of information about duck manure and litter conditions in the barn on house fly development. Gaining knowledge about the moisture conditions of duck manure that *M. domestica* favour for oviposition, larval development, and adult emergence can help producers identify fly “hot spots” in manure, in order to modify the substrate before fly populations establish or before adult emergence occurs. The objectives of the study were to 1) evaluate the effectiveness of additional ventilation (circulation fans) on the moisture content of the litter in duck barns, and 2) evaluate the most suitable and least suitable litter moisture levels for fly development and egg-laying in duck manure.
3.2 MATERIALS AND METHODS

Litter collection in barns

Three two-floored Muscovy duck barns in Beamsville, ON were used as the sample sites. Sample collection was performed at week 4, 6, and 8 (three random periods selected in the grow-out period) on each test floor within the duck barns to assess the dry matter content and percent moisture of the litter. Two types of management (the treatment) were compared: use of circulation fans and non-use of circulation fans. Six circulating fans (Exacon CF18LP – 47.5 cm in diameter) were installed per floor in a serpentine configuration, as per the manufacturer’s recommendations, for the ‘use of circulation fans’ floors. Differences in litter moisture content between treatments were assessed, by collecting litter at three specific locations on each floor: under feed lines; under water lines; and in alleyways. The three locations were chosen to represent potential differences in moisture content inside a duck barn. Five samples were collected from each of the three locations per floor (for a total of 15 samples per floor) to give a fair representation of moisture content in various areas. A representative sample of 40-50 g of manure was collected at each sampling site (5-10 cm below the manure surface) wearing nitrile gloves, and placed in a small, labelled Ziploc bag. The samples were stored at 4°C. Additionally, at each sampling site, 1 L of duck litter was collected from the surface of the barn floor using a clean, plastic 1 L milk jug to determine the number of house fly larvae. The manure was poured into a shallow tray for larvae counting.
**Litter moisture determination (use of circulation fans and non-use of circulation fans in barn)**

Litter samples were processed < 7 d after collection to assess the moisture content. Each sample was conveniently assigned a crucible and crucibles were then weighed. Approximately 15 g of well-mixed litter was placed in each crucible and weighed in grams (initial weight). Samples were then placed in a drying oven at 105°C for a minimum of 4 h (samples were dried no longer than 12 h). Crucibles containing dried litter were removed from the oven and weighed again (final weight).

The percent moisture was calculated as follows:

\[
\text{% moisture} = \left( \frac{B - A}{B} \right) \times 100
\]

Where, \( A \) = Final weight and \( B \) = Initial weight

**Adult house fly emergence of larvae reared in duck manure of different moisture contents (laboratory)**

Additional samples of duck manure were collected from test floor 1 from the farm described in section 3.2.1. Bedding material (wood shavings) is added daily to the barn floor, and birds defecate on top of the bedding material; samples collected were therefore a mixture of shavings and manure (hereafter referred to as *manure*), although wet areas of the barn were targeted to maximize the amount of fecal material in each sample. Samples were frozen for 24-48 h to kill any house fly eggs, larvae, pupae, or other arthropods, and processed within 72 h of collection. Each 2 L sample of manure was dried completely in a drying oven (minimum of 24 h; samples were dried no longer
than 36 h). The water content of the manure (the *treatment*) was adjusted to the desired moisture level (40-90% in 5% increments) by adding the appropriate amount of distilled water. Two hundred grams of each treated sample was then placed in a 1.9 L plastic container. Twenty-five neonate *M. domestica* larvae (24-36 h old) were placed on the surface of the manure and reared until adult emergence. Observations on the manure characteristics (consistency, presence of molds) and larval behaviour (location in the manure and inhabitual movement) was assessed at day 1, 5, 10, and 15. Each sample was gently mixed every 48 h and emergence of adult flies was recorded until there were five consecutive days with no emergence. Containers were placed at room temperature (22 ± 2°C) and three replicates of each treatment were performed.

**Adult house fly landing preference and egg-laying in duck manure of different moisture contents (laboratory)**

Fifteen grams of each treated sample (the same samples as described in section 3.2.3) was transferred into glass petri dishes 15 min before the beginning of the experiment. A 3 cm x 3 cm square of brown recycled paper towel was placed on top of the manure in the centre of each petri dish to encourage oviposition. The paper was lightly moistened with distilled water before placement. The petri dishes were then randomly placed (uncovered) side-by-side in a fly cage (described in Chapter 2) containing 50 *M. domestica* females (10 d old) that were provided with sugar water for 48 h before the beginning of the experiment. Females used for testing were previously in contact with males to ensure the possibility of copulation. The sugar water was removed at the beginning of the experiment. The experiment was conducted at room temperature (22 ±
Observations on landing and oviposition were recorded to assess house fly preference. Landing preference was observed at T = 0, 15, 30, 45, 60, 75, 90, 105, and 120 min. The number of flies landing on the treated manure at each time was recorded for each petri dish. Counts for landing on the manure and landing on the paper towel were grouped together and averaged to reduce the variability of punctual observation points. Oviposition was assessed by counting all eggs on the manure treatment and paper towel for all three replicates after T = 120 min using a microscope at 10 X magnification.

3.2.1 DATA ANALYSIS

*Litter moisture content*

The data were analyzed using a generalized linear model (PROC GLM) and repeated measures analysis (SAS Institute, 2010). Differences among the treatment groups (use of circulation fans and non-use of circulation fans) at each location (under waterline, under feedline and side alley) and for each observation time (4, 6 and 8 weeks of age) were analyzed using Tukey’s multiple means comparison in PROC GLM (SAS Institute, 2010). Differences among treatments at each observation time were considered at a significance level of $\alpha = 0.05$.

*Adult emergence and landing preference in duck manure of different moisture contents*

The PROC UNIVARIATE was run for the experiments and the results of the Shapiro-Wilk and Kolmogorov-Smirnov D tests were used to verify the assumptions of normality.
The square root transformation was carried out to percentage data by computing $\sqrt{\%\text{emergence}/100}$. The mean adult emergence for each treatment group (manure moisture content) was analyzed using a Tukey’s multiple means comparison in PROC GLM (SAS Institute, 2010). Differences among treatments were considered to be significant at $\alpha = 0.05$. The same analysis was used to compare landing preference, but the log transformation was carried out to satisfy the assumptions of normality. The untransformed means are presented in graphs.

### 3.3 RESULTS

**Litter moisture content (use of circulation fans and non-use of circulation fans in duck barns)**

No significant differences ($P = 0.9569$) in the moisture content of the litter were found between treatment groups (floors with fans versus floors with no fans) or between collection times (week 4, 6 and 8) (Figure 3.1). At each observation period, the litter under the feed lines always had significantly lower moisture content (19.2–36.7% moisture) than the litter under the water lines (59.2–70.3%) and in the alleyways (55.1–60.5%). For most of the weeks, the litter under the water lines had significantly higher moisture content than the litter in the alleyways. In total, only 17 *M. domestica* larvae were collected from all of the 1 L manure samples (15 samples x 3 collection times).
Adult house fly emergence of larvae reared in duck manure of different moisture contents (laboratory)

Adult emergence rates were highest in duck manure with moisture contents of 65-85% (Figure 3.2). At 75% moisture, emergence of 100% was observed over the three replicates. Lower adult emergence rates were observed in duck manure that was 40-55 or 90% moisture compared to the 65–85% range \((P < 0.0001)\). However, the number of adult flies emerging at lower moisture contents was at least 70% of the initial number of neonates placed on the manure.

Adult landing preference and egg-laying in duck manure of different moisture contents (laboratory)

The average number of adult flies landing on the manure was low, and no statistical differences \((P = 0.8433)\) were identified between duck manure of different moisture contents (Figure 3.3). Oviposition was very infrequent. Over the four replications, a total of 22 eggs were laid on manure that was 65, 70, 75, and 80% moisture (6, 3, 6 and 7 eggs, respectively); therefore, no statistical analysis was performed for oviposition.

3.4 DISCUSSION

Poultry manure is a heterogeneous substrate that is often warm and moist while fermenting, providing an ideal habitat for the development of large populations of *M. domestica* (Axtell, 1986). Kaufman et al. (2000) suggested that the use of supplemental fans in a barn might reduce the suitability of the manure for house fly reproduction by reducing its moisture content. In poultry barns, the running speed and duration of
operation of exhaust fans and circulation fans are determined by the weather (temperature and humidity). For this research, although circulation fans were running continuously at the time of the experiments in the spring and fall months, no significant differences were found in the moisture content of the litter between floors with and without circulation fans. Further testing would need to be conducted before recommending the installation of this type of circulating fan into duck barns.

As birds approach market weight, they consume more feed and therefore produce more feces; thus, it was important to determine if there was an effect of the time of litter collection on the moisture content. No significant differences in the moisture content of the litter were identified between weeks 4, 6 and 8. However, the moisture content of the litter varied greatly with the location in the barn, ranging from an average of 19.2% moisture to 70.3%. Ducks are known to “play” with their drinking nipples, often breaking or causing physical damage to them, enabling water to drip onto the litter, thereby creating a wetter environment underneath the water lines. The moisture content of the litter under the water lines in our study (65 to 85%) was similar to moisture levels identified by other researchers (Stafford and Bay, 1987; Axtell, 1999) as being appropriate for larval development and adult fly emergence in poultry manure. Perhaps sampling manure deeper (e.g. 15-20 cm under the surface), where less litter is present in the manure, would also impact the manure moisture contents at the various locations. Ducks do not typically defecate around their feeders, providing a plausible explanation for the much lower moisture content under the feed lines. Because drier manure typically discourages oviposition and larval development (Stafford and Bay, 1987), it
was important to determine if circulation fans had an effect on the larval population size in the manure. Although fairly large (1 L) manure samples were collected each week at each location, we were unsuccessful at locating larvae, even though the barn had a fairly high number of adult flies. It is possible that larvae in the barn were so heterogeneously distributed that our random samples ended up with no larvae. A different technique and/or method should be employed in future studies to locate more larvae, in order to determine the effect of circulation fans on larval populations.

Farkas et al. (1998) successfully reared *M. domestica* in poultry manure with moisture levels of 50-80%, and Kaufman et al. (2000) reported successful house fly development in manure with moisture contents of 50-85%. Our research used duck manure instead of chicken manure, and neonate larvae were successfully reared to adults at moisture levels of 40-90%. The highest adult emergence rates were observed in manure with moisture contents of 65-85%, with an average adult emergence of 100% when the manure moisture content was 75%. Similar to our study, Stafford and Bay (1987) found house fly population numbers to be highest when the chicken manure moisture content was between 70% and 79%. Although the average adult emergence was lower for the manure that was 40-55 or 90% moisture, a considerable number of adults still emerged (73.3–84%). Decreasing the manure moisture content might reduce *M. domestica* population numbers; however, other methods of control (such as physical or chemical control methods) will need to be utilized in tandem to fully control house fly populations.
In our study, no significant differences between manure moisture content were identified for the adult landing preference experiment, and no conclusive results for oviposition site selection preference could be made because oviposition was very infrequent. In contrast, Fatchurochim et al. (1988) assessed oviposition of adult house flies in chicken manure of different moisture contents (40, 50, 60, 70, 80, and 90%) and recovered a total of 2,845 eggs, 76% of which were found on the manure that was 70% moisture. Thus, future research evaluating oviposition on duck manure would likely benefit from a larger female population size, a longer observation period, and/or conditions more conducive to oviposition. Fatchurochim et al. (1988) identified chicken manure of < 40% moisture as an unattractive substrate for oviposition. Further, Hogsette (1996) found a direct relationship between the duration of larval and pupal states of house flies, and the moisture content of chicken manure. Due to the limited amount of research conducted on duck manure to-date, further studies should be conducted to assess adult landing preference and oviposition studies on duck manure of 40-55% moisture, and to determine the duration of each life stage in duck manure of different moisture contents.

Poultry producers should attempt to keep the manure moisture in the barn at a level that is least conducive to fly reproduction. This research identified lower adult emergence rates in duck manure of 40-55 and 90% moisture. Maintaining moisture content of 90% in a poultry barn is impractical; water would have to be added to the manure to obtain this level and the manure would be almost completely liquefied. In addition to unpleasant work conditions, wet manure might harbour harmful pathogens that could negatively impact the health and welfare of the birds (Schefferle, 1965). Aiming to keep manure drier is a much more feasible option, and can be accomplished using barn
management and cultural control methods (e.g. fixing water leaks, adding naturally-occurring substances or other drying agents to the manure, using drier wood chips). Good manure management techniques must be implemented, as moisture control can play a key role in house fly control (Farkas et al., 1998). Manure management has the advantage of diminishing fly populations before they reach adulthood (the nuisance stage). Targeting adult house flies will not eliminate a fly infestation; the removal or modification of attractive reproductive areas (i.e., areas of ideal moisture content) will contribute to an overall successful fly control program in poultry facilities.
CHAPTER 4: Effectiveness of naturally-occurring substances added to duck manure at reducing survivorship and repelling house flies

4.1 INTRODUCTION

Commercial poultry barns provide an ideal breeding environment for flies due to the controlled temperatures and manure accumulation within the housing facilities (Kaufman et al., 2000). Poultry manure provides a suitable substrate for house fly oviposition, larval development, and adult feeding (Axtell, 1999).

The current control of pest flies in poultry facilities relies primarily on synthetic insecticides (most commonly used as a spray or included in baits) (Kaufman et al., 2000). The overuse and/or improper use of insecticides has enabled house flies to develop resistance to many active ingredients (Geden et al., 1995), and increases the risk of contaminating table eggs or poultry meat with chemical residues (Kaufman et al., 2010). In the United States, Larvadex® (N-cyclopropyl-l,3,5-triazine-2,4,6-triamine) is a commercially available insect growth-regulating pesticide that is incorporated into poultry feed. The active ingredient, cyromazine, is digested by the bird and then excreted, controlling the growth of fly larvae from developing in the area of defecation. Although a relatively new product, reports of resistance to cyromazine in the UK were reported in 2010 (Bell et al., 2010), emphasizing the need for alternative methods of fly control that avoid the use of harmful insecticides that have a limited duration of effectiveness. Moreover, the insecticides almost always target the adult stage. Techniques or products that reduce manure suitability for house flies could help in controlling pest populations in poultry barns (Malik et al., 2007). House fly development
is dependent on the presence of bacteria, suggesting that fly development in a natural environment is supported by a complex microbial community (Zurek et al., 2000). House fly larvae are commonly found in spilled or spoiled silage; however, composted silage provides unsuitable conditions for larval development due to the lack of oxygen and low pH (Zurek et al., 2000). The modification of manure characteristics (e.g. pH) may render the medium less attractive for house fly development, reducing oviposition, larval development and/or survival, and adult emergence. For example, Calvo et al. (2010) treated calf manure with an acidifier to reduce the abundance of bacteria, and results indirectly revealed a decrease in house fly larval survival. Moreover, a change in manure characteristics could influence oviposition, as female stable flies (Stomoxys calcitrans (L.)) used microbial-derived stimuli to select a suitable site for oviposition and larval development (Romero et al., 2006). Further research should explore the application of naturally-occurring substances to poultry manure, to examine their potential as house fly control agents in poultry housing facilities.

Naturally-occurring substances (NOS) are compounds that are present naturally in the environment. NOS can be used to control a wide variety of livestock and crop pests, and are relatively safe for use around animals, poultry, and humans. For example, formic and oxalic acid can be used to control Varroa destructor (Anderson) (Varroa mite) in honey bee colonies, citric acid can assist in the control of Aphis craccivora (Koch) (cowpea aphid), diatomaceous earth can control Rhyzopertha dominica (Fabricius) (lesser grain borer), and hydrated lime can control Alphitobius diaperinus (Tenebrionidae) (darkling beetle) quite efficiently (Imdorf et al., 1996; Abd El-Baset El-
kady et al., 2010; Korunić and Mackay, 2000; Watson et al., 2003). In addition to controlling *Blattella germanica* (L.) (German cockroaches) (Gore and Schal, 2004), *Atta sexdens rubropilosa* Forel (worker ants) (Sumida et al., 2010), and *Anopheles quadrimaculatus* Say (adult mosquitoes) (Xue and Barnard, 2003), boric acid has been incorporated into baits and solutions to control *M. domestica* (Hogsette and Koehler, 1994; Hogsette et al., 2002). Hogsette and Koehler (1992) successfully controlled house flies using liquid formulations of boric acid combined with 10% sucrose; flies that fed on 2.25% boric acid concentrations died within 3-4 hours. Repellent effects were also observed from substrates treated with higher concentrations of boric acid, as fewer flies landed on those substrates (Hogsette and Koehler, 1994). Due to dust accumulation in barns, liquid formulations of boric acid baits might not be practical in agricultural settings, as dust can settle on the traps, preventing house flies from consuming the necessary dose needed for death (Hogsette et al., 2002).

Vinegar, another of the NOS, is comprised of acetic acid and water. Qain et al. (2013) evaluated a commercial diluted vinegar product (ChinKiang®) and found that it was highly attractive to adult house flies. Seven volatile compounds (acetic acid, furfural, butanoic acid, isovaleric acid, hexanoic acid, 2-phenylethanol, and p-cresol) elicited significant responses from the antennae of female and male house flies (Qain et al., 2013).

In addition to acting as an attractant, the application of NOS to manure could potentially alter the pH of the manure, making it less suitable as a reproductive substrate for house
flies, discouraging fly development, and potentially limiting the number of flies dispersing to neighbouring residents (Heinrich et al. 2010). To our knowledge, there has been no research conducted on the direct application of NOS to livestock manure (more specifically duck manure) as a method of house fly control. The objective of this study was to evaluate the impact of NOS (acetic acid, boric acid, citric acid, hydrated lime, diatomaceous earth) added to duck manure on house fly egg-laying and growth.

4.2 MATERIALS AND METHODS

Manure collection

Two L of duck manure (a mixture of feces and wood shavings) was collected from a commercial two-floored Muscovy duck farm in Beamsville, ON < 72 h prior to the experiments, from 5-10 locations in the poultry barns. The manure was collected when the birds were 6-8 weeks into the grow-out period, and frozen for 24-48 h to kill any house fly eggs, larvae, pupae, or other arthropods. The frozen manure was thawed, allowed to reach room temperature and mixed before further use. The dry content of the manure was assessed by drying three 15 g aliquots at 105°C in a drying oven for a minimum of 4 h (samples were dried no longer than 12 h). Stafford and Bay (1987) found house fly population numbers to be highest when the chicken manure moisture content was between 70 and 79%; therefore, the moisture content of duck manure used in our experiment was adjusted to 75% by adding the appropriate amount of water.
Naturally-occurring substances applied to duck manure

Manure samples (200 g of manure in 1.9 L plastic containers) were treated with one of two concentrations (1.9 or 4.7%) of acetic acid, boric acid, citric acid, hydrated lime, or diatomaceous earth. Each of the products was mixed with 10 mL of distilled water in 100 mL beakers for 30 sec before being poured onto the manure samples. The treated manure was mixed with a glass stirring rod for 30 sec to ensure homogeneity. The remaining product in the beaker was rinsed twice with 5 mL of distilled water, and the rinsed water was then poured onto the manure and the manure was mixed again. The same amount of distilled water (20 mL) was added to the control (untreated) manure.

Adult house fly emergence of larvae reared in duck manure treated with NOS

Twenty-five neonate *M. domestica* larvae (1-2 d old) were placed on the surface of each manure sample (treated and control) using a fine-tip paintbrush and reared until adult emergence. Observations on the manure characteristics (consistency, presence of molds) and larval behaviour (location in the manure and inhabitual movement) were assessed at day 1, 5, 10, and 15. The pH of the manure samples (treated and control) was tested on days 1, 5, and 10; the pH of each NOS treatment was also measured at T = 0 before they were mixed with distilled water or manure. Each manure sample was gently mixed every 48 h and emergence of adult flies was recorded until there were five consecutive days with no emergence. Containers were placed at room temperature (22 ± 2°C) and four replicates of each treatment were performed.
Adult house fly landing preference and egg-laying in duck manure treated with NOS

Fifteen grams of each treated manure sample was transferred into glass petri dishes (80 mm x 15 mm) 15 min prior to the start of the experiment. Fifteen grams of a larval rearing media (4.65 mL water, 0.75 mL blood meal, 1.35 mL powdered milk, 0.15 mL brewer’s yeast, and 8.1 mL wheat bran) and 15 g of untreated manure served as controls. A 3 cm² square of brown paper towel was placed in the centre of each petri dish to encourage oviposition. The paper was lightly moistened with distilled water (3-5 drops) before placement. The petri dishes were then placed randomly in a fly cage containing 50 M. domestica females (10 d old) that were provided with only sugar water for 48 ± 2 h before beginning the experiment. Females used for testing were maintained in contact with males to ensure mating and the sugar water was removed from the cage < 5 min before starting the experiment. The experiment was conducted at room temperature (22 ± 2°C). Observations on landing and oviposition were recorded to assess house fly preference. Landing preference was performed at T = 0, 15, 30, 45, 60, 75, 90, 105, and 120 min. The number of flies landing on the treated manure at each time was recorded for each petri dish, and averaged to reduce the variability of punctual observation points. Oviposition was assessed at 120 min using a microscope at 10X magnification. Four replications were performed.

Dose response of acetic and boric acid applied to duck manure

Following the results of the adult emergence experiment (section 4.2.3), a dose-response study was undertaken with acetic and boric acid. Following the same
experimental procedure as described in section 4.2.2, seven concentrations of boric acid (0.25, 0.50, 1.0, 1.5, 2.0, 3.25, and 4.5%) and seven of acetic acid (0.5, 1.0, 2.0, 3.25, 4.5, 5.75, and 7.0%) were applied to manure samples and 25 neonate larvae were reared in each sample (treated and a control). The same boric acid and acetic acid treatments were used in a dose-response landing preference and oviposition study following the same experimental procedure as described in section 4.2.5.

4.2.1 DATA ANALYSIS

*The pH of duck manure treated with NOS*

The data (pH of manure treated with 1.9 or 4.7% acetic acid, boric acid, citric acid, hydrated lime, or diatomaceous earth) were analyzed using a generalized linear model (PROC GLM) with repeated measures analysis (SAS Institute, 2010). Means for each treatment were separated using Tukey’s multiple means comparison, and differences among treatments were considered significant at a level of $\alpha = 0.05$. The assumption of sphericity was verified for repeated measures analysis. If the data did not satisfy the assumption of sphericity, an adjustment was performed in PROC GLM and appropriate significance levels associated with the $F$-test (SAS Institute, 2010).

*Adult Emergence/ Landing Preference*

The PROC UNIVARIATE was run for all experiments and the results of the Shapiro-Wilk and Kolmogorov-Smirnov D tests were used to verify the assumptions of normality (SAS Institute, 2010). The tangent transformation was carried out to percentage adult house fly emergence from duck manure treated with NOS by computing tangent
(\%{emergence}+1/100), to meet the assumptions of normality. The log transformation was carried out on the landing numbers. Mean adult emergence and the average landing number for each manure treatment was analyzed using Tukey's multiple means comparison in PROC GLM (SAS Institute, 2010). Differences among treatments were considered significant at a level of \( \alpha = 0.05 \). Untransformed data are presented in graphs.

The lethal concentration 50 and 90\% (LC\(_{50}\) and LC\(_{90}\)) and the lower and upper 95\% fiducial limits were calculated for acetic acid and boric acid using probit analysis (PROC PROBIT) of SAS version 9.2. Due to high mortality of larvae with the doses selected for boric acid, the LC\(_{50}\) value has very wide fiducial limits.

4.3 RESULTS

The pH of duck manure treated with NOS

The pH of the three acids applied to the manure samples was < 3.2; the pH was 8.8 for diatomaceous earth and 12.15 for hydrated lime (Table 4.1). On day 1 of the experiment, the pH of manure treated with boric acid or DE was not significantly different than the pH of untreated manure (Figure 4.1). On days 5 and 10, the pH of most of the treated manures tended to change towards the initial pH of the control manure (untreated).

In the dose-response study, although the pH of acetic acid treated manure tended to change towards the initial pH of the control manure as the experiment progressed
(Figure 4.2), the pH of all acetic acid treated manure samples was significantly lower than the control manure. The pH of all boric acid treated manure samples was also significantly lower than the control manure on days 1, 5, and 10 (Figure 4.3), although to a lesser extent than the acetic acid.

**Adult house fly emergence from larvae reared in duck manure treated with naturally-occurring substances, and lethal concentrations of acetic and boric acid**

Manure treated with 1.9 or 4.7% acetic or boric acid had significantly lower adult emergence than the control manure ($P < 0.0001$) (Figure 4.4). When boric acid was applied to duck manure at a concentration of 4.7%, the adult fly emergence was 0%. For acetic acid, manure treated with the lower concentration (1.9%) had an average fly emergence of 40%, whereas an average fly emergence of 23% was observed for manure treated with the higher concentration (4.7%).

In the dose-response study, a decrease in emergence was observed with increased concentrations of acetic acid applied to duck manure (Figure 4.5). Manure treated with 2.0, 3.25, 4.5, 5.75, or 7.0% acetic acid reduced adult house fly populations by > 50% (i.e., by 57.3, 62.7, 76.0, 85.3, and 92.0%, respectively) compared to the control manure. Manure treated with 1.5, 2.0, or 4.5% boric acid had no adult emergence (Figure 4.6). The other concentrations had very low emergence (Figure 4.6).

The LC$_{50}$ for boric acid was estimated to be 0.01% (Table 4.2), lower than for acetic acid (1.51%). Similarly, the LC$_{90}$ of boric acid is 0.12%, which is lower than the LC$_{90}$ of acetic acid, with an estimated value of 10.03%.
Adult house fly landing preference and egg-laying in duck manure treated with NOS (laboratory)

Although no statistical differences were identified between the average number of flies landing on the different manure treatments (Figure 4.7), significantly more flies landed on the rearing media than the other substrates ($P < 0.0001$). No eggs were found on any of the substrates after 120 min.

In the dose-response study, manure treated with 7.0% acetic acid had significantly fewer adult flies landing on it compared to the untreated control manure ($P < 0.0001$) (Figure 4.8). Over the four replications, 24 eggs were laid in total; 15 on the control manure, 6 on the 0.5% acetic acid treated manure, and 3 on the 1.0% treated manure; thus, no statistical analysis was performed.

For boric acid, no statistical differences in the number of adults landing were identified between treatment groups (manure treated with seven concentrations of boric acid), including the control group (untreated manure) (Figure 4.9). Over the four replications, 13 eggs were laid in total; 8 on the control manure, 2 on the 1.0% boric acid treated manure, and 3 on the 3.25% treated manure; thus, no statistical analysis was performed.

4.4 DISCUSSION

This research examined the effectiveness of five NOS (acetic acid, boric acid, citric acid, hydrated lime, and diatomaceous earth) as alternatives to conventional insecticides for house fly control in duck barns. Two of the substances tested (boric acid and acetic acid) showed promising results in their potential to decrease *M. domestica*
populations in duck manure. These two NOS could potentially be implemented, and used to control existing fly infestations, as well as prevent future ones from establishing.

Considered relatively safe with low toxicity to mammals, boric acid has previously been used in granular and sugar baits to control house fly populations in poultry and swine facilities (Hogsette and Koehler, 1994; Balme et al., 2013). Boric acid must be ingested by house flies to cause death; it acts a stomach poison by causing dehydration and starvation, and inhibiting the insect’s ability to absorb nutrients (Hogsette et al., 2002). Boric acid also has a rough, granular composition that can cause damage to an insect’s exoskeleton. Larvae will crawl up to 15 m to a drier, cooler place to transform into pupae (Keilding, 1986); therefore, the direct application of boric acid to duck manure has potential as a fly control method as well, because crawling larvae might come in contact with the product if present in the manure. Both concentrations of boric acid tested (1.9 and 4.7%) significantly reduced adult fly emergence; the 4.7% treatment reduced adult fly emergence by 100%. Because boric acid was the most effective NOS at controlling house fly emergence, further dose response testing was performed to identify a more economically sensible (i.e., lower) dose that could be applied in the barn. The doses selected for the two products that underwent further dosage testing (boric and acetic acid), were almost similar for practical reasons and based on the previous two doses tested. A lower dose was selected for boric acid based on higher efficacy. All seven concentrations of boric acid significantly reduced adult house fly emergence (ranging from 0 to 5.3% emergence). These results indicate that boric acid added to duck manure at a concentration as low as 0.25% might be effective at reducing the number of
house flies emerging from manure in a barn setting, creating a more financially-friendly, practical solution for producers. To determine a more precise LD$_{50}$ for the flies, lower doses (< 0.25 %) of boric acid should have been selected and tested. Notwithstanding, although boric acid significantly reduced adult fly emergence, the adult flies were not repelled from landing on any of the boric acid treated manure.

Acetic acid treatments of 1.9 and 4.7% significantly reduced adult fly emergence. The mechanism by which acetic acid controls house fly development and emergence in manure is not fully understood; however, it is possible that the effect is due to its low pH. Manure treated with acetic acid had lower pH values on days 1, 5, and 10 than most of the other NOS tested. Although the manure tended toward neutral over the test period, its initial low pH (4.43 for the lower concentration and 4.11 for the higher concentration) might have been responsible for the significant reduction in emergence of adults.

Qain et al. (2013) examined a commercial vinegar product (ChinKiang®) and found it to be highly attractive to adult house flies. Further, the researchers found that a combination of seven volatile compounds (acetic acid, furfural, butanoic acid, isovaleric acid, hexanoic acid, 2-phenylethanol, and p-cresol) elicited significant responses from the antennae of female and male house flies; however, when tested alone, each compound (including acetic acid) did not attract house flies (Qain et al., 2013). In our dose-response study, only the highest concentration of acetic acid (7.0%) repelled flies from landing on the manure. However, it is difficult to draw conclusions from the
experiment because the number of adults landing on manure samples was low for all of the landing preference studies conducted in the lab, and few to no eggs were found on any of the tested manure samples. Conducting the experiments earlier in the day (i.e., when flies are more active) instead of the afternoon would may generated higher landing and oviposition numbers (World Health Organization, 1991). The light intensity or room temperature might also have influenced the flies’ behaviour; individual variables can be manipulated in future studies to identify any impact on landing preference and egg-laying. Continued research could also include a larger female population size and/or a longer observation period, as it has been shown that semiochemicals released by previous feeding of ovipositing flies can elicit egg-laying (Brodie, 2014).

In our study, hydrated lime added to duck manure at the concentrations tested (1.9 or 4.7%) did not significantly reduce adult house fly emergence or repel flies from landing compared to untreated manure. In contrast, Calvo et al. (2010) applied hydrated lime to calf bedding at 17.7 and 26.5 g/0.05 m² and found that house fly larval survival were reduced by 99 and 100%, respectively. Perhaps the differences in house fly control between studies could be attributed to the different components of the manure (feces plus wood shavings for the duck manure vs. feces plus straw bedding for the calf manure), or the concentration of hydrated lime applied to the two different types of manure. Laboratory and barn studies should study the application of higher concentrations of hydrated lime to duck manure to mitigate house fly populations.
In our study, diatomaceous earth added to duck manure at the concentrations tested (1.9 or 4.7%) did not significantly reduce adult house fly emergence or repel flies from landing compared to untreated manure. In contrast, Korunić and Mackay (2000) reduced populations of the rice weevil (*Sitophilus oryzae* (L.)), the red flour beetle (*Tribolium castaneum* (Herbst)), and the lesser grain borer (*Rhyzopertha dominica* (Fabricius)) by 98 to 100%, when diatomaceous earth was applied to hard red spring wheat (*Triticum aestivum* (L.)). It is likely that the moisture content of the manure used in our experiments (i.e., adjusted to 75%) was too wet to allow the diatomaceous earth to express its full insecticidal potential, as house fly larvae seek drier portions of the bedding material (e.g. near the surface and edges of manure) to pupate (Axtell and Arrends, 1990). Further research on diatomaceous earth may be conducted to determine if it is more effective at controlling house flies at the pupal stage, or when the larvae are crawling to drier portions of the litter to pupate. However, the other NOS investigated had higher rates of success at reducing adult emergence and therefore might have more potential than diatomaceous earth at reducing fly populations.

Abd El-Baset El-kady et al. (2010) prepared soluble citric acid powder using sodium dodecyl sulphate (SDS) as a wetting agent to decrease cow pea aphid (*Aphis craccivora*) populations. Citric acid showed effectiveness against all tested stages for this major pest of leguminous crops. In our research, citric acid applied to duck manure at 1.9 or 4.7% did not reduce adult emergence or repel flies from landing on the manure. Higher doses of citric acid might provide some control, although further high dosage testing would need to assess its effectiveness and economical value. Similar to
diatomaceous earth, the moisture content of the duck manure used in our experiments might have been too high to allow citric acid to function at its full insecticidal potential.

In the landing preference trial, the rearing media (RM) was identified as being the most attractive to adult house flies, as more flies landed on this substrate compared to any of the other NOS treated manure. The RM is composed of all organic substances (water, blood meal, powdered milk, and wheat bran) and active yeasts, making it very attractive for house fly reproduction as it provides a good source of nutrients (proteins, carbohydrates, and fats) and microorganisms for developing larvae to feed on. Fly landing numbers were low for the landing preference study; however, flies were more attracted to the RM (possibly due to the strong-smelling volatiles) than any of the other manure treatments. This experiment should be repeated without the rearing media, as it may draw most of the flies to it.

The application of acetic or boric acid to duck manure to control house fly populations appears promising. Most conventional insecticides for house fly mitigation must be sprayed when no birds are present in the barn. Acetic or boric acid could potentially be sprinkled on manure while the birds are still in the barn, helping to control fly populations during the grow-out period, instead of post-shipping. Muscovy ducks (Cairina moschata L.) are known to feed readily on fly pupae and have been identified as effective predators of M. domestica, and are sometimes used in dairy barns as a biological fly control agent (Glofcheskie and Surgeoner, 1990). The LD<sub>50</sub> of each NOS for small mammals is listed in Table 4.3; research is needed to ensure that
poultry products originating from birds reared on manure treated with NOS do not contain residues. Because the experiments conducted in this research were laboratory-based, further research needs to be conducted in a barn environment to assess effectiveness in a production situation. A commercial product (Farm and Ranch Darkling Beetle Dust® (Agrium Advanced Technologies RP Inc.)) containing 98 % boric acid is already available for the control of darkling beetles in poultry productions.

The practicality and cost of acetic or boric acid applications also need to be assessed before they can be used in the field. The cost of applying acetic or boric acid to the entire surface of a commercial duck barn at the concentrations and method of application used in this study would be prohibitive and impractical. Instead of sprinkling or spraying the entire surface of the manure with one of these NOS, producers could treat only the areas of manure with higher moisture content (i.e., underneath the water lines) (Chapter 3) where flies are known to reproduce. Treating a smaller area and using a lower concentration of acetic or boric acid will use a lower quantity of the NOS, creating a more economically-feasible solution for producers. The application of either acetic or boric acid should also be used in tandem with other fly control methods, to contribute to an overall successful integrated pest management (IPM) program in duck production facilities.
CHAPTER 5: Conclusions and recommendations

Musca domestica L., the house fly, is described as the most abundant and pestiferous fly species in poultry facilities globally, creating both nuisance and health concerns for poultry, barn workers and residential areas in proximity to poultry facilities (Axtell, 1990; Axtell and Arrends, 1990). Poultry manure provides an ideal growth substrate for the house fly because it is typically warm and moist making it very attractive to adults (Axtell 1986; Kaufman et al., 2000). Fly populations can readily establish and increase rapidly within poultry facilities (Axtell, 1990; Mwamburi et al., 2011).

The objectives of this study were to determine whether duck manure is a suitable reproductive medium for the house fly, evaluate the impact of moisture content of duck manure on house fly reproduction, and verify the effectiveness of various naturally-occurring substances (i.e., acetic acid, boric acid, citric acid, hydrated lime and diatomaceous earth) in controlling house fly life stages.

All poultry manures appear to be excellent substrates for M. domestica, as between 90-98 % of neonate larvae placed on duck, layer, turkey and broiler manure emerged as adults in laboratory bioassays. Although low oviposition and low numbers of larvae were observed in duck manure, high emergence rates from all poultry manures indicate these substrates provide an attractive environment for house fly reproduction. Therefore, it is essential to implement management techniques that render the manure less suitable in an effort to reduce existing fly infestations in poultry facilities, and prevent future populations from rapidly proliferating.
Moisture management can play a key role in house fly control. Adult emergence percentages were highest in duck manure with moisture contents of 65-85%; producers should attempt to keep manure moisture at a level that is sub-optimal for house fly reproduction in poultry facilities. My research identified lower adult emergence rates in duck manure of 40-55%. Aiming to keep manure drier can be accomplished using various cultural control methods such as identifying and removing fly breeding “hot spots” in manure (i.e., areas of higher moisture content), and the elimination of leaky water nipples which can potentially contribute to a higher manure moisture content. The number of flies landing was not affected by manure moisture content. However, results were inconclusive with the oviposition preference since oviposition was very low.

Low numbers of adults landing on the different manure treatments in the landing preference bioassay was due in part to the low number (50) of adults in the testing cage and the short period of time they were in the cage. Only a few to no eggs were observed on the tested manures. Further research on oviposition should include a larger female population size, a longer observation period, and conditions more conducive to oviposition. Perhaps if this research was conducted in a barn (i.e., a non-controlled environment), more flies may have been attracted to the manure treatments.

Acetic acid (AA) and boric acid (BA) added to duck manure at 1.9 and 4.7%, respectively, greatly reduced adult fly emergence. However, flies were not repelled from landing on the manure. The insecticide action of AA may be due to its lower pH, while
BA is a stomach poison to adult house flies (Hogsette et al., 2002) and will destroy the water balance of the feeding insects. Applying a lower concentration of AA or BA is more cost effective for a producer. Further analysis of seven concentrations of AA (0.5, 1.0, 2.0, 3.25, 4.5, 5.75, 7.0 %) and BA (0.25, 0.50, 1.0, 1.5, 2.0, 3.25 and 4.5 %) was performed to determine an LC₅₀ and LC₉₀. A significant reduction in adult emergence was observed from all treatments tested. However, the LC₉₀ for AA is much higher than for BA (10.03 and 0.12 respectively), meaning a larger quantity of AA needs to be applied to the manure to achieve the same results as BA. The LC₅₀ for BA was estimated to be 0.01%, lower than for AA (1.51%). The cost of AA ($6.50/L – 99.7% pure; FisherScientific) is about 1/10th of the cost of BA ($61.50/kg – 99.9% pure; FisherScientific) but the effectiveness of BA was found to be significantly better than AA, thus likely more economically feasible. Performing a full economic assessment is difficult, due to the different management strategies implemented into the barns, as well as differences observed between barn structures. Although the effectiveness of boric acid was proven as a result of my research, a formulated product must be developed and registered for fly control before boric acid is commercially available. BorActin® (Rockwell Labs Ltd.) is an insecticidal powder used to control a wide variety of crawling insects (i.e., different cockroach and ant spp.), however it has not been approved for the control of *M. domestica* or any other pest fly species.

The practicality of using AA or BA applications should be further assessed in an effort to reduce fly populations in these facilities by outlining the most economically sensible method. The AA used in this study was a 99% laboratory grade liquid formulation.
Common household vinegar (primarily AA and water) is more easily accessible to producers, however its concentration is typically only ~5%. A much larger quantity would need to be applied to the manure to achieve the required concentrations in manure and the results observed in the laboratory. Since it is also a liquid, and not a powder like the other NOS tested, the risk of increasing the moisture content of manure is also a concern. As it would be used as an insecticide, it also needs to be registered by the Pest Management Regulatory Agency (PMRA) before it would be available for use in Canadian agricultural settings.

Instead of applying AA or BA to the entire surface of the manure, further research should identify methods to easily identify house fly breeding “hot spots” within duck production facilities (i.e., under water lines where moisture content is the highest) where NOS could be applied. Research on the method of application should also be conducted to assess the most effective, simple and economically-feasible control technique. The powder could be dispersed on the manure surface and then mixed into the manure, or combined with water in a backpack sprayer. Although NOS are relatively safe with low toxicity to mammalian species, the placement of more bedding (i.e., wood chips) on top of the NOS-treated manure could provide protection for the birds’ feet as these products could be corrosive.

Adult house fly preference for organic substrates in a barn bioassay determined that the rearing media (RM) was most attractive. Rearing media is composed the organic substances - water, blood meal, powdered milk, brewer's yeast and wheat bran -
making it very attractive for house fly reproduction as it provides a good source of nutrients (proteins, carbohydrates and fats) for developing larvae. The RM was also preferred by house flies in the laboratory bioassay, as significantly more flies landed on it compared to other NOS-treated manures. Molasses was also significantly attractive for adults. Both RM and molasses have the potential to be incorporated into physical (i.e., a lure-kill trapping mechanism) or chemical (i.e., combined with a spinosyn or neonicotinoid) control methods, to mitigate house fly populations in poultry facilities. Rearing media does not store well, and dries out in poultry facilities making it less attractive to house flies and limiting its commercial use. Molasses has a longer shelf-life and is readily available; it could potentially be incorporated into traps indoors and at residential areas, as it has a much more pleasant odour than many of the commercially available baits (Quinn et al., 2007).

Many of the results discussed were generated in laboratory bioassays and need to be verified in a field/barn situation. Manure moisture was adjusted to 75 % in laboratory bioassays, however the manure moisture content is not consistent throughout a production facility (i.e., higher % moisture under waterlines, lower % moisture under feedlines). It is expected that more flies would be produced in manure in wetter parts of the production facility. The effectiveness of BA and AA will likely be affected depending on the moisture content of bedding associated with the manure. In my research, the addition of circulating fans to barn floors did not significantly impact the moisture content of manure.
Since producers implement different management practices (i.e., type/amount of bedding) at their facilities, manure samples should be collected from multiple barn locations to generate representative samples of typical manure characteristics in each type of poultry production. Duck manure was primarily used for this research, as little was known about its suitability for house fly reproduction. However, knowledge gained from this study could easily be transferred to other poultry commodities, providing innovative methods of house fly control for producers raising other types of birds.

Targeting adult house flies will not eliminate a fly infestation; the removal or modification of attractive reproductive sites in a production facility will limit the source of adult flies from developing and establishing, contributing to an overall successful fly management program. This research provides evidence that moisture management and naturally-occurring additives to manure could be used to decrease house fly numbers in poultry barns; however, results need to be verified in a field setting.
Table 2.1 Adult house fly landing preference on non-fed-on & fed-on duck manure.

<table>
<thead>
<tr>
<th>Type of Manure</th>
<th>Time</th>
<th>Avg. # of flies landing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Non-fed-on</td>
<td>2.0 ± 0.8</td>
<td>3.6 ± 0.8</td>
</tr>
<tr>
<td>Fed-on</td>
<td>3.6 ± 0.8</td>
<td>3.2 ± 0.8</td>
</tr>
<tr>
<td>p-value</td>
<td>P=0.207 3</td>
<td>P=0.734 0</td>
</tr>
</tbody>
</table>

Data analyzed using a repeated measures ANOVA followed by Tukey’s test (p < 0.05).
Table 2.2 Nutrient content of different types of poultry manure.

<table>
<thead>
<tr>
<th>Type of Manure</th>
<th>Dry Matter (%)</th>
<th>K (% wet)</th>
<th>P (% wet)</th>
<th>Total Kjeldahl N (% wet)</th>
<th>Ammonium-N (mg/kg wet)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscovy Duck</td>
<td>34.9</td>
<td>0.65</td>
<td>0.29</td>
<td>1.05</td>
<td>2,760</td>
<td>9.15</td>
</tr>
<tr>
<td>Mule Duck</td>
<td>45.5</td>
<td>0.85</td>
<td>0.90</td>
<td>1.16</td>
<td>1,910</td>
<td>9.06</td>
</tr>
<tr>
<td>Turkey</td>
<td>55.7</td>
<td>1.35</td>
<td>0.73</td>
<td>4.69</td>
<td>16,300</td>
<td>8.00</td>
</tr>
<tr>
<td>Layer Chicken</td>
<td>30.6</td>
<td>0.67</td>
<td>0.49</td>
<td>2.67</td>
<td>5,270</td>
<td>8.54</td>
</tr>
<tr>
<td>Broiler Chicken</td>
<td>29.5</td>
<td>0.90</td>
<td>0.61</td>
<td>1.39</td>
<td>3,030</td>
<td>9.01</td>
</tr>
</tbody>
</table>
### Table 4.1 Measured pH and physical characteristics of NOS

<table>
<thead>
<tr>
<th>Product</th>
<th>State</th>
<th>Measured pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic Acid ($C_2H_4O_2$)</td>
<td>Liquid</td>
<td>1.52</td>
</tr>
<tr>
<td>Boric Acid ($H_3BO_3$)</td>
<td>Solid Powder</td>
<td>3.18</td>
</tr>
<tr>
<td>Citric Acid ($C_6H_8O_7$)</td>
<td>Solid Powder</td>
<td>1.89</td>
</tr>
<tr>
<td>Hydrated Lime ($Ca(OH)_2$)</td>
<td>Solid Powder</td>
<td>12.15</td>
</tr>
<tr>
<td>Diatomaceous Earth ($SiO_2$)</td>
<td>Solid Powder</td>
<td>8.80</td>
</tr>
</tbody>
</table>
Table 4.2 Relative toxicity estimates of acetic acid and boric acid, added to manure, against the house fly.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chi-square</th>
<th>Slope</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>95% FL</th>
<th>LC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>95% FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>7.24</td>
<td>1.51</td>
<td>1.42a</td>
<td>1.09 - 1.75</td>
<td>10.03a</td>
<td>7.39 - 15.60</td>
</tr>
<tr>
<td>Boric acid</td>
<td>4.03</td>
<td>1.01</td>
<td>0.01b</td>
<td>6.50&lt;sup&gt;a9&lt;/sup&gt; - 0.05</td>
<td>0.12b</td>
<td>6.62&lt;sup&gt;a4&lt;/sup&gt; - 0.28</td>
</tr>
</tbody>
</table>

Values followed by the same letters are not significantly different as determined by overlap of 95% fiducial limits. LC lethal concentration, FL fiducial limits.
Table 4.3 LD$_{50}$ of the naturally-occurring substances acetic acid, boric acid, citric acid, hydrated lime, and diatomaceous earth.

<table>
<thead>
<tr>
<th>Product</th>
<th>Toxicity to Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic Acid (C$_2$H$_4$O$_2$)</td>
<td>Acute oral toxicity (LD$_{50}$): 3310 mg/kg [Rat]</td>
</tr>
<tr>
<td></td>
<td>Acute dermal toxicity (LD$_{50}$): 1060 mg/kg [Rabbit]</td>
</tr>
<tr>
<td>Boric Acid (H$_3$BO$_3$)</td>
<td>Acute oral toxicity (LD$_{50}$): 2660 mg/kg [Rat]</td>
</tr>
<tr>
<td>Citric Acid (C$_6$H$_8$O$_7$)</td>
<td>Acute oral toxicity (LD$_{50}$): 5040 mg/kg [Mouse]</td>
</tr>
<tr>
<td></td>
<td>Acute oral toxicity (LD$_{50}$): 3000 mg/kg [Rat]</td>
</tr>
<tr>
<td>Hydrated Lime (Ca(OH)$_2$)</td>
<td>Acute oral toxicity (LD$_{50}$): 7300 mg/kg [Mouse]</td>
</tr>
<tr>
<td>Diatomaceous Earth (Na$_2$CO$_3$)</td>
<td>LD$_{50}$: n/a</td>
</tr>
</tbody>
</table>

Source: www.sciencelab.com/msds
Figure 1.1 Livestock cash receipts in Canada (Agriculture and Agri-Food Canada, 2012).
Figure 1.2 The provincial distribution of duck farms in Canada (Statistics Canada, 2006).
Figure 1.3 An adult house fly (*Musca domestica* L.). (Source: www.flycontrol.novartis.com).
Figure 1.4 Life cycle of the house fly - *Musca domestica*. Source: Eggs - www.arkive.org; Larvae – www.arkive.org; Pupae - www.mantiskingdom.com; Adult- www.nature.ca
Figure 1.5 – Parasitoid wasps depositing eggs in fly pupae. (Source: www.buglogical.com)
Figure 2.1 Adult house fly emergence of larvae reared in different types of poultry manure. Manure types with the same letter are not significantly different. An ANOVA followed by a Tukey’s test was used at $\alpha = 0.05$ ($n = 4$ replicates).
Figure 2.2 Adult house fly landing preference on different types of poultry manure. Manure types with the same letter are not significantly different. An ANOVA followed by a Tukey's test was used at α = 0.05 (n = 4 replicates).
Figure 2.3 Adult house fly landing preference on different substrates placed in a duck barn. Substrates with the same letter are not significantly different. An ANOVA followed by Tukey's test was used at $\alpha = 0.05$ (n = 3 replicates).
Figure 3.1 Litter moisture content in duck barns with and without circulating fans and at three different locations. Treatments with the same letter within the same observation time are not significantly different, using a repeated measures ANOVA followed by a Tukey’s test at $\alpha = 0.05$ (n = 3 replicates).
Figure 3.2 Adult house fly emergence from duck manure of different moisture contents. Data with the same letter are not significantly different using an ANOVA followed by Tukey’s test at $\alpha = 0.05$ ($n = 3$ replicates).
Figure 3.3 Adult house fly landing preference on duck manure of different moisture contents. Substrates with the same letter are not significantly different. An ANOVA followed by Tukey’s test was used at $\alpha = 0.05$ ($n = 3$ replicates).
Figure 4.1 Mean pH of duck manure treated with two concentrations of the naturally-occurring substances acetic acid, boric acid, citric acid, hydrated lime, or diatomaceous earth (DE) on days 1, 5, and 10 after treatment. Data with the same letter within the same observation period are not significantly different using a repeated measures ANOVA followed by Tukey’s test at $p < 0.05$ (n = 3 replicates).
Figure 4.2 Mean pH of duck manure treated with different concentrations of acetic acid on days 1, 5, and 10 after treatment. Data with the same letter within the same observation period are not significantly different using a repeated measures ANOVA followed by Tukey's test at p < 0.05 (n = 3 replicates).
Figure 4.3 Mean pH of duck manure treated with different concentrations of boric acid on days 1, 5, and 10 after treatment. Data with the same letter within the same observation period are not significantly different using a repeated measures ANOVA followed by Tukey’s test at p < 0.05 (n = 3 replicates).
Figure 4.4 Adult house fly emergence from larvae reared in duck manure treated with one of two concentrations of the naturally-occurring substances acetic acid (AA), boric acid (BA), citric acid (CA), hydrated lime (HL), or diatomaceous earth (DE). Data with the same letter are not significantly different using an ANOVA followed by Tukey’s test at p < 0.05 (n = 4 replicates).
Figure 4.5 Adult house fly emergence from larvae reared in duck manure treated with different concentrations of acetic acid (n = 3 replicates).
Figure 4.6 Adult house fly emergence of larvae reared in duck manure treated with different concentrations of boric acid (n = 3 replicates).
Figure 4.7 Adult house fly landing preference on duck manure treated with one of two concentrations of the naturally-occurring substances acetic acid (AA), boric acid (BA), citric acid (CA), hydrated lime (HL), or diatomaceous earth (DE). Data with the same letter are not significantly different using an ANOVA followed by Tukey’s test at p < 0.05 (n = 4 replicates).
Figure 4.8 Adult house fly landing preference on duck manure treated different concentrations of acetic acid. Data with the same letter are not significantly different using an ANOVA followed by Tukey’s test at p < 0.05 (n = 3 replicates).
Figure 4.9 Adult house fly landing preference on duck manure treated with different concentrations of boric acid. Data with the same letter are not significantly different using an ANOVA followed by Tukey’s test at $p < 0.05$ (n = 3 replicates).
References


