Compatibility of biological and cultural control in dairy heifer group housing

A Master's Thesis
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

Jessica L. Starcevich

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Dr. Roger D. Moon

January 2016

Acknowledgements

Advisory committee
Dr. Roger D. Moon
Dr. William D. Hutchison
Dr. Hugh Chester-Jones

Useful discussions
Dr. Bill Clymer
Dr. Tom Halbach
Dr. Rebecca Merica
Dr. Russell Bey

Assistance
Joelle Olson
Stacy Reyna
Jodi Browning
Katie Flodquist
Kyle Kuehlwein
Benjamin Tutt

Organic Valley
Doran Holm
Dan Pearson
Matt Grimm
Rich Holman
John and Lisa Zweber
Larry and Ruth Coffing
Jeff and Pam Riesgraf

Table of Contents

List of Tables

Chapter 2

Chapter 3

List of Figures

Chapter 2

Chapter 3

Chapter I: Biology of filth flies (*Stomoxys calcitrans* (L.) and *Musca domestica* (L.)) and pest management through cultural and biological control on confinement dairies: A review of the literature

Introduction

Stable flies (*Stomoxys calcitrans* (L.)) and house flies (*Musca domestica* (L.)) are serious pests of the dairy industry. Stable flies bite cattle, which can reduce feed efficiency, weight gain, and milk production leading to economic loss for dairymen (Freeborn et. al 1925, Bruce and Decker 1947, Cheng and Kesler 1961, Wieman et. al 1992, Taylor et. al 2012). House flies are a nuisance to cattle and workers and can potentially spread pathogenic bacteria to both humans and animals (Lissant-Cox et. al 1912, Sanders 1940, Lindsay and Scudder 1959, Graczyk et. al 1999, De Jesus et. al 2004, Förster et. al 2009, Talley et. al 2009, Wasala et. al 2013).

Filth flies can also cause dairymen trouble when they become a nuisance to workers and neighbors. Urban encroachment is putting suburban neighborhoods closer to large livestock operations; a dairy that is producing large numbers of flies can quickly become a point of controversy and lawsuits due to flies being both a nuisance and a potential health hazard.

Dairy husbandry

Most conventional dairies breed their cattle year round to ensure they always have lactating cows producing milk. These cows are usually kept in confined barns with individual beds made out of a variety of materials including, sand, straw, recycled manure, matts, etc. These beds are generally the length of the cow, so most urine and feces is dropped into an isle that gets scraped with a tractor or flushed with water on a regular basis. This waste ends up in a storage lagoon that will sometimes develop a thick crust on top. Lagoons are generally only emptied once a year to be spread on fields.

Once a cow's milk begins to dry up, she is moved to another location for 'dry cows' where she will finish out her current pregnancy or become pregnant. Once a calf is born, it is typically removed from the mother within 2 days and hand reared and the lactating cow is moved back into the main milk barn.

The calves are then moved to individual pens for about 60-90 days where they are kept on bedding to keep them warm and dry. Bedding materials often include straw and stover, but may also include materials such as sawdust, sand, rice hulls, etc. What type of bedding is used depends on cost and availability. Calf bedding often gets formed into packs, where fresh bedding is added on top of older soiled bedding to keep calves healthy, then periodically the whole pack is removed.

Later, the older calves are then grouped together in group pens of varying sizes where they are started on grain and hay. Most of these group calves still have a bedding pack on which to lay. Once heifers (female calves that have not yet had their own calf) are old enough to breed, they are usually moved to a pasture or to the dry cow barn.

Why flies are a problem on dairies

Filth flies create problems on dairies by biting and bothering cows which causes the animals to bunch, not eat, and expend extra energy. These actions result in cattle that are not converting feed efficiently, which leads to reductions in weight gain and milk production (Freeborn et. al 1925, Bruce and Decker 1947, Cheng and Kesler 1961, Wieman et. al 1992, Taylor et. al 2012). Flies can also spread pathogenic bacteria and fungi that can contaminate milk as well as human and animal food (Lissant-Cox et. al 1912, Sanders 1940, Lindsay and Scudder 1959, Graczyk et. al 1999, De Jesus et. al 2004, Förster et. al 2009, Talley et. al 2009, Wasala et. al 2013).

Filth fly biology

In northern temperate locations, filth flies become abundant at dairies during the summer, or when temperatures exceed 15°C. These flies develop through a complete life cycle. Females lay eggs in moist decaying organic matter, eggs hatch into larvae that will feed on the bacteria in the substrate, larvae move to drier areas to pupate, and adult flies eventually emerge. Egg to adult development can be less than 2 weeks, depending on temperature (Barnard & Geden 1993).

Current fly control methods

To help control fly populations, farmers commonly use sanitation, traps, feed through larvacides, adult insecticides, and pteromalid pupal parasitoids for biological control (Lazarus et. al 1989, Geden et. al 1992, Miller et. al 1993, Skovgard and Nachman 2004).

Mechanical control

Fly traps are commonly used on dairies and come in a few varieties. Some traps have a sticky glue that catches and holds flies that land on its surface, while other traps have an odor attractant that draws flies in and drowns them in water. These types of traps do not include any insecticides and are generally safe for use on organic and conventional farms.

Chemical control

Another option for conventional dairies is to use chemical control. Chemical controls include insecticidal baits, fogs, premise sprays, and feed though larvacides. Pyrethroids, pyrethrins, carbamates, organophosphates, neonicotinoids, and insect growth regulators are commonly used.

Cultural control

Sanitation. Cultural control of flies can include sanitation, conservation of beneficial predators and parasitoids, and the choice of animal bedding. Stable flies and house flies develop in moist decaying organic material such as soiled animal bedding and spilled

grain, and will readily colonize soiled calf bedding on dairy farms (Schmidtmann 1988, Stafford 2008). Because we know where flies develop, and because they require a minimum amount of time to develop, routine sanitation practices can vastly reduce fly populations. Cleaning up spilled feed, removing soiled bedding regularly, and cleaning up manure missed by scrapers and flush systems will reduce the amount of material available to breed flies. Feed, manure, and soiled bedding that have been cleaned up are often spread thinly on fields, stock piled on concrete or dirt pads, composted, or dumped into storage lagoons.

Conservation of beneficial parasitic wasps. Many flies are killed by beneficial organisms such as predatory mites and insects as well as parasitic wasps. Often, these beneficials are more susceptible to insecticides than the pest flies (Scott et. al 1988 and Geden et. al 1992), so it is important to use insecticides conservatively and only in areas where beneficials are unlikely to come into contact with the insecticide. Farmers can also find areas that support large numbers of beneficials but do not breed large numbers of flies, then conserve these areas to allow for the proliferation of beneficials.

Possible effects of bedding choice on filth flies. Bedding choice can also have a large impact on fly production on dairies. It is common practice on dairies to build bedding packs, particularly around calves. These bedding packs provide an ideal environment for fly breeding. Surveys of dairies have shown that soiled bedding in calf hutches is a primary source of filth fly populations (Meyer and Petersen 1983, Smith and Rutz 1991,

Olbrich 2003), which suggests that proper choice of calf bedding materials may reduce filth fly production, and thereby reduce overall fly populations on dairy farms. The following review summarizes previous research on alternative bedding materials and ways they could affect filth flies around confinement animal facilities. Cultural control through bedding management may also be an effective way to control filth fly populations on dairy farms.

Physical and chemical properties of bedding materials. A variety of materials may be used for animal bedding, including small grain straw, wood chips, wood shavings, sawdust, sand, ground corn cobs, and corn and bean stover. These materials vary in heat capacity, moisture content, bulk density, pH, and microbial communities, all of which may affect numbers of filth flies emerging from soiled bedding. Percent moisture increases more rapidly in straw than in wood shavings, requiring greater amounts of straw to maintain the same level of animal cleanliness. Ward et. al (2000) and Ward et. al (2001) found that over a 5 day period, the percent moisture of straw increased 5.5 fold as compared to a 4.5 fold increase in percent moisture of wood shavings. Although temperatures, moisture contents, and bulk densities of bedding materials are variable, after being soiled by urine and feces, pH values were basic (between 8 and 9) and varied little between bedding materials (Godden et. al 2008, Ward et. al 2001).

Physical properties vary among materials available for bedding calves. These differences may affect filth flies directly by affecting their growth rates, or indirectly by affecting

food abundance or efficacy of the flies' natural enemies, but these aspects of different bedding materials remain to be studied fully.

Microbial properties of bedding materials and possible effects on filth fly production

Microbial communities in different bedding materials vary in abundance. These differences may be due to differences in temperature, moisture, nutrient composition, and antimicrobial properties of the materials. Bedding material made from soft woods such as pines and firs are high in terpenes which have proven to be antimicrobial (Himejima et. al 1992, Andrews et. al 1980, Lindberg et. al 2004). Different types of bedding materials, once soiled with urine and feces, develop higher overall bacterial counts than other materials. The following materials are listed in descending order of bacterial counts: straw, ground corncobs, hardwood chips, softwood sawdust, and sand (Zehner et. al 1986, Hogan et. al 1990, Godden et. al 2008, LeJeune and Kauffman 2005).

Soft woods contain antimicrobial terpenes which can affect filth fly oviposition and development. Maganga et al. (1996) identified four terpenes (myrcene, ρ-cymene, γ-terpinene, and linalool) from pine oil via gas chromatography and tested them to determine their effect on feeding and oviposition by female house flies. Linalool deterred both feeding and oviposition. Flies laid an average of only 368 eggs on linalool treated cotton wicks as compared to 2,792 on untreated wicks. These experiments suggest that active microbial communities are essential to stimulate oviposition in gravid female filth flies and that some substrates that would otherwise invite oviposition may become

undesirable due to the chemical properties of certain bedding materials. Differences in microbial activity in different bedding materials may affect oviposition and thus affect overall fly production in the bedding.

Also, filth fly larvae require bacteria for successful development and different bedding materials support varying species and quantities of bacteria. Choice of bedding material may therefore affect the nutritional suitability for filth fly larvae, making choice of bedding material a possible method for filth fly control or a source of filth fly production.

Ovipositional preferences of female filth flies. Female filth flies will oviposit in some substrates while refusing to do so in others based on the nutritional quality of the substrate for the larvae. The ability to cue in on substrates that will support larval growth allows filth flies to maximize the survival of their offspring. Romero et. al (2006) assessed the importance of microbial communities and specific bacteria in oviposition by gravid female stable flies. A variety of field collected substrates were presented in choice tests to gravid females, with one choice being autoclaved and the other not autoclaved. More than 95% of eggs were oviposited on the non-sterile, microbially active substrate. This finding suggests that gravid females are able to detect and choose to oviposit in substrates with active microbial communities. Romero et. al (2006) also isolated bacteria from field substrates and offered them in choice tests to determine if females showed a preference for different kinds of bacteria. Of nine isolates, all but two attracted greater oviposition than a sterilized control.

Nutritional requirements of larval filth flies. A holidic diet for filth fly larvae has not been defined, but it is known that filth fly larvae are able to digest and require bacteria to complete their development (Lysyk et. al 1999). Levinson (1960) and Watson et al. (1993) determined that although larval survival was greater on live microbial communities, house fly larvae could develop in media inoculated with desiccated *E. coli*. In 2004, Rochon et. al (2004) traced *E. coli* through the guts of both house fly and stable fly larvae and found that *E. coli* concentrations decreased over time in house flies, but remained constant in stable flies. The authors also found that while house fly larvae survived well (62%) on isolated *E. coli* colonies, stable fly larvae did not survive as well (25%). Espinoza-Fuentes and Terra (1987) used dye to track consumed material through the gut of third instar house fly larvae. Dissections of the larval gut showed that pH decreased in the mid-midgut and that lysozyme and pepsin became present. This combination served to kill bacteria, which were then digested in the posterior midgut. A further study conducted by McGaughey and Nayduch (2009) tracked GFP-expressing *Aeromonas hydrophila* through the digestive track of larval house flies. The bacteria were shown to be lysed in the midgut. Live bacteria were found only in the foregut and cultures could not be obtained from feces, demonstrating that enzymes within the larval gut are capable of killing and digesting bacteria. The changes in enzymes along the larval mid-gut demonstrate that house fly larvae can digest some bacteria.

Although bacteria are essential for larval development, the specific bacteria needed are unclear. The guts of house fly larvae collected from two different breeding sites (turkey bedding and corn silage) yielded 25 bacterial species, only one of which (*Providencia rettgeri*) occurred in larvae at both sites (Zurek et. al 2000). Larvae were able to reach pupation on most of the bacterial isolates, suggesting that no specific bacteria are required; however, larvae were most successful when reared on a mix of multiple bacterial isolates (Schmidtmann and Martin 1992 Lysyk et. al 1999). Of six bacterial isolates (*Serratia fanticola, Citrobacter freundii, Enterococcus spp., Psuedomonas spp., Aeromonus spp. and S. marcescens*) extracted from a natural substrate supporting filth fly growth, larvae could complete development on four (*Serratia fanticola, Citrobacter freundii, Enterococcus spp., and Psuedomonas spp.*). Eighty four percent of larvae pupated when media contained all bacterial isolates, but fewer pupated when reared on a single isolate (Romero et. al 2006).

Field studies of different bedding substrates and their effect on filth fly production. Few field studies have examined how choice of bedding substrate affects larval densities of filth flies. In 1962, MacCreary and Haenlein compared densities of house fly larvae in peanut-hull and sawdust bedding. Concrete floored pens were cleaned and bedded with either sawdust or peanut-hulls, and then four calves were kept on the bedding. After 25 days, 1,000 in^3 of bedding was collected and the numbers of larvae, pupae, and adult flies were counted. At the time of collection, sawdust bedding was more compact and remained cooler and drier than peanut-hull bedding. As a result, peanut-hulls contained

24 times as many house flies as sawdust bedding. Later, Schmidtmann et. al (1989) and Schmidtmann (1991) compared larval densities of house flies and stable flies in calf hutches bedded for 6 weeks with sand, gravel, ground corn cobs, straw, pine shavings or wood chips. Each week a one liter bedding sample was collected and placed in Berlese-Tungren units to extract and count 2nd and 3rd instar larvae. Schmidtmann found that house fly larvae were most abundant in straw, whereas stable fly larvae were equally abundant in both straw and pine shavings. Sand and gravel produced very few larvae of either kind, but both materials compacted and soiled quickly, reducing calf cleanliness. Sawdust produced more larvae than sand and gravel, but significantly fewer than straw, and calves remained clean (Schmidtmann et. al 1989, Schmidtmann 1991, Watson et. al 1996). Of the studies conducted sawdust seemed to be the best bedding choice, because it supported fewer filth fly larvae and calves remained clean and healthy. Calves also remained clean on straw bedding, but straw also supported significantly more filth fly larvae than sawdust. These studies indicate that compared to straw, some degree of filth fly control could be achieved by the use of sawdust bedding.

Biological control

Pupal parasitoids offer another alternative for filth fly control. Pupal parasitoids consist of several species of naturally occurring wasps, some of which can also be bought commercially. These parasitoids lay eggs inside the fly puparium and the wasp larvae feed on and ultimately kill the fly.

Field studies on biological control of filth flies by pteromalid pupal parasitoids. Several studies have been conducted to evaluate the effectiveness of pteromalid pupal parasitoids for the biological control of filth flies on dairies with mixed results. In some studies, the regular release of parasitoids resulted in a significant increase in the percent parisitism of filth flies while others did not. Sustained releases of *Spalangia cameroni* were found to increase the percent parasitism of house flies and stable flies on organic and confinement dairies in Denmark (Skovgard 2004, Skovgard 2005, Skovgard 2006). However, despite large regular releases of *Nasonia vitripennis* on dairies in Manitoba, few were ever recovered (McKay & Galloway 1999). Even though an increase in parasitism was seen in other studies, no measurable effect could be found in the population of adult flies. In west-central Nebraska, combined releases of *S. nigroaenea* and *M. raptor* increased field parasitism of stable flies, but failed to reduce adult fly populations (Andress and Campbell 1994). Meyer et. al (1990) also found parasitoid releases to increase parasitism rates overall, but found no effect on adult fly populations on the farm. When releases of parasitoids were combined with an integrated pest management program, Geden et. al (1992) found that *M. raptor* did significantly reduce the number of house flies on dairies in Maryland and New York. Dairies where *M. raptor* was released found that fewer pesticide applications were needed when compared to control dairies in the same area.

Many factors may contribute to the success or failure of biological control. Commercial availability of pteromalid parasitoids is convenient but sometimes unreliable in quantities and species purity (Andress and Campbell 1994, Geden 2005). The immigration of adult

flies from untreated areas may also result in high adult fly populations despite increased parasitism in a treated area (Birkemoe et. al 2008). Adverse weather may also affect pteromalids released for biological control (Petersen et. al 1992). The quantity and form of parasitoid release may also affect the success of biological control, as Petersen and Currey (1996) found multiple releases of *M. raptorellus* to result in greater parasitism than single releases.

Possible effects of bedding choice on pupal parasitoids. Bedding materials vary in temperature and moisture, both of which should affect the growth rates and survival of both filth flies and their pupal parasitoids. Optimal temperature for rapid growth of house fly larvae and pupae is between 32°C and 38°C; however, they will survive at temperatures as low as 14°C (Sanchez-Arroyo and Capinera 2008). Barnard and Geden (1993) showed that developmental rate of house fly larvae and pupae peaked at 32°C. Although filth fly larvae prefer larval substrates in moisture ranges of 60-75%, pupae are most commonly found in drier areas where the moisture content is 50% or lower (Schmidtmann 1991, Smith and Rutz 1991). Several studies have shown that choice of bedding material will significantly affect density of filth flies (MacCreary and Haenlein 1962, Schmidtmann et. al 1989, Schmidtmann 1991). Differences in filth fly densities in different types of bedding material may be caused by ovipositional preferences of female flies, nutritional suitability for maggots, egg to adult survival, and/or efficacy of parasitoids.

Parasitoids that attack filth fly pupae demonstrate peak fecundity between 27°C and 30°C (Ables and Shepard 1976, Legner 1977, Pawson and Peterson 1990, and Geden 1996). They can generally forage in substrates with a wide range of moisture levels, with *Spalangia* spp. demonstrating the widest range; however, they are most successful at parasitizing hosts at lower moisture levels where their hosts are most commonly found (Geden 1999, Legner 1977). The range of microhabitat conditions created by different bedding materials may affect where parasitoid hosts are found, and the depth at which maggots pupate may affect parasitoid search success. *Muscidifurax* spp. have difficulty penetrating into substrates to find their hosts, especially when the substrates have a high moisture content (Legner 1977, Rueda and Axtell 1985, Geden 2002). *Spalangia* spp. however are generally able to penetrate more deeply in their search for hosts below the surface (sometimes up to 10cm) (Legner 1977, Rueda and Axtell 1985, Geden 2002).

Schmidtmann (1991) found greater densities of filth fly larvae in calf hutches bedded with straw as compared to those bedded with sand, gravel, or sawdust; therefore, bedding substrates may also affect the density of host pupae for parasitoids. Some bedding materials, such as peanut hulls and straw, reach such high host densities that it is unlikely parasitoids would be able to suppress filth fly populations (MacCreary and Haenlein 1962, Schmidtmann et. al 1989, Schmidtmann 1991). Ables and Shepard (1974) compared the functional response to host density by two parasitoid species. *Splangia endius* was able to increase the number of hosts attacked over 72 hours as host density increased from 30 to 80, indicating that the host density fox maximum attack rate for this

species exceeds 80 pupae/female wasp. *Muscidifurax raptor* also continued to attack more hosts as host density increased; however, the response curve began to level as it approached a density of 80 pupae/female wasp, indicating that the maximum attack rate occurs around 80 pupae/female wasp.

Conclusions and implications for future work

Dairy farmers and other livestock managers use a variety of natural materials as bedding substrates for their livestock. Those materials differ in plant origin, particle size, and bulk density. Consequently, bedding packs formed with those materials may differ in suitability for growth by aerobic and anaerobic bacteria. Although mechanisms are not clear, experimental evidence from field studies suggests bedding materials may also differ in their suitability for ovipositing females and developing immatures of stable flies and house flies. In particular, two studies (Schmidtmann et al. 1989, Schmidtmann 1991) demonstrated that bedding packs in individual calf hutches consistently yielded greater numbers of filth fly larvae when straw was used as a bedding substrate rather than other materials. This result suggests that more general use of alternatives to straw might be a cultural method to reduce on-farm production of filth flies. However, before this practice can be recommended, three important issues should be addressed.

Schmidtmann (1988) and Schmidtmann (1991) found that larval densities were greatest in straw in individual calf hutches during the first six weeks of occupancy. Commercial practice is to move calves from individual hutches to small group housing at about six

weeks of age, and to maintain those groups in bedded pens until the calves can be moved into larger dry lot pens or onto pasture. It is unknown if greater larval densities in straw would also occur in bedding packs occupied by weaned, older and heavier animals, and if differences would persist as bedding packs aged beyond six weeks. Furthermore, it is unknown if emergence rates of adult flies from different substrates would parallel densities of larvae in the different substrates, or if rates of pupal mortality would vary among materials. It is possible, for example, that temperatures from composting and trampling by animals could differentially kill fly pupae in one or another substrate, due to the physical differences in the parent materials. If so, then pupal mortality could magnify or cancel differences in larval densities. Most interestingly, compatibility of cultural and biological control methods should be examined directly. Different bedding materials could vary in attractiveness to searching parasitoids, and wasps in the different materials could be more or less effective at finding and killing prey pupae.

For these reasons, I conducted two studies to further develop the basis for filth fly integrated pest management (IPM) on commercial dairies. To lay a foundation, I conducted a survey of the fly and parasitoid faunas on commercial organic dairies to document habitat locations of developing filth fly species and associated parasitoids in the absence of insecticides and larvicides. My hypothesis was that calf bedding would be a principal source of house flies and stable flies, but that other drier, older substrates would be the principal habitats of *Spalangia* spp. and *Muscidifurax* spp. Knowledge of the sources of filth flies and associated parasitoids could help determine if soiled bedding

was a major sources of flies, and if choice of bedding substrate could materially affect fly numbers on the premises. The results of this survey are summarized in chapter 2 of this thesis. The second study was a 2-year field experiment to assess net fly production and parasitoid efficacy in experimental bedding packs formed from three commonly used materials: small grain straw, pine shavings, and hardwood sawdust. Results of this study are summarized in chapter 3.

References

Ables, J.R. and M. Shepard. 1976. Influence of temperature on oviposition by the parasites *Spalangia endius* and *Muscidifurax raptor*. Environ. Entomol. 5: 511-513.

Andrews, R.E., L.W. Parks, K.D. Spense. 1980. Some effects of douglas fir terpenes on certain microorganisms. Appl. Environ. Micro. 40: 301-304.

Barnard, D.R. and C.J. Geden. 1993. Influence of larval density and temperature in poultry manure on development of the house fly (Diptera: Muscidae). Environ. Entomol. 22: 971-977.

Bruce, W.N. and G.C. Decker. 1947. Fly control and milk flow. J. Econ. Entomol. 40: 530-536.

Cheng, Tien-His and E.M. Kesler. 1961. A three-year study on the effect of fly control on milk production by selected and randomized dairy herds. J. Econ. Entomol. 54: 751-757.

Coats, S. 1976. Life cycle and behavior of *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae). Ann. Entomol. Soc. Am. 69: 772-780.

De Jesus, A., A.R. Olsen, J.R. Bryce, and R.C. Whiting. 2004. Quantitative contamination and transfer of *Escherichia ecoli* from foods by house flies, *Musca domestica* L. (Diptera: Muscidae). Int. J. Food Microbiol. 93:259-262.

Espinoza-Fuentes F.P. and W.R. Terra. 1987. Physiological adaptations for digesting bacteria: water fluxes and distribution of digestive enzymes in *Musca domestica* larval midgut. Ins. Biochem. 17: 809-817.

Förster, M., K. Sievert, S. Messler, S. Klimpel, and K. Pfeffar. 2009. Comprehensive study on the occurrence and distribution of pathogenic microorganisms carried by synanthropic flies caught at different rural locations in Germany. J. Med. Entomol. 46: 1164-1166.

Freeborn, S.B., W.M. Regan, and A.H. Folger. 1925. The relation of flies and fly sprays to milk production. J. Econ. Entomol. 18: 779-791.

Geden, C.J., D.A. Rutz, R.W. Miller, and D.C. Steinkraus. 1992. Suppression of house flies (Diptera: Muscidae) on New York and Maryland dairies using releases of *Muscidifurax raptor* (Hymenoptera: Pteromalidae) in an integrated management program. Environ. Entomol. 21: 1419-1426.

Geden, C.J., D.A. Rutz, J.G. Scott, and S.J. Long. 1992. Susceptability of house flies (Dipter: Muscidae) and five pupal parasitoids (Hymenoptera: Pteromalidae) to abamectin and seven commercial insecticides. J. Econ. Entomol 85: 435-440.

Geden, C.J. 1996. Modeling host attacks and progeny production of *Spalangia gemina*, *Spalangia cameroni*, and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) at constant and variable temperatures. Bio. Contr. 7: 172-178.

Geden, C. 1999. Host location by house fly (Diptera: Muscidae) parasitoids in poultry manure at different moisture levels and host densities. Environ. Entomol. 23: 755-760.

Geden, C. 2002. Effect of habitat depth on host location by five species of parasitoids (Hymenoptera: Pteromalidae, Chalcididae) of house flies (Diptera: Muscidae) in three types of substrates. Environ. Entomol. 31: 411-417.

Gerling, D. and E. Legner. 1968. Developmental history and reproduction of *Spalangia cameroni*, parasite of synanthropic flies. Ann. Entomol. Soc. Am. 61: 1436-1443.

Godden, S., R. Bey, K. Lorch, R. Farnsworth, and P. Rapnicki. 2008. Ability of organic and inorganic bedding materials to promote growth of environmental bacteria. J. Dairy Sci. 91: 151-159.

Graczyk, T.K., M.R. Cranfield, R. Fayer, and H. Bixler. 1999. House flies (*Musca domestica*) as transport hosts of *Cryptosporidium parvum*. Am. J. Trop. Med. Hyg. 61: 500-504

Himejima, M., K.R. Hobson, T. Otsuka, D.L. Wood, I. Kubo. 1992. Antimicrobial terpenes from oleoresin of ponderosa pine tree *Pinus ponderosa*: a defense mechanism against microbial invasion. J. Chem. Ecol. 18: 1809-1818.

Hogan, J.S., K.L. Smith, D.A. Todhunter, P.S. Schoenberger. 1990. Bacterial counts associated with recycled newspaper bedding. J. Dairy Sci. 73: 1756-1761.

Hogsette, J., J. Ruff, and C. Jones. 1987. Stable fly biology and control in northwestern Florida. J. Agric. Entomol. 4: 1-11.

Lazarus, W.F., D.A. Rutz, R.W. Miller, and D.A. Brown. 1989. Costs of existing and recommended manure management practices for house fly and stable fly (Diptera: Muscidae) control on dairy farms. J. Econ. Entomol. 82: 1145-1151.

Legner, E.F. 1977. Temperature, humidity and depth of habitat influencing host destruction and fecundity of muscoid fly parasites. Entomophaga 22: 199-206.

LeJeune, J.T. and M.D. Kauffman. 2005. Effect of sand and sawdust bedding materials on the fecal prevalence of *Escherichia coli* O157:H7 in dairy cows. App. Environ. Micro. 71: 326-330.

Levinson, Z.H. 1960. Food of housefly larvae. Nature 188: 427-428.

Lindberg, L.E., S.M. Willfor, B.R. Holmbom. 2004. Antibacterial effects of knotwood extractives on paper mill bacteria. Ind. Micro. Biotech. 31: 137-147.

Lindsay, D.R. and H.I. Scudder. 1959. Nonbiting flies and disease. Ann. Rev. Entomol. 1:323-346.

Lissant Cox, G., F.C. Lewis and E.E. Glynn. 1912. The number and varieties of bacteria carried by the common house-fly in sanitary and insanitary city areas. J. Hyg. 12: 290-319.

Lysyk, T.J., L. Kalishuk-Tymensen, L.B. Selinger, R.C. Lancaster, L. Wever, K.J. Cheng. 1999. Rearing stable fly larvae (Diptera: Muscidae) on an egg yolk medium. J. Med. Entomol. 36: 382-388

MacCreary, D. and G.F.W. Haenlein. 1962. House fly breeding in oak sawdust and peanut hulls used as bedding in calf pens. J. Econ. Entomol. 55: 419.

Maganga, M.E., G. Gries, R. Gries. 1996. Repellency of various oils and pine oil constituents to house flies (Diptera: Muscidae. Environ. Entomol. 25: 1182-1187.

McGaughey, J. and D. Nayduch. 2009. Temporal and spatial fate of GFP-expressing motile and nonmotile *Aeromona hydrophila* in the house fly digestive tract. J. Med. Entomol. 46: 123-130.

Meyer, J.A. and J.J. Petersen. 1983. Characterization and seasonal distribution of breeding sites of stable flies and house flies (Diptera: Muscidae) on eastern Nebraska feedlots and dairies. J.
Econ. Entomol. 76: 103-108.

Miller, R.W., D.A. Rutz, L.G. Pickens, and C.J. Genden. 1993. Evaluation of traps and the parasitoid *Muscidifurax raptor* Girault and Sanders to manage house flies and stable flies on dairy farms. J. Agri. Entomol. 10: 9-19.

Olbrich, D.L. and B.H. King. 2003. Host and habitat use by parasitoids (Hymenoptera: Pteromalidae) of house fly and stable fly (Diptera: Muscidae) pupae. Great Lakes Entomol. 36: 179-190.

Pawson, B.M. and J.J. Petersen. 1990. Temperature preferences and effects of photoperiod on oviposition behavior of five pteromalid wasps (Hymenoptera: Pteromalidae) using house fly (Diptera: Muscidae) pupae as hosts. Environ. Entomol. 19: 1452-1456.

Pitzer, J., P. Kaufman, C. Geden, J. Hogsette. 2011. The ability of selected pupal parasitoids (Hymenoptera: Pteromalidae) to locate stable fly hosts in a soiled equine bedding substrate. Environ. Entomol. 40: 88-93.

Rochon, K., T.J. Lysyk, L.B. Selinger. 2004. Persistence of *Escherichia coli* in immature house fly and stable fly (Diptera: Muscidae) in relation to larval growth and survival. J. Med. Entomol. 41: 1082-1089.

Romero, A., A. Broce, L. Zurek. 2006. Role of bacteria in the oviposition behavior and larval development of stable flies. Med. Vet. Entomol. 20: 115-121.

Rueda, L.M. and R.C. Axtell. 1985. Effect of depth of house fly pupae in poultry manure on parasitism by six species of Pteromalidae (Hymenoptera). J. Entomol. Sci. 20: 444-449.

Sanders, D.A. 1940. *Musca domestica* a vector of bovine mastitis. J. Am. Vet. Med. Assoc. 97:120-122.

Schmidtmann, E.T. 1988. Exploitation of bedding in dairy outdoor calf hutches by immature house and stable flies (Diptera: Muscidae). J. Med. Entomol. 25: 484-488.

Schmidtmann, E.T., R.W. Miller, and R. Muller. 1989. Effect of experimental bedding treatments on the density of immature *Musca domestica* and *Stomoxys calcitrans* (Diptera: Muscidae) in outdoor calf hutches. J. Econ. Entomol. 82: 1134-1139.

Schmidtmann, E.T. 1991. Suppressing immature house and stable flies in outdoor calf hutches with sand, gravel, and sawdust bedding. J. Dairy Sci. 74: 3956-3960

Schmidtmann, E.T. and P.A.W. Martin. 1992. Relationship between selected bacteria and the growth of immature house flies, *Musca domestica*, in an axenic test system. J. Med. Entomol. 29: 232-235.

Scott, J.G., D.A. Rutz, and J. Walcott. 1988. Comparative toxicity of seven insecticides to adult *Spalangia cameroni* Perkins. J. Agric. Entomol 5: 139-145.

Skovgard, H. and G. Nachman. 2004. Biological control of house flies *Musca domestica* and stable flies *Stomoxys calcitrans* (Diptera: Muscidae) by means of inundative releases of *Spalangia cameroni* (Hymenoptera: Pteromalidae). Bull. Entomol. Res. 94: 555-567.

Smith, L. and D. Rutz. 1985. The occurrence and biology of *Urolepis rufipes* (Hymenoptera: Pteromalidae), a parasitoid of house flies in New York dairies. Environ. Entomol. 14: 365-369.

Smith, L. and D. Rutz. 1991. Relationship of microhabitat to incidence of house fly (Diptera: Muscidae) immatures and their parasitoids at dairy farms in central New York. Environ. Entomol. 20: 669-674.

Stafford III, K.C. 2008. Fly management handbook: A guide to biology, dispersal, and management of the house fly and related flies for farmers, municipalities, and public health officials. Conn. Agric. Exp. St, New Haven, CT. Bulletin 1013.

Talley, J.L., A.C. Wayadande, L.P. Wasala, A.C. Gerry, J. Flethcher, U. DeSilva, and S.E. Gilliland. 2009. Association of *Escherichia coli* O157:H7 with filth flies (Muscidae and Calliphoridae) captured in leafy greens fields and experimental transmission of E. coli O157:H7 to spinach leaves by house flies (Diptera: Muscidae). J. Food Prot. 72: 1547-52.

Ward, P.L., J.E. Wohlt, P.K. Zajac, K.R. Cooper. 2000. Chemical and physical properties of processed newpaper compared to wheat straw and wood shavings as animal bedding. J. Dairy Sci. 83: 359-367.

Ward, P.L., J.E. Wohlt, S.E. Katz. 2001. Chemical, physical, and environmental properties of pelleted newspaper compared to wheat straw and wood shavings as bedding for horses. J. Anim. Sci. 79: 1359-1369.

Wasala, L., J.L. Talley, U. DeSilva, J. Fletcher, and A. Wayadande. 2013. Transfer of *Escherichia coli* O157:H7 to spinach by house flies, *Musca domestica* (Diptera: Muscidae). Phytopathology 103: 373-380.

Wieman, G.A., J.B. Campbell, J.A. DeShazer, and I.L. Berry. 1992. Effects of stable flies (Diptera: Muscidae) and heat stress on weight gain and feed efficiency of feeder cattle. J. Econ. Entomol. 85: 1835-1842.

Chapter II: Survey of filth flies and their pupal parasitoids on six organic dairies in east-central Minnesota and west-central Wisconsin.

Abstract

A survey of six organic dairies in east-central Minnesota and west-central Wisconsin was conducted to determine what species of flies and their associated pupal parasitoids occurred on the dairies, and in which habitats they most commonly occurred. Six species of Diptera were encountered. House flies (*Musca domestica (L.)*) and stable flies (*Stomoxys calcitrans (L.)*) made up 88% of the fly specimens collected. These flies were found primarily in the soiled bedding in young calf housing and cow loafing barns. Among parasitoids, *Spalangia* spp. were the most abundant and parasitized 30% of the stable fly puparia and 52% of the house fly puparia. These results suggest that filth fly populations may be reduced by removing soiled bedding from young calf housing and cow loafing barns more frequently, and conserving and augmenting parasitoids in those areas may also help reduce fly populations.

Introduction

Filth flies such as house fly (*Musca domestica*) and stable fly (*Stomoxys calcitrans*) can spread pathogenic bacteria and reduce dairy farm profits (Lissant-Cox et. al 1912, Freeborn et. al 1925). The most important method of controlling filth flies is source reduction through breeding site sanitation.

Filth flies on dairies develop in moist decaying organic material such as soiled animal bedding and spilled grain (Schmidtmann 1988, Stafford 2008). Surveys of dairies have shown that soiled bedding from calf hutches is a primary contributor of filth fly populations (Meyer and Petersen 1983, Smith and Rutz 1991, Olbrich 2003).

It could be beneficial to know where the greatest numbers of flies are developing on a given premise, so that sanitation can be targeted to those areas. Biological control using pupal parasitoids could also help reduce filth fly populations (Geden et. al 1992, Skovgard and Nachman 2004). Olbrich (2003) found that parasitoid species vary with habitat and host species on dairy farms; therefore, areas where parasitoids are abundant can be protected to help conserve populations.

We surveyed six organic dairies in west-central WI and east-central MN in August and September 2010 to determine which species of flies and parasitoids were breeding on the farms and in which habitats they occurred. The purpose of this study is to aid dairy farmers in determining which areas to concentrate sanitation practices to reduce fly breeding, and which areas are important to conserve parasitoids for biological control.

Materials and Methods

A convenient sample of six organic dairies was surveyed in August and September 2010. Three dairies were in west-central Wisconsin and the other three were in east-central

Minnesota. This allowed us to compare farms near each other as well as farms from different states.

Each farm manager gave us a brief walking tour of their farm, after which all areas with conditions that were likely to support fly breeding were sampled (Appendix A). Each area was photographed and then later categorized by animal age, housing type, and substrate (Table 1). Fly larval media from each habitat was excavated with trowels and placed in 5 gallon buckets. Substrate was dug for 10 minutes or until approximately 500 puparia were collected. Puparia were extracted from the material by flotation and up to 500 puparia were collected and brought back to the lab to be reared. When possible at least 100 puparia from each site sampled on the farm were reared; however, some sites did not yield enough puparia. Habitats were retrospectively categorized based on parent material, age of animal housed, and age of material. Collected puparia were placed in individual wells of a 96 flat bottomed well BD Falcon assay plates (Becton, Dickinson and Company, Franklin Lakes, NJ). Puparia were incubated at 26°C for six weeks to allow adult flies and associated parasitoids to emerge. Flies, un-eclosed puparia, and parasitoids were identified to family and species when possible. Adult flies were identified using the Manual of Nearctic Diptera (McAlpine et. Al 1981). Parasitoid identification was made using Gary Gibson's Illustrated key to the native and introduced chalcidoid parasitoids of filth flies in America north of Mexico (Hymenoptera:Chalcidoidea) and voucher specimens were verified by Gary Gibson and

deposited in the University of Minnesota Insect Museum. Host puparia were identified via The Biology of the Muscidae of the World (Skidmore 1985).

Analyses were restricted to viable puparia only, defined as a puparium that yielded either an adult fly or parasitoid. Because not all habitats were present at all locations, analyses are restricted to data collected from calf housing and loafing areas. Analyses were conducted to determine how habitat and state affected species of flies, numbers of viable puparia, and numbers of parasitoids. Abundance of different fly species among different habitats on farms were compared using Friedmans; 2-way non-parametric test. Subsequent counts of individual fly species attacked by different parasitoids in different habitats were analyzed as a binomial outcome with a General Linear Model in R.

Results

Farms. All six dairy farms sampled were organically operated and milked fewer than 75 cows. Replacement heifer calves were reared on the farms either in individual calf hutches and pens and/or small groups. Each farm had areas where fly breeding was present, so we categoriazed those areas into 4 habitats. Habitats were categorized into 1) calf housing, which included both individual calf hutches and group housing, 2) loafing areas where adult cows had periodic access, 3) manure lagoons and similar manure storage areas, 4) barnyard lots and alleyways traveled by cattle on the way to and from the milking parlor, and 5) spilled or rotten feed. Viable filth fly pupae were generally

most abundant in samples from calf housing and cow loafing areas, and less so at manure storage areas or lots and alleyways (Table 1).

A total of six species of flies and nine species of pupal parasitoids were recovered from 1,107 viable puparia collected from the six farms combined (Table 2). House flies (*Musca domestica*) and stable flies (*Stomoxys calcitrans*) collectively represented 92% of the total flies collected. When comparing counts of emerged house and stable flies, there was no significant difference between species or habitat (P=0.80). Other fly species included *Lespi nasoni, Hydrotea aenecsens* from the family Muscidae, *Physiophora sp.* from the family Otitidae, and an unidentified species of Sepsidae. Parasitoid species recovered were *Spalangia endius, S. cameroni, S. nigroaenea, S. nigra, Muscidifurax raptor, M. zaraptor, M. raptorellus, Urolepis rufipes* from the family Pteromalidae and *Phygadeuon sp* from the family Ichneumondiae. *Spalangia* and *Muscidifurax* species collectively represented 88% of the total parasitoids collected. Counts of emerged parasitoids did vary significantly among farms (F=18.9; df=5,15; P<0.01) but did not vary among host fly species (F=4.5; df=1,15; P=0.06) or habitat (F=1.1; df=1,15; P=0.32).

House Flies. A total of 379 viable house fly pupae were collected from the six dairies (Table 3). Of the total house fly puparia collected 50% produced adult flies, 50% produced either a *Spalangia* spp. or *Muscidifurax* spp. parasitoid (Table 3). House fly puparia yielding adult flies were most commonly collected from loafing barns (15%) and

young calf housing (11%) (Fig. 2). Parasitoids exerted the most control over house fly populations along lagoon edges, in young calf housing, and in loafing barns with 75%, 56%, and 51% house fly pupal mortality respectively (Fig. 2).

Stable flies. A total of 643 viable stable fly puparia were collected. Stable fly puparia were most commonly collected from calf pens (32%) and loafing barns (15%) (Figure 2). Of the collected stable fly puparia, 70% produced adult flies, 30% produced *Spalangia* spp. or *Muscidifurax* spp. (Table 4). Parasitoids exerted the most control over stable fly populations along lagoon edges, barn lots, and calf pens with 97%, 80%, and 76% pupal mortality respectively (Figure 2).

Discussion

Our results are the first survey of filth flies and associated parasitoids at dairies in the upper Midwest. Our results revealed few flies in manure storage areas but instead found the majority of stable flies breeding in calf pens and most house flies breeding in loafing barns. This differed from Meyer and Petersen (1983) who found that house flies and stable flies were breeding most frequently in stored manure and secondly in soiled straw bedding. Soiled straw bedding collected by Meyer and Petersen was obtained from around the edges of stalls from milking cows rather than from calf pens which may lead to different fly breeding capabilities of the material.

In our survey, *S. endius* made up the majority of parasitoids collected from house fly puparia, which agreed with similar results from Legner et. al (1967). Other surveys of parasitoids attacking sentinel house fly puparia showed *M. raptor* to be the most commonly occurring parasitoid; however, surveys of parasitoids from naturally formed puparia suggest *S. endius, S. nigra*, and *S. nigroaenea* to be more common (Floate et al. 1999, Legner et. al 1967, Lysyk 1995, Smith and Rutz 1991). Another survey conducted in Canada found *P. fumator* to be the most frequently occurring parasitoid on house fly and stable fly puparia (McKay and Galloway 1999, Noronha et al 2007).

Spalangia endius was also the most abundant parasitoid collected from stable fly puparia in our survey, followed by *S. cameroni* and *S. nigra*. We did not collect any *U. rufipes* from stable fly puparia. This differed from surveys of sentinel and naturally formed stable fly puparia that suggested *S. nigra* is one of the most commonly collected parasitoids (Smith et. al 1987, Gibson and Floate 2004). Gibson and Floate (2004) also demonstrated that *S. nigra*, *P. fumator*, and *U. rufipes* have a preference for stable fly puparia.

House flies and stable flies were readily found in soiled calf bedding, making young calf housing a major source of flies on the farm. Winter loafing barns were also a major source of flies. Sanitation efforts that are focused on these areas could potentially reduce fly populations. Young calf housing should be cleaned once a week and the material spread thin or composted to prevent flies from completing their life cycle. Material from

winter loafing barns should be removed and spread or composted in the spring before flies become abundant to prevent them from becoming breeding sites for flies. Other areas where flies were found to be breeding such as barnyard lots and manure piles/lagoons were not producing significant numbers of flies, but were being attacked by parasitoids. Sanitation could be more relaxed in these areas as they do not pose a significant fly risk but can act as reservoirs for parasitoids. Learning where flies are breeding and where their parasitoids are most abundant can help dairymen use labor for sanitation more effectively to help control fly populations on their farm.

Acknowledgements
We gratefully acknowledge the Grimm, Holman, Pearson, Coffings, Riesgraf and Zweber farms who are members of Organic Valley Cooperative who graciously permitted access to their properties and made possible this study. We also thank Katie Flodquist and Kyle Kuehlwein for their assistance in the field and in the laboratory. We thank the CERES Trust, UM Entomology summer block grant fellowship and teaching assistantship, and Minnesota Agriculture Experiment Station project MN-050 for helping to fund this survey.

References cited

Floate, K., B. Khan, G. Gibson. 1999. Hymenopterous parasitoids of filth fly (Diptera: Muscidae) pupae in cattle feedlots. Can. Entomol. 131: 347-362.

Freeborn, S.B., W.M. Regan, and A.H. Folger. 1925. The relation of flies and fly sprays to milk production. J. Econ. Entomol. 18:779-791.

Geden, C.J., D.A. Rutz, R.W. Miller, and D.C. Steinkraus. 1992. Suppression of house flies (Diptera: Muscidae) on New York and Maryland dairies using releases of *Muscidifurax raptor* (Hymenoptera: Pteromalidae) in an integrated management program. Environ. Entomol. 21:1419-1426.

Gibson, G., K. Floate. 2004. Filth fly parasitoids on dairy farms in Ontario and Quebec, Canada. Can. Entomol. 136: 407-417.

Legner, E.F., E.C. Bay, and E.B. White. 1967. Activity of parasites from Diptera: Musca domestica, Somoxys calcitrans Fannia canicularis, and F. femoralis at sites in the Western Hemisphere. Ann. Entomol. Soc. Am. 60: 462-468.

Lissant Cox, G., F.C. Lewis and E.E. Glynn. 1912. The number and varieties of bacteria carried by the common house-fly in sanitary and insanitary city areas. J. Hyg. 12:290-319.

Lysyk, T.J. 1995. Parasitoids (Hymenoptera: Pteromalidae, Ichneumonidae) of filth fly (Diptera: Muscidae) pupae at dairies in Alberta. J. Econ. Entomol. 88(3): 659-665.

McKay, T. and T.D. Galloway. 1999. Survey and release of parasitoids (Hymenoptera) attacking house and stable flies (Diptera: Muscidae) in dairy operations. Can. Entomol. 131(6): 743-756.

Meyer, J.A. and J.J. Petersen. 1983. Characterization and seasonal distribution of breeding sites of stable flies and house flies (Diptera: Muscidae) on eastern Nebraska feedlots and dairies. J. Econ. Entomol. 76:103-108.

Noronha, C., G. Gibson, K. Floate. 2007. Hymenopterous parasitoids of house fly and stable fly puparia in Prince Edward Island and New Brunswick, Canada. Can. Entomol. 139: 748-750.

Olbrich, D.L. and B.H. King. 2003. Host and habitat use by parasitoids (Hymenoptera: Pteromalidae) of house fly and stable fly (Diptera: Muscidae) pupae. Great Lakes Entomol. 36(3-4): 179-190.

Schmidtamnn, E.T. 1988. Exploitation of bedding in dairy outdoor calf hutches by immature house and stable flies (Diptera: Muscidae). J. Med. Entomol. 25:484-488.

Skovgard, H. and G. Nachman. 2004. Biological control of house flies *Musca domestica* and stable flies *Stomoxys calcitrans* (Diptera: Muscidae) by means of inundative releases of *Spalangia cameroni* (Hymenoptera: Pteromalidae). Bull. Entomol. Res. 94:555-567.

Smith, J.P., R.D. Hall, and G.D. Thomas. 1987. Field parasitism of the stable fly (Diptera: Muscidae). Ann. Entomol. Soc. Am. 80(3): 391-397.

Smith, L. and D. Rutz. 1991. Relationship of microhabitat to incidence of house fly (Diptera: Muscidae) immatures and their parasitoids at dairy farms in central New York. Environ. Entomol. 20(2): 669-674.

Stafford III, K.C. 2008. Fly management handbook: A guide to biology, dispersal, and management of the house fly and related flies for farmers, municipalities, and public health officials. Conn. Agric. Exp. St, New Haven, CT. Bulletin 1013.

T**able 1**. Number of viable puparia collected from different habitats at six organic dairies in WI and MN in 2010

State	Date	Young calf housing	Loafing area	Lagoons	Barnyard lot	Rotten feed	Total
WI	19 Aug 2010	223	0	5	na	13	241
WI	23 Aug 2010	31	99	na	na	na	130
WI	26 Aug 2010	52	64	0	na	0	116
MN	16 Aug 2010	0	93	74	na	8	175
MN	30 Aug 2010	112	19	na	34	na	165
MN	2 Sept 2010	94	100	na	na	90	284
	Total	512	375	79	34	111	1,111

Table 2. Numbers and species of adult flies collected as puparia from different habitats at six organic dairies in WI and MN in 2010.

State	Date	Viable puparua	*M. domestica*	*S. calcitrans*	Other[a]
WI	19 Aug 2010	236	26	193	5
WI	23 Aug 2010	162	3	116	3
WI	26 Aug 2010	113	13	24	1
MN	16 Aug 2010	162	31	41	79[b]
MN	30 Aug 2010	154	21	56	1
MN	2 Sept 2010	284	94	20	0
	Total	727	188	450	89

[a]Other species include *Lespi nasoni,* Sepsidae*, Physiophora sp.,* and *Hydrotea aenscens*
[b]77 *Physiophora sp*. from fermenting grain inside a shed

Table 3. Numbers and species of pteromalid wasps reared from puparia of *M. domestica*

State	Date	Viable puparia[a]	*S. endius*	*S. camer-oni*	*S. nigroa-enea*	*S. nigra*	*M. raptor*	*M. zaraptor*	*M. raptorellus*
WI	19 Aug 2010	31	2	0	0	0	0	1	2
WI	23 Aug 2010	3	0	0	0	0	0	0	0
WI	26 Aug 2010	19	3	1	0	2	0	0	0
MN	16 Aug 2010	40	1	4	3	0	0	1	0
MN	30 Aug 2010	37	8	8	0	0	0	0	0
MN	2 Sept 2010	249	138	1	2	0	9	5	0
	Total	379	152	14	5	2	9	7	2

[a] All viable fly puparia collected

Table 4. Numbers and species of pteromalid wasps reared from puparia of *S. calcitrans*

State	Date	Viable puparia	*S. endius*	*S. camer-oni*	*S. nigroae-nea*	*S. nigra*	*M. raptor*	*M. zarap-tor*	*M. raptorell-us*
WI	19 Aug 2010	200	4	3	0	0	0	0	0
WI	23 Aug 2010	156	29	11	0	0	0	0	0
WI	26 Aug 2010	93	21	15	0	31	2	0	0
MN	16 Aug 2010	43	1	1	0	0	0	0	0
MN	30 Aug 2010	116	37	23	0	0	0	0	0
MN	2 Sept 2010	35	7	4	3	0	0	1	0
	Total	643	99	57	3	31	2	1	0

Figure 1: Habitat categorization of fly breeding sites.

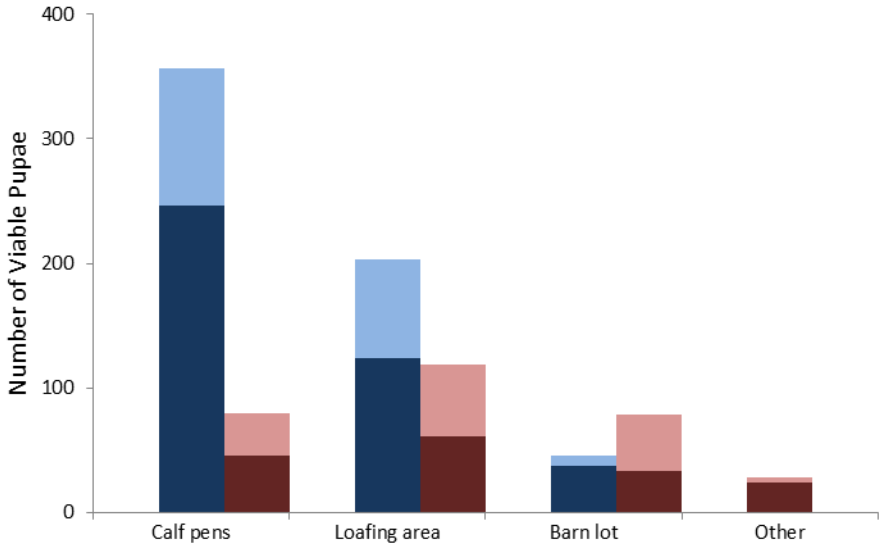

Figure 2: Pupal mortality caused by parasitoids is shown for house fly (right side of paired column) and stable fly (left side of paired column). The full length of the column (dark plus light) is the number of viable pupae collected, the dark colored part of the column is the number of adult flies that emerged and the light colored part is the number of pupae killed by parasitoids.

Chapter III: Filth fly production and parasitism in heifer rearing pens bedded with straw, hardwood sawdust, and pine shavings

J. Starcevich[1], R. Moon[1], B. Clymer[2], H. Chester-Jones[1], and D. Ziegler[1]

[1] University of Minnesota and [2] Spalding Labs

Abstract. Filth fly production and efficacy of their pupal parasitoids was examined in three different commercially available bedding materials in a heifer rearing facility in southern Minnesota in 2009 and 2010. Of the more than 12,000 pupae examined, 99% were either house fly (*Musca domestica L.*) or stable fly (*Stomoxys calcitrans L.*). Pupae from house flies were most abundant in straw and least abundant in sawdust; however, numbers of emerging adult house flies did not differ among bedding materials. Pupae and adult stable flies were most abundant in pine shavings. Six species of pteromalid parasitoids were collected from house fly and stable fly pupae: *Spalangia endius, Spalangia cameroni*, *Muscidifurax zaraptor, Muscidifurax raptorellus, Nasonia vitripennis,* and *Pachycrepoideus vindemia,* but the first two species comprised 99% of the total. Parasitoids killed a greater percentage of fly pupae in straw than in either shavings or sawdust, resulting in high mortality of pupae developing in straw due to parasitoid induced mortality.

Introduction

Two filth flies are prominent pests around dairies and other livestock facilities. Biting stable flies (*Stomoxys calcitrans L.*) reduce animal feed efficiency, weight gain, and milk

production, leading to economic loss (Freeborn et. al 1925, Bruce and Decker 1958, Cheng and Kesler 1961, Wieman et. al 1992, Taylor et. al 2011). House flies (*Musca domestica L*.) spread pathogenic bacteria (Talley et. al 2009**)**. Both flies develop in moist decaying organic materials that accumulate around livestock such as spilled feed, manure, and soiled bedding. Replacement dairy heifers are usually raised in individual hutches or group pens, and dry bedding is usually added as older material becomes soiled with urine and feces. The resulting bedding packs are readily colonized by filth flies. Surveys of dairies have shown that soiled bedding packs in calf hutches are leading sources of filth flies (Meyer and Petersen 1983, Schmidtmann 1988, Olbrich 2003).

Dairymen have a variety of bedding materials they can choose from, including straw, shavings, sawdust, sand, recycled manure solids, ground corn cobs, and corn stover. Bedding packs formed from these materials will vary in heat capacity, moisture content, bulk density, pH, and microbial communities, all of which have the potential to affect the survival of filth fly maggots by both direct and indirect effects and the success of their pupal parasitoids.

A study by Barnard and Geden (1993) showed that developmental rate of house fly larvae and pupae peaked at 32°C. Parasitoids that attack the pupae of filth flies demonstrate peak fecundity between 27°C and 30°C (Ables 1976, Legner 1977, Pawson 1990, and Geden 1996). Although filth fly larvae prefer higher moisture ranges (60-75%), pupae are most commonly found in drier areas of bedding where the moisture content is 50% or

lower (Schmidtmann 1991, Smith & Rutz 1991). Parasitoids can generally manage in a wide range of moisture levels, with *Spalangia* spp. demonstrating the widest range; however, they are most successful at parasitizing hosts at lower moisture levels where their hosts are most commonly found (Geden 1999, Legner 1977).

The range of microhabitat conditions created by bedding choice, affects where parasitoid hosts are found. The depth at which maggots pupate can have a significant effect on parasitoid search success. Studies have shown that *Muscidifurax* spp. have difficulty penetrating into substrates to find their hosts, especially when they have a high moisture content (Legner 1977, Rueda 1985, Geden 2002). *Spalangia* spp. however are generally able to penetrate substrates and search for hosts below the surface (sometimes up to 10cm) (Legner 1977, Rueda and Axtell 1985, Geden 2002). The microhabitat created by bedding choice also affects the density of parasitoid hosts. A study by Ables & Shepard (1974) compared the functional response of host density for two parasitoid species (*Spalangia endius* and *Muscidifurax raptor*). *S. endius* was able to continually increase the number of hosts attacked over 72 hours as host density increased from 30 to 80, indicating that the optimal host density for this species exceeds 80 pupae/female wasp. *M. raptor* also continued to attack more hosts as host density increased; however, the response curve began to level as it approached a density of 80, indicating that optimal host density lies around 80. Several studies have shown that bedding choice will significantly affect host density. Some bedding choices, such as peanut hulls and straw, reach such high host densities that it is unlikely parasitoids would be able to suppress filth fly populations (Schmidtmann 1989 & 1991). Differences in filth fly densities in

different types of bedding material may be caused by ovipositional preferences of female flies, nutritional suitability for maggots, egg to adult survival, and/or efficacy of parasitoids. In 1962, MacCreary and Haenlein compared larval densities in peanut hull and sawdust bedding. Sawdust bedding was found to remain cooler and drier than peanut hull bedding and sawdust was found to become more compact. As a result, bedding packs of peanut hulls contained 24 times as many house flies as sawdust bedding packs. Later, Schmidtmann (1989 and 1991) compared densities of house fly and stable fly in calf hutches bedded with sand, gravel, ground corn cobs, straw, and wood chips. House fly larvae were most abundant in straw, while stable fly larvae were most abundant in both straw and pine shavings. Sand and gravel produced very few larvae, but compacted and soiled quickly, reducing calf cleanliness. Sawdust produced more larvae than sand and gravel, but significantly fewer than straw and calves remained clean (Schmidtmann 1989, Schmidtmann 1991).

To assess the compatibility of bedding choice and biological control for filth fly integrated pest management (IPM), we conducted the present study to test the hypotheses that choice of bedding material affects (1) numbers of filth flies developing in calf pen bedding packs, and (2) the efficacy of naturally occurring pupal parasitoids.

Materials and Methods

The study was conducted at a heifer rearing facility at University of Minnesota Southern Research and Outreach Center (SROC) in Waseca, MN, and was conducted under an approved IACUC protocol (Univ. of MN, 0905A66161). The facility was a naturally

ventilated barn (Fig. 1) with a concrete floor and 10 calf pens on each the north side and south side, separated by an alley down the middle. Pens were 7.3 m long and 3.6 m wide, with individual water fountains. Feed was offered twice per day in the alley outside pen fronts. Front halves of the pens were scraped weekly to remove manure and feed debris, but soiled bedding was allowed to accumulate into bedding packs in the back halves of pens. Nine-week old calves entered the barn on a staggered schedule, were penned in sets of seven of similar age, and remained in the barn for another 16 weeks before transfer elsewhere. Vacated pens were restocked with incoming 9 week old calves the same week.

Experimental design. The experiment was conducted in June of 2009 and 2010 in nine adjacent pens along the barn's south wall. Existing bedding packs were removed, plywood dividers were inserted to prevent lateral spread of bedding and insect inhabitants, and then the pens were started anew with different bedding materials. Materials were long stemmed wheat straw grown on site, pine shavings (Nature's Nest, Rice, MN), or hardwood sawdust (#24 C grade, P.J. Murphy Forest Products, Montville, NJ), and three replicates of each material were assigned to pens at random. Over the next 12 weeks each year, measured amounts of fresh straw, shavings or sawdust were added twice per week as needed to keep calves dry and clean. Pens were sampled periodically to characterize the developing bedding packs and associated filth flies and parasitoids. Weather data at SROC were obtained from the weather station in Waseca, MN (Fig. 2).

Calf growth and cleanliness. Calves were weighed on a CBR Livestock Systems digital platform scale with ±1 lb precision (Pearson Livestock Equipment, Thedford, NE) upon entry, and either at end of the study or earlier if calf maturity dictated replacement during the study. All calves were photographed when weighed to assign a cleanliness score from 1-5, with 1 being completely clean and 5 being heavily soiled (Fig. 3).

Bedding materials and bedding packs. Fresh bedding materials were sampled each year to assess bulk density, moisture content, and pH. Bulk density was measured by tapping subsamples into 1-liter containers and weighing (± 0.1 g) with an Acculab V-1200 balance scale (Acculab, Edgewood, NY). Moisture content was determined gravimetrically, and bedding pH was measured (± 0.1 pH) by mixing 100 mL material into 100 mL of distilled water and then probing the solution electronically (model PH52 probe, Milwaukee, Rocky Mount, NC).

Bedding packs were sampled at week 12 in 2009 and at weeks 3, 7, and 11 in 2010 to measure bulk density, moisture content and pH using the same methods as before. Depth and temperature were measured bi-weekly in 2009 and weekly in 2010. In 2009, pen floors were stratified into edge and center regions (Fig. 4, edge is wall and corner combined), and samples for analysis of bulk density, moisture content, and pH were excavated from 10 x 20 cm areas at two randomly chosen points along each side and back wall, and two from the center, for a total of eight points. In 2010, samples were further stratified to include extractions from pen corners (Fig. 4). Pack depths were measured (± 6 mm) at the same locations in both years with a metal probe inserted to underlying

concrete, and temperatures were measured (± 0.1 °C) 5 cm under the bedding surface with a digital thermometer (Mini-thermometer, Cole-Palmer, Vernon Hills, IL).

Adult fly abundance. Adult fly abundance in the barn was measured using spot cards (Lysyk and Axtell 1985, Lysyk and Axtell 1986) and leg counts in 2009 and 2010 to gauge overall abundance during the study each year. Twenty white 15x20cm cards were hung 180cm above the pen floor and each week cards were retrieved to count fly spots. Stable flies on calves were measured as "leg counts," which were measured by averaging the numbers of flies per pair of legs for seven calves in each of the 20 pens. Counts in 2009 were erratic due to cool temperatures. In 2010, leg counts were not made until temperatures during observation days exceeded 20°C.

Fly populations in bedding packs. Bedding packs were sampled every two weeks in 2009 and 2010. In 2009, pen floors were stratified into edge and center regions (Fig. 4, edge is wall and corner combined), and samples were excavated from 10x20cm areas at six points (two along each wall) from the walls and two points from the center, for a total of eight points. In 2010, floors were stratified into wall, corner, and center regions (Fig. 4). Samples were taken from three points along the walls (one from each wall), each of the two corners, and two points from the center, for a total of 7 points. Samples from the same strata within a pen were mixed together and divided into subsamples of equal size. One subsample was transferred to a bucket with an emergence funnel to capture emerging adults flies (Fig. 5), and the other subsample was washed through sieves to

collect intact puparia, so they could be reared individually in 96-well plates at 24°C for six weeks. Identifications of adult flies were made using keys in McAlpine et al (1981, 1987).

Parasitoids in bedding packs. Mortality to filth fly pupae caused by parasitoids were measured in three ways. First, extracted pupae were incubated for 6 weeks to allow for parasitoid emergence, after which the numbers and species of parasitoids were recorded. Second, sentinel bags of 50 pupae each were stapled just below the bedding pack surface along the side and back walls of each pen (two along each wall in 2009, one in 2010). Bags were retrieved and replaced once per week and the pupae were reared at 24°C for 6 weeks. Third, sentinel bags were placed in perforated metal cages and buried at the bottom in the center of the bedding pack in 2009. These sentinels were treated the same as those placed along the walls. Pupae from the perforated metal cages drown in 2009, so this method was not repeated in 2010. Parasitoids were identified using Gibson's Illustrated key to the native and introduced chalcidoid parasitoids of filth flies in America north of Mexico (Hymenoptera: Chalcidoidea) (2000) and host puparia were identified by Skidmore's The Biology of the Muscidae of the World (1985). Voucher specimens of parasitoids were sent to Gary Gibson for verification of identification.

Survival of preimaginal flies. Egg to adult survival was estimated in 2010 by artificially planting eggs from house flies with the double recessive genes for ochre eyes and brown bodies. Females from a lab colony of house flies were presented with lab media wrapped

in a moist black cloth and eggs were washed from the cloth every 6 hours for 3 days and stored at 7°C to prevent hatching. Groups of 150 eggs on filter paper were planted throughout each of the nine pens in weeks 3, 7, and 11 at about 11,000 eggs per m^2. Adult flies with ochre eyes and brown bodies were recorded from both emergence traps and washed pupae.

Data analysis. Differences among treatment means for average daily gain of calves and calf cleanliness were analyzed with analysis of variance (ANOVA) for a randomized block design, repeated in the two years, including interactions between treatments and years. Moisture content and pH of the bedding packs were analyzed with ANOVA in 2009 and with repeated measures ANOVA in 2010. Depths, temperatures, densities of pupae, densities of emerging adult flies, and density of parasitoids were analyzed with repeated measures ANOVA for a randomized block design, repeated in the two years, including interactions between treatments and years.

Results

Calf growth and cleanliness. Calves gained an average of 0.90 kg ±0.01 kg per day across bedding materials. Calves remained clean and healthy overall in both 2009 and 2010. Average cleanliness scores were 2 for straw, shavings, and sawdust.

Bedding materials and bedding packs. More straw was required to keep calves clean and dry than shavings or sawdust in both years (Table 1). The bulk density of the parent

materials did not change between years and was 28.2, 91.2, and 192.7 grams per liter for straw, shavings, and sawdust respectively (Table 2). In 2010, the bulk densities of soiled material were 312.4, 323.6, and 465 grams per liter for straw, shavings, and sawdust respectively (Table 2). Straw compacted to a greater degree after being soiled than either shavings or sawdust (Table 2). Moisture varied by treatment ($P<0.01$) and by stratum ($P<0.01$) (Fig. 6). Straw was wettest, shavings were intermediate, and sawdust was driest. Top strata in pens were drier than bottom strata. Once soiled with urine and feces, all bedding materials became basic, averaging 9.1pH in 2009 and 8.1pH in 2010. In 2009 we did not measure temperatures or depths until the third week of the study, bedding pack temperatures peaked at about 35°C in early August (Fig. 7) and did not vary among treatments ($P=0.11$). Depths of the bedding packs did vary significantly among treatments ($P=0.01$) with straw being the deepest, shavings intermediate, and sawdust the shallowest (Fig. 8). In 2010, bedding pack temperatures did vary among treatments ($P=0.04$) with straw being the warmest at 33°C and sawdust being the coolest at 29.7°C (Fig. 7). Depths of the bedding packs also varied significantly among treatments ($P=0.02$) with straw being the deepest at 15.8cm and sawdust being the shallowest at 11.6cm (Fig. 8).

Fly populations in bedding packs. Spot cards indicated fly abundance in the barn peaked in July and began to decline in August of 2009 and 2010 (Fig. 9). Numbers of house and stable fly pupae reached peak levels within the first three weeks of pack formation in both years (Fig. 10).

In 2009, 2,040 pupae were collected over 12 weeks via washing: 99% were either house fly (*Musca domestica*) or stable fly (*Stomoxys calcitrans*). In 2010, 10,830 total pupae were collected. The average number of house fly pupae varied significantly between treatments and weeks ($P<0.01$ for both). There was no difference between the amount of house fly pupae developing in sawdust and shavings ($P = 0.32$), but significantly more pupae developing in straw ($P<0.01$) (Fig. 10). The average number of stable fly pupae varied between treatments and weeks ($P = 0.01$ and $P = 0.02$ respectively). For stable flies, there were significantly more pupae in shavings and straw than in sawdust ($P < 0.01$ and $P = 0.04$ respectively) (Fig. 10).

Average numbers of adult house flies that emerged per pen did not differ significantly among treatments ($P = 0.3$) but did differ between weeks ($P<0.01$) (Fig. 11). Average numbers of adult stable flies that emerged per pen differed significantly between treatments and weeks (P=0.02 for both). Average number of adult stable flies did vary between sawdust and shavings ($P<0.01$) but not between sawdust and straw ($P = 0.16$) (Fig. 11). Emergence trap and washed samples were correlated for all bedding types and species in both years, demonstrating that pupae were not killed during the washing process.

Parasitoids in bedding packs. In 2009, a total of 430 adult parasitoids were collected from reared pupae, which were comprised primarily of naturally occurring *Spalangia endius* and *Spalangia cameroni*; these two species comprised 99% of all species collected. Trace numbers of *Muscidifurax zaraptor, Muscidifurax raptorellus, Nasonia*

vitripennis, and *Pachycrepoideus vindemia* made up the remaining species collected. In 2010, a total of 1,022 adult parasitoids were collected from reared pupae, which were comprised primarily of naturally occurring *S. endius;* these species comprised 84% of all species collected. *S. endius* was the most prevalent species in both hosts parasitizing 78% of house fly pupae and 84% of stable fly pupae. Unfortunately pupae in sentinel bags along pen walls centers were crushed and/or drowned by urine and did not yield results. All 6 species were collected from house fly pupae with *S. endius* and *S. cameroni* being the most abundant and equally successful. Only *S. endius* and *S. cameroni* were successful in parasitizing stable fly pupae, with *S. endius* being the most prevalent in this host. The average number of parasitoids reared from house fly pupae varied significantly between treatments, weeks, and host density ($P<0.01$, $P = 0.02$, and $P<0.01$ respectively). Parasitoids killed house fly pupae equally well in sawdust and shavings ($P = 0.73$) but killed significantly more house fly pupae in straw than in sawdust ($P =0.02$) (Fig. 12). The average number of parasitoids reared from stable fly pupae varied slightly between treatments and weeks, but not host density ($P = 0.04$, $P = 0.01$, and $P=0.06$ respectively) (Fig. 12). Parasitoids killed stable fly pupae equally well in sawdust as they did in shavings and straw ($P=0.12$ and $P=0.2$ respectively).

Survival of preimaginal flies. Insufficient numbers of mutant adult house flies were recovered for viable analysis. A total of 2, 1, and 3 adults were recovered from eggs of the phenotypically mutant house flies planted in weeks 3, 7, and 11 respectively. These

adults were collected from pens bedded with sawdust (weeks 3 and 7) and straw (week 11).

Discussion

Different environments created by bedding choice affected the success of both filth flies and their pupal parasitoids. Although house fly pupal densities were highest in straw bedding packs, intermediate in shavings, and lowest in sawdust, the number of adult flies emerging from collected pupae did not vary among bedding choice. Stable fly pupal density was highest in pine shavings, intermediate in straw, and lowest in sawdust. More adult stable flies emerged from pupae collected in pine shavings than in either sawdust or straw. We found that increased parasitoid activity in straw counter balanced the house fly production potential of straw, but seemed to have a minimal effect on stable flies. Schmidtmann (1988 and 1989) and Schmidtmann et. al (1991) only measured numbers of fly larvae, and like them we found more filth flies on average developing in straw than any other bedding; however, we reared out adults from collected pupae and found that the net numbers of adult house flies emerged were not different between beddings although adult stable flies were highest from pine shavings. Previous work also did not account for possible differences in natural mortality to developing flies based on bedding type. In this study, we found that choice of bedding material affects the efficacy of pupal parasitoids as well as potential fly production. Parasitoids were more successful at finding and attacking house fly pupae that were in straw than those in shavings or sawdust, which resulted in greater house fly pupal mortality in straw bedding. Greater

success of parasitoids in straw could be a functional response to greater numbers of host pupae in straw, or to better search efficacy of parasitoids in a more porous substrate.

These results indicate that cultural control through bedding choice could reduce numbers of developing filth flies in calf rearing pens. Sawdust bedding showed a significant reduction in all filth fly breeding; however, parasitoids were not successful at finding and attacking the few flies that did develop in sawdust. Straw bedding had the potential to produce the greatest numbers of house flies, but net production of adults was equal to that of the other beddings because pupal mortality was highest in straw. Shavings had the potential to produce the greatest numbers of stable flies. Cultural control through use of sawdust bedding could significantly reduce fly problems on dairies; however, when straw is a more economical choice, the conservation or augmentation of naturally occurring parasitoids could counterbalance the house fly production potential of straw. An economic analysis is needed to assess the profitability of different calf bedding materials, with and without naturally occurring or commercially produced parasitic wasps.

Acknowledgements

We thank Spalding Labs for their financial support of this study, and we would like to thank the staff at the University of Minnesota's Southern Research and Outreach Center for their assistance in maintaining the calves and pens. Also, we would like to thank Stacy Reyna, Katie Flodquist, and Kyle Kuehlwein (Undergraduate Research Assistants, University of Minnesota) for their help in conducting this study.

References Cited

Ables, J.R. and M. Shepard. 1976. Influence of temperature on oviposition by the parasites *Spalangia endius* and *Muscidifurax raptor*. Environ. Entomol. 5(3): 511-513.

Bruce, W.N. and G.C. Decker. 1947. Fly control and milk flow. J. Econ. Entomol. 40:530-536.

Cheng, Tien-His and E.M. Kesler. 1961. A three-year study on the effect of fly control on milk production by selected and randomized dairy herds. J. Econ. Entomol. 54:751-757.

Freeborn, S.B., W.M. Regan, and A.H. Folger. 1925. The relation of flies and fly sprays to milk production. J. Econ. Entomol. 18:779-791.

Geden, C. 2002. Effect of habitat depth on host location by five species of parasitoids (Hymenoptera: Pteromalidae, Chalcididae) of house flies (Diptera: Muscidae) in three types of substrates. Environ. Entomol. 31(2): 411-417.

Legner, E.F. 1977. Temperature, humidity and depth of habitat influencing host destruction and fecundity of muscoid fly parasites. Entomophaga 22(2): 199-206.

MacCreary, D. and G.F.W. Haenlein. 1962. House fly breeding in oak sawdust and peanut hulls used as bedding in calf pens. J. Econ. Entomol. 55: 419.

Meyer, J.A. and J.J. Petersen. 1983. Characterization and seasonal distribution of breeding sites of stable flies and house flies (Diptera: Muscidae) on eastern Nebraska feedlots and dairies. J. Econ. Entomol. 76:103-108.

Olbrich, D.L. and B.H. King. 2003. Host and habitat use by parasitoids (Hymenoptera: Pteromalidae) of house fly and stable fly (Diptera: Muscidae) pupae. Great Lakes Entomol. 36(3-4): 179-190.

Pitzer, J., P. Kaufman, C. Geden, J. Hogsette. 2011. The ability of selected pupal parasitoids (Hymenoptera: Pteromalidae) to locate stable fly hosts in a soiled equine bedding substrate. Environ. Entomol. 40(1):88-93.

Rueda, L.M. and R.C. Axtell. 1985. Effect of depth of house fly pupae in poultry manure on parasitism by six species of Pteromalidae (Hymenoptera). J. Entomol. Sci. 20(4): 444-449.

Schmidtamnn, E.T. 1988. Exploitation of bedding in dairy outdoor calf hutches by immature house and stable flies (Diptera: Muscidae). J. Med. Entomol. 25:484-488.

Schmidtmann, E.T., R.W. Miller, and R. Muller. 1989. Effect of experimental bedding treatments on the density of immature *Musca domestica* and *Stomoxys calcitrans* (Diptera: Muscidae) in outdoor calf hutches. J. Econ. Entomol. 82(4): 1134-1139.

Schmidtmann, E.T. 1991. Suppressing immature house and stable flies in outdoor calf hutches with sand, gravel, and sawdust bedding. J. Dairy Sci. 74: 3956-3960

Talley, J.L., A.C. Wayadande, L.P. Wasala, A.C. Gerry, J. Flethcher, U. DeSilva, and S.E. Gilliland. 2009. Association of *Escherichia coli* O157:H7 with filth flies (Muscidae and Calliphoridae) captured in leafy greens fields and experimental transmission of E. coli O157:H7 to spinach leaves by house flies (Diptera: Muscidae). J. Food Prot. 72:1547-52.

Wieman, G.A., J.B. Campbell, J.A. DeShazer, and I.L. Berry. 1992. Effects of stable flies (Diptera: Muscidae) and heat stress on weight gain and feed efficiency of feeder cattle. J. Econ. Entomol. 85:1835-1842.

Table 1: Usage of bedding materials (kg) in 2009 and 2010

	Straw	**Shavings**	**Sawdust**
2009	140	122	132
2010	141	119	137

Table 2: Bulk density and compaction of materials in 2009 and 2010

	Straw		Shavings		Sawdust	
	2009	2010	2009	2010	2009	2010
Bulk density of fresh material (g/L)	28.2	28.5	91.2	91.5	192.7	190.9
Bulk density of soiled material g/L	--[a]	312.4	--	323.6	--	465
Percent compaction	95	95	80	84	68	74

[a] not measured in 2009

Figure 1: Interior of experimental replacement heifer barn. Calves in pens at left where being fed, while calves in pens at right were temporarily crowded onto bedding packs to allow cleaning of pen fronts.

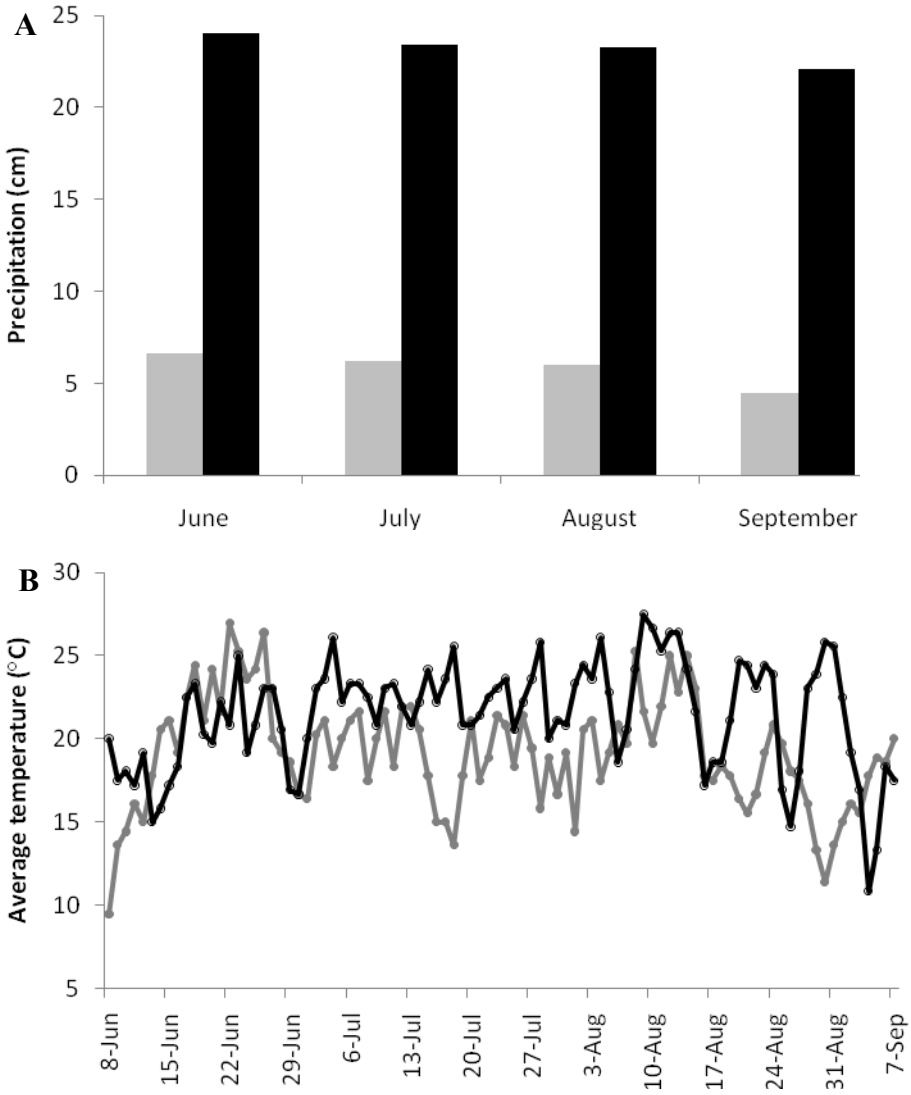

Figure 2: Average monthly precipitation (A) and daily average temperatures (B) as recorded at SROC, Waseca, MN, June 8-September 7, 2009 (grey) and June 8-September 7, 2010 (black).

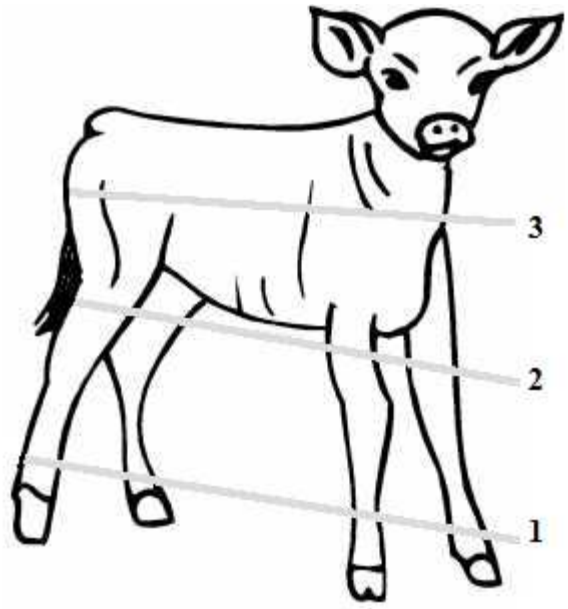

Figure 3: Cleanliness Scores 1 – 5; 1- little manure below line 1, 2 – little manure below line 2, 3 – manure present in most areas below line 3, 4 - manure over much of the body and especially thick manure on legs, 5 - thick manure coating the belly and legs as well as areas of manure over much of the body.

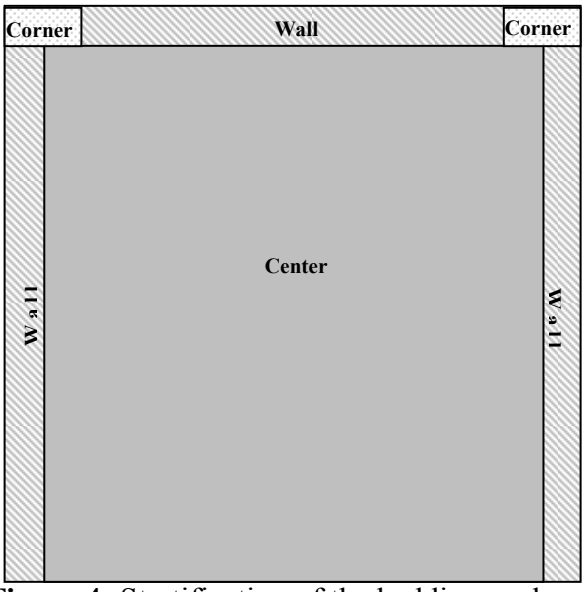

Figure 4: Stratification of the bedding pack

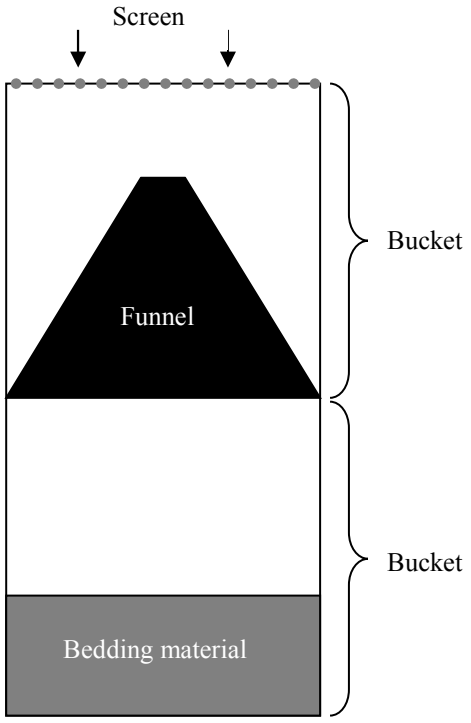

Figure 5: Emergence trap construction

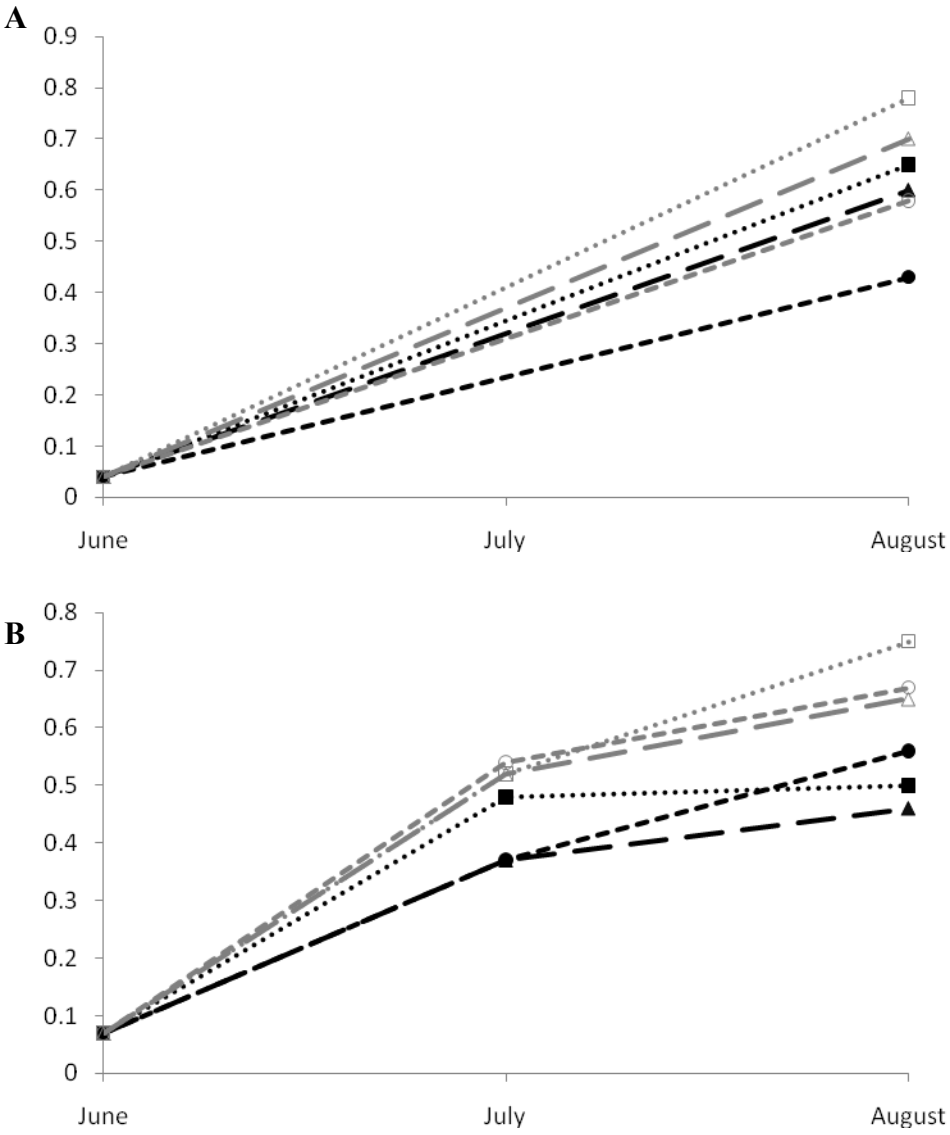

Figure 6. Change in moisture content of the top (black lines, filled markers) and bottom (grey lines, open marker) strata of straw (dotted lines, square markers), shavings (long dashed lines, triangle markers), and sawdust (short dashed lines, circle markers) in 2009 (A) and 2010 (B).

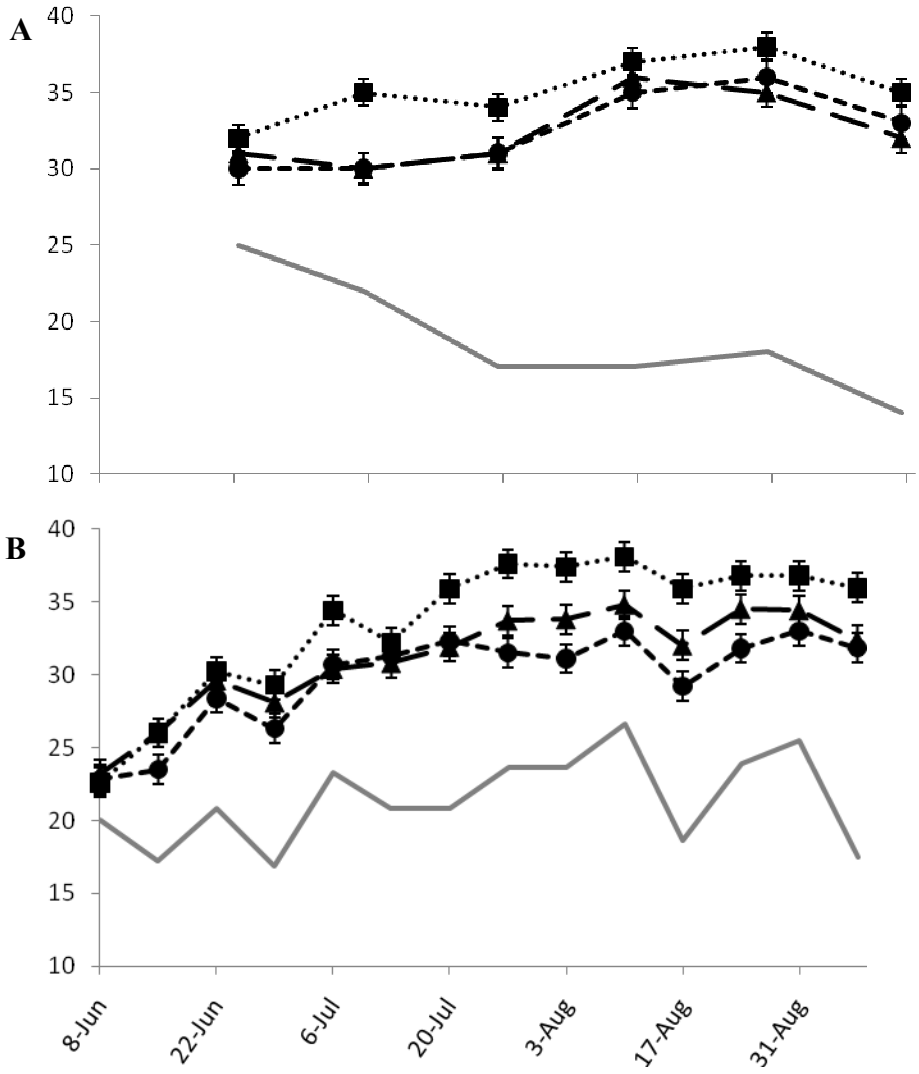

Figure 7: Temperature (°C) of straw (dotted line, square marker), shavings (long dashed line, triangle marker), sawdust (short dashed line, circle marker), and ambient air temperature (grey line, no marker) in 2009 (A) and 2010 (B).

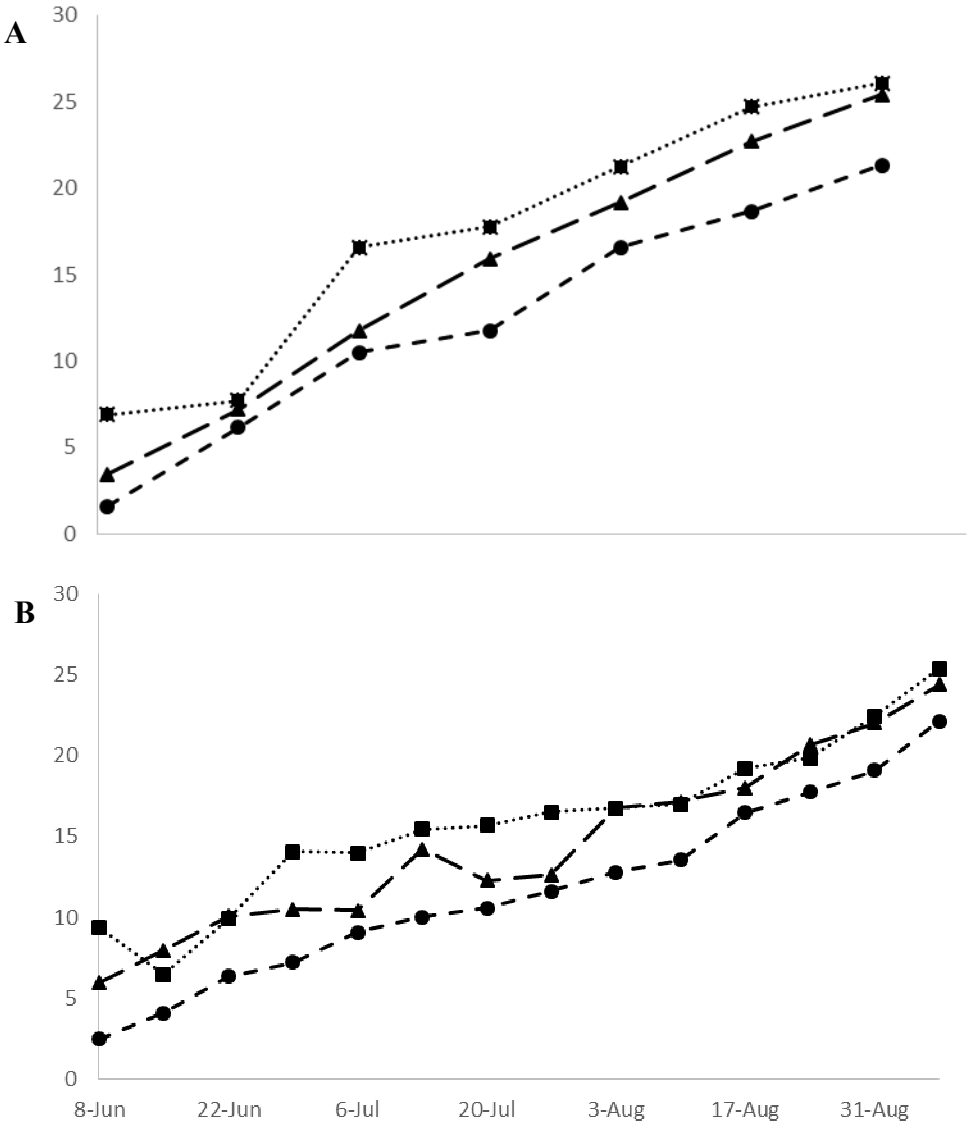

Figure 8: Depth (cm) of straw (dotted line, square marker), shavings (long dashed line, triangle marker), sawdust (short dashed line, circle marker) in 2009 (A) and 2010 (B).

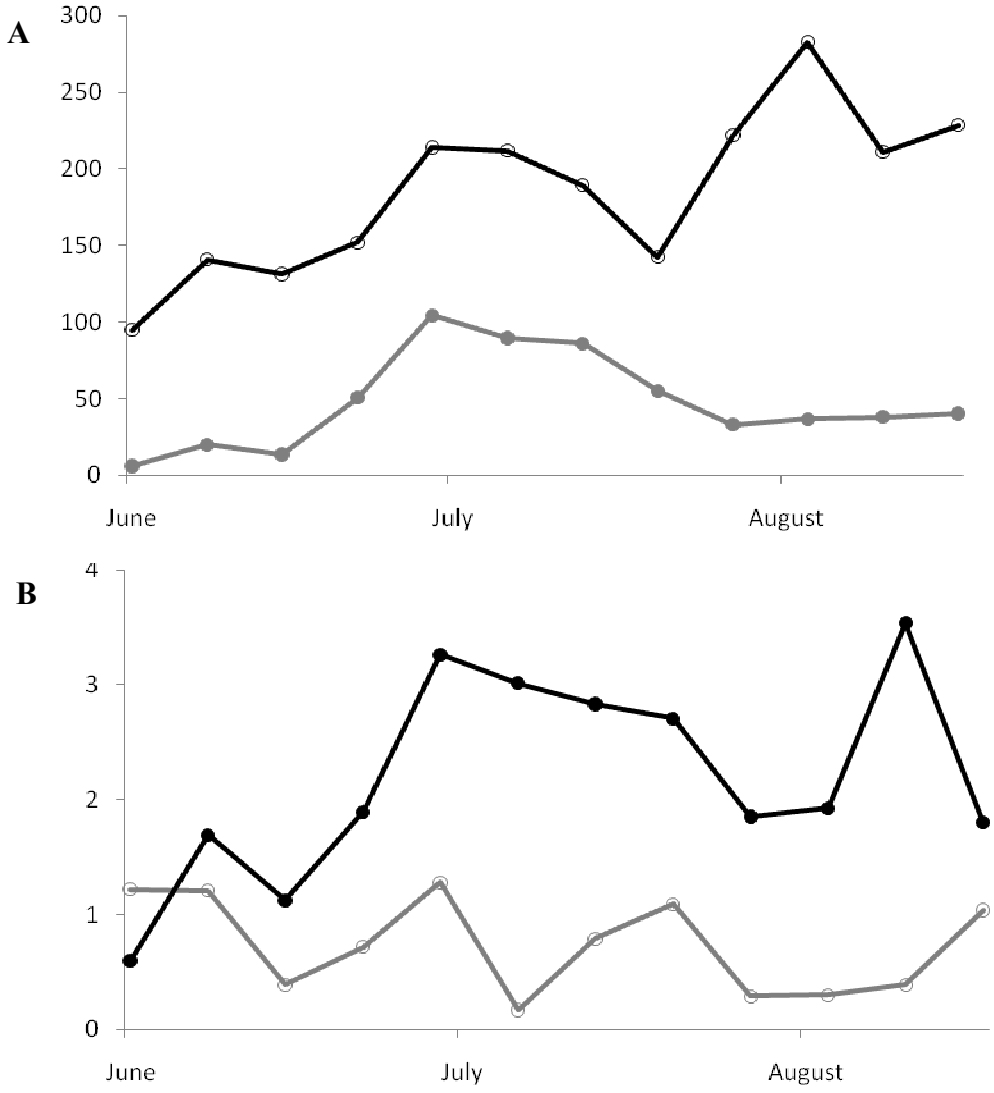

Figure 9: Fly abundance indices in the grower barn, as measured with spot cards (A, average spots per card) and leg counts (B, average flies per pair of legs) in 2009 (grey) and 2010 (black)

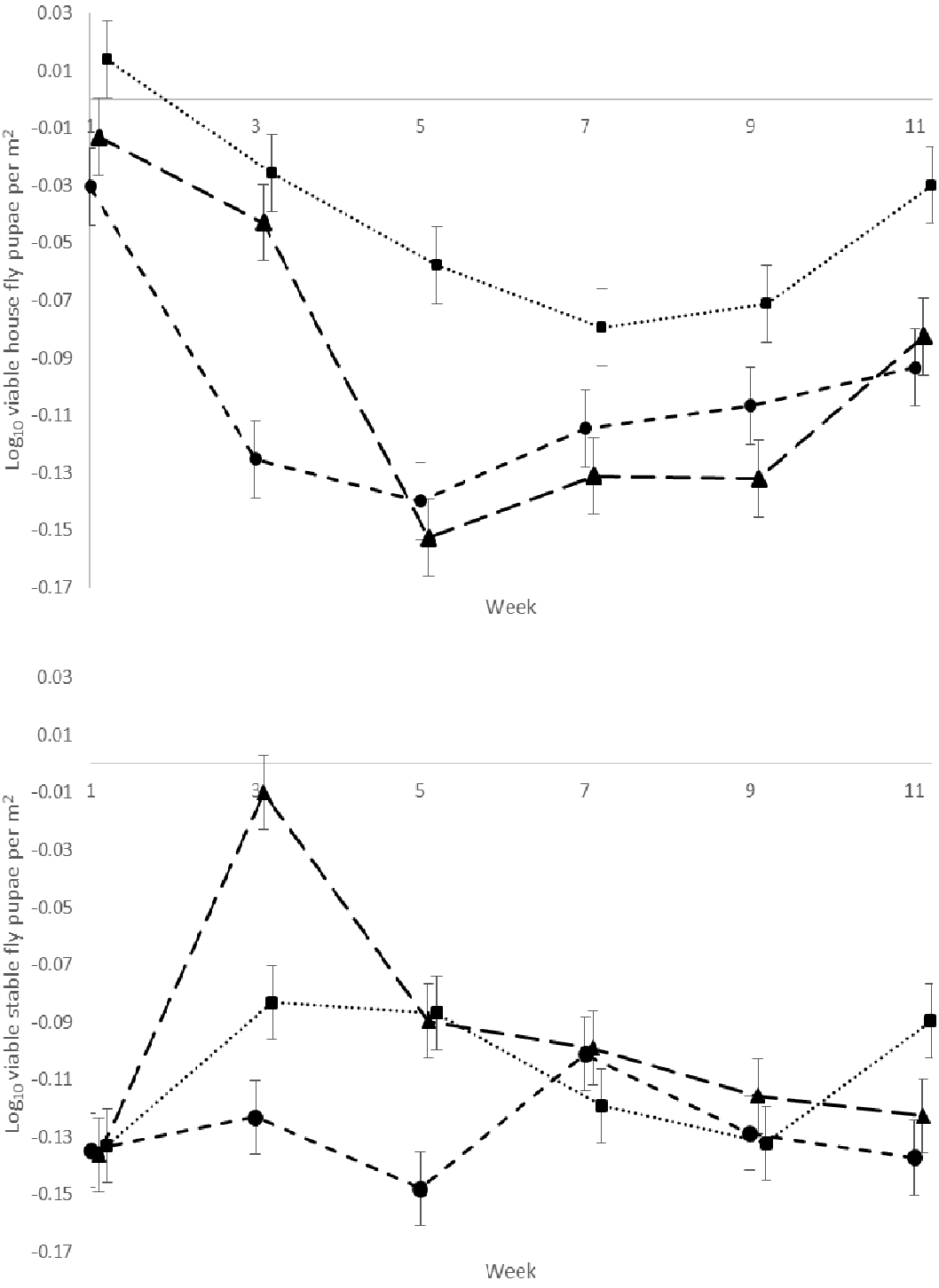

Figure 10: Average number of $\log_{10}$ viable house fly (top) and stable fly (bottom) pupae per m^2 collected from sawdust (short dashed line, circle marker), shavings (long dashed line, triangle marker), and straw (dotted line, square marker) with ½ LSD error bars.

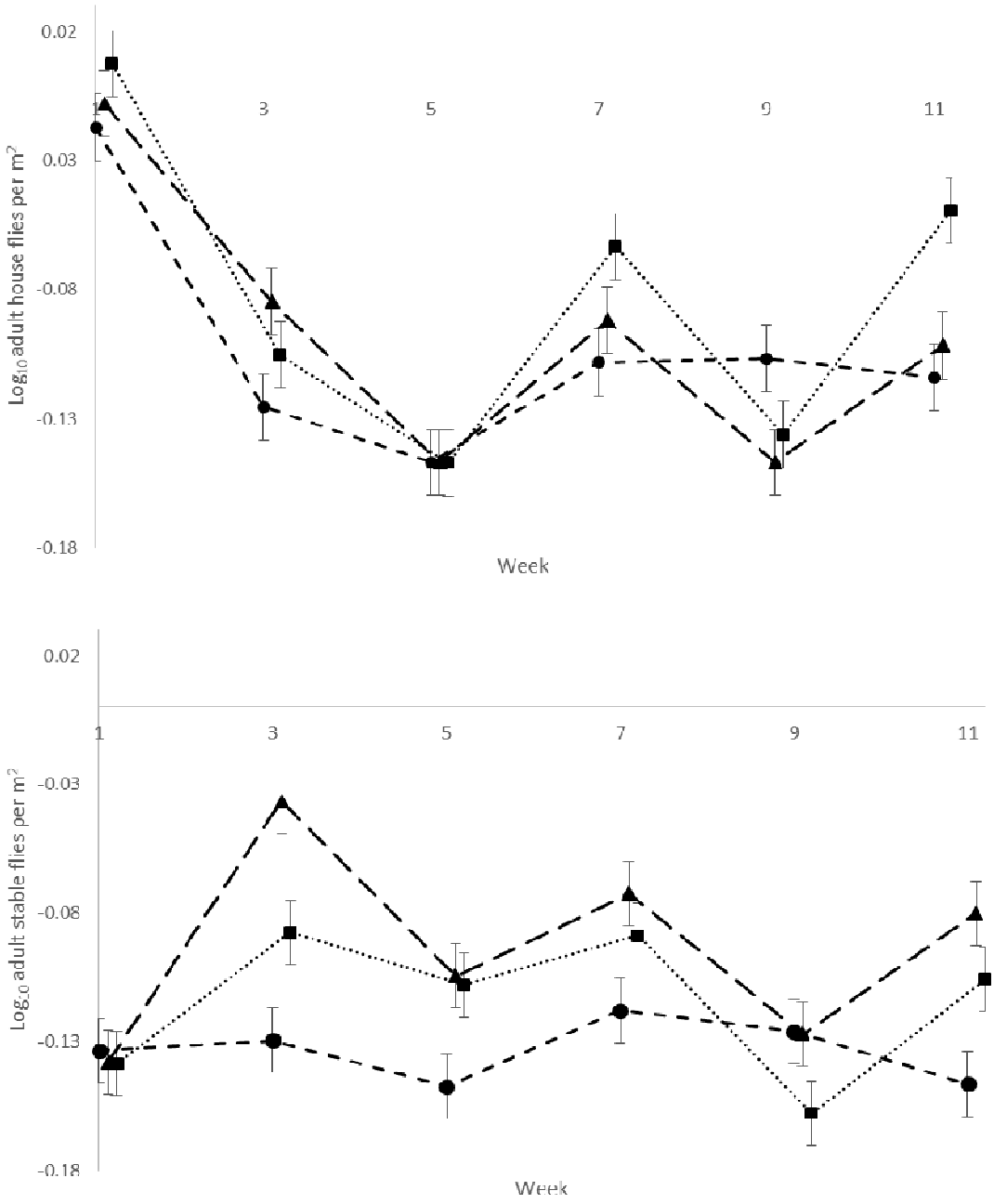

Figure 11: Average number of $\log_{10}$ adult house flies (top) and stable flies (bottom) per m^2 collected sawdust (short dashed line, circle marker), shavings (long dashed line, triangle marker), and straw (dotted line, square marker) with ½ LSD error bars.

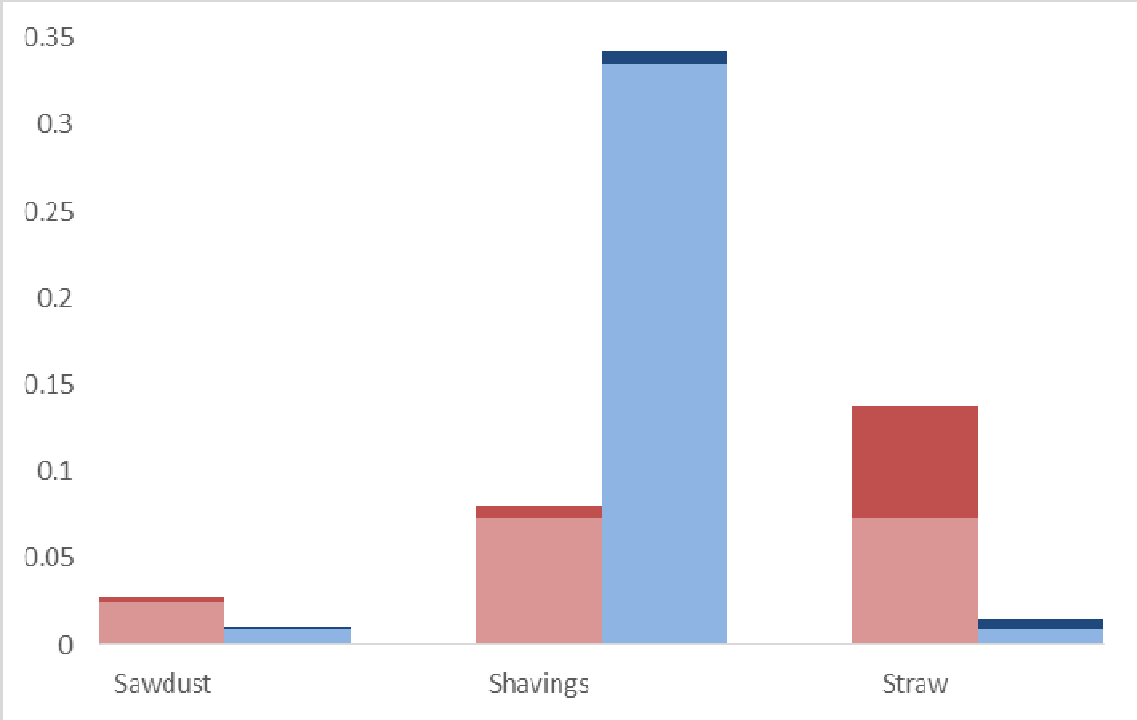

Figure 12: Numbers of viable house fly (left side of paired column) and stable fly (right side of paired column) pupae collected and the numbers of adult flies (light portions of the columns) the pupae yielded. The dark parts of the columns demonstrate the adult fly reduction due to pupal parasitoids for house fly and stable fly respectively.

Bibliogrophy

Ables, J.R. and M. Shepard. 1976. Influence of temperature on oviposition by the parasites *Spalangia endius* and *Muscidifurax raptor*. Environ. Entomol. 5: 511-513.

Andrews, R.E., L.W. Parks, K.D. Spense. 1980. Some effects of douglas fir terpenes on certain microorganisms. Appl. Environ. Micro. 40: 301-304.

Barnard, D.R. and C.J. Geden. 1993. Influence of larval density and temperature in poultry manure on development of the house fly (Diptera: Muscidae). Environ. Entomol. 22: 971-977.

Bruce, W.N. and G.C. Decker. 1947. Fly control and milk flow. J. Econ. Entomol. 40: 530-536.

Cheng, Tien-His and E.M. Kesler. 1961. A three-year study on the effect of fly control on milk production by selected and randomized dairy herds. J. Econ. Entomol. 54: 751-757.

Coats, S. 1976. Life cycle and behavior of *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae). Ann. Entomol. Soc. Am. 69: 772-780.

De Jesus, A., A.R. Olsen, J.R. Bryce, and R.C. Whiting. 2004. Quantitative contamination and transfer of *Escherichia ecoli* from foods by house flies, *Musca domestica* L. (Diptera: Muscidae). Int. J. Food Microbiol. 93:259-262.

Espinoza-Fuentes F.P. and W.R. Terra. 1987. Physiological adaptations for digesting bacteria: water fluxes and distribution of digestive enzymes in *Musca domestica* larval midgut. Ins. Biochem. 17: 809-817.

Floate, K., B. Khan, G. Gibson. 1999. Hymenopterous parasitoids of filth fly (Diptera: Muscidae) pupae in cattle feedlots. Can. Entomol. 131: 347-362.

Förster, M., K. Sievert, S. Messler, S. Klimpel, and K. Pfeffar. 2009. Comprehensive study on the occurrence and distribution of pathogenic microorganisms carried by synanthropic flies caught at different rural locations in Germany. J. Med. Entomol. 46: 1164-1166.

Freeborn, S.B., W.M. Regan, and A.H. Folger. 1925. The relation of flies and fly sprays to milk production. J. Econ. Entomol. 18: 779-791.

Geden, C.J., D.A. Rutz, R.W. Miller, and D.C. Steinkraus. 1992. Suppression of house flies (Diptera: Muscidae) on New York and Maryland dairies using releases of

Muscidifurax raptor (Hymenoptera: Pteromalidae) in an integrated management program. Environ. Entomol. 21: 1419-1426.

Geden, C.J., D.A. Rutz, J.G. Scott, and S.J. Long. 1992. Susceptability of house flies (Dipter: Muscidae) and five pupal parasitoids (Hymenoptera: Pteromalidae) to abamectin and seven commercial insecticides. J. Econ. Entomol 85: 435-440.

Geden, C.J. 1996. Modeling host attacks and progeny production of *Spalangia gemina*, *Spalangia cameroni*, and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) at constant and variable temperatures. Bio. Contr. 7: 172-178.

Geden, C. 1999. Host location by house fly (Diptera: Muscidae) parasitoids in poultry manure at different moisture levels and host densities. Environ. Entomol. 23: 755-760.

Geden, C. 2002. Effect of habitat depth on host location by five species of parasitoids (Hymenoptera: Pteromalidae, Chalcididae) of house flies (Diptera: Muscidae) in three types of substrates. Environ. Entomol. 31: 411-417.

Gerling, D. and E. Legner. 1968. Developmental history and reproduction of *Spalangia cameroni*, parasite of synanthropic flies. Ann. Entomol. Soc. Am. 61: 1436-1443.

Gibson, G., K. Floate. 2004. Filth fly parasitoids on dairy farms in Ontario and Quebec, Canada. Can. Entomol. 136: 407-417.

Godden, S., R. Bey, K. Lorch, R. Farnsworth, and P. Rapnicki. 2008. Ability of organic and inorganic bedding materials to promote growth of environmental bacteria. J. Dairy Sci. 91: 151-159.

Graczyk, T.K., M.R. Cranfield, R. Fayer, and H. Bixler. 1999. House flies (*Musca domestica*) as transport hosts of *Cryptosporidium parvum*. Am. J. Trop. Med. Hyg. 61: 500-504

Himejima, M., K.R. Hobson, T. Otsuka, D.L. Wood, I. Kubo. 1992. Antimicrobial terpenes from oleoresin of ponderosa pine tree *Pinus ponderosa*: a defense mechanism against microbial invasion. Chem. Ecol. 18: 1809-1818.

Hogan, J.S., K.L. Smith, D.A. Todhunter, P.S. Schoenberger. 1990. Bacterial counts associated with recycled newspaper bedding. J. Dairy Sci. 73: 1756-1761.

Hogsette, J., J. Ruff, and C. Jones. 1987. Stable fly biology and control in northwestern Florida. J. Agric. Entomol. 4: 1-11.

Lazarus, W.F., D.A. Rutz, R.W. Miller, and D.A. Brown. 1989. Costs of existing and recommended manure management practices for house fly and stable fly (Diptera: Muscidae) control on dairy farms. J. Econ. Entomol. 82: 1145-1151.

Legner, E.F., E.C. Bay, and E.B. White. 1967. Activity of parasites from Diptera: Musca domestica, Somoxys calcitrans Fannia canicularis, and F. femoralis at sites in the Western Hemisphere. Ann. Entomol. Soc. Am. 60: 462-468.

Legner, E.F. 1977. Temperature, humidity and depth of habitat influencing host destruction and fecundity of muscoid fly parasites. Entomophaga 22: 199-206.

LeJeune, J.T. and M.D. Kauffman. 2005. Effect of sand and sawdust bedding materials on the fecal prevalence of *Escherichia coli* O157:H7 in dairy cows. App. Environ. Micro. 71: 326-330.

Levinson, Z.H. 1960. Food of housefly larvae. Nature 188: 427-428.

Lindberg, L.E., S.M. Willfor, B.R. Holmbom. 2004. Antibacterial effects of knotwood extractives on paper mill bacteria. Ind. Micro. Biotech. 31: 137-147.

Lindsay, D.R. and H.I. Scudder. 1959. Nonbiting flies and disease. Ann. Rev. Entomol. 1:323-346.

Lissant Cox, G., F.C. Lewis and E.E. Glynn. 1912. The number and varieties of bacteria carried by the common house-fly in sanitary and insanitary city areas. J. Hyg. 12: 290-319.

Lysyk, T.J. 1995. Parasitoids (Hymenoptera: Pteromalidae, Ichneumonidae) of filth fly (Diptera: Muscidae) pupae at dairies in Alberta. J. Econ. Entomol. 88(3): 659-665.

Lysyk, T.J., L. Kalishuk-Tymensen, L.B. Selinger, R.C. Lancaster, L. Wever, K.J. Cheng. 1999. Rearing stable fly larvae (Diptera: Muscidae) on an egg yolk medium. J. Med. Entomol. 36: 382-388

MacCreary, D. and G.F.W. Haenlein. 1962. House fly breeding in oak sawdust and peanut hulls used as bedding in calf pens. J. Econ. Entomol. 55: 419.

Maganga, M.E., G. Gries, R. Gries. 1996. Repellency of various oils and pine oil constituents to house flies (Diptera: Muscidae. Environ. Entomol. 25: 1182-1187.

McGaughey, J. and D. Nayduch. 2009. Temporal and spatial fate of GFP-expressing motile and nonmotile *Aeromona hydrophila* in the house fly digestive tract. J. Med. Entomol. 46: 123-130.

McKay, T. and T.D. Galloway. 1999. Survey and release of parasitoids (Hymenoptera) attacking house and stable flies (Diptera: Muscidae) in dairy operations. Can. Entomol. 131(6): 743-756.

Meyer, J.A. and J.J. Petersen. 1983. Characterization and seasonal distribution of breeding sites of stable flies and house flies (Diptera: Muscidae) on eastern Nebraska feedlots and dairies. J. Econ. Entomol. 76: 103-108.

Miller, R.W., D.A. Rutz, L.G. Pickens, and C.J. Genden. 1993. Evaluation of traps and the parasitoid *Muscidifurax raptor* Girault and Sanders to manage house flies and stable flies on dairy farms. J. Agri. Entomol. 10: 9-19.

Noronha, C., G. Gibson, K. Floate. 2007. Hymenopterous parasitoids of house fly and stable fly puparia in Prince Edward Island and New Brunswick, Canada. Can. Entomol. 139: 748-750.

Olbrich, D.L. and B.H. King. 2003. Host and habitat use by parasitoids (Hymenoptera: Pteromalidae) of house fly and stable fly (Diptera: Muscidae) pupae. Great Lakes Entomol. 36: 179-190.

Pawson, B.M. and J.J. Petersen. 1990. Temperature preferences and effects of photoperiod on oviposition behavior of five pteromalid wasps (Hymenoptera: Pteromalidae) using house fly (Diptera: Muscidae) pupae as hosts. Environ. Entomol. 19: 1452-1456.

Pitzer, J., P. Kaufman, C. Geden, J. Hogsette. 2011. The ability of selected pupal parasitoids (Hymenoptera: Pteromalidae) to locate stable fly hosts in a soiled equine bedding substrate. Environ. Entomol. 40: 88-93.

Rochon, K., T.J. Lysyk, L.B. Selinger. 2004. Persistence of *Escherichia coli* in immature house fly and stable fly (Diptera: Muscidae) in relation to larval growth and survival. J. Med. Entomol. 41: 1082-1089.

Romero, A., A. Broce, L. Zurek. 2006. Role of bacteria in the oviposition behavior and larval development of stable flies. Med. Vet. Entomol. 20: 115-121.

Rueda, L.M. and R.C. Axtell. 1985. Effect of depth of house fly pupae in poultry manure on parasitism by six species of Pteromalidae (Hymenoptera). J. Entomol. Sci. 20: 444-449.

Sanders, D.A. 1940. *Musca domestica* a vector of bovine mastitis. J. Am. Vet. Med. Assoc. 97:120-122.

Schmidtmann, E.T. 1988. Exploitation of bedding in dairy outdoor calf hutches by immature house and stable flies (Diptera: Muscidae). J. Med. Entomol. 25: 484-488.

Schmidtmann, E.T., R.W. Miller, and R. Muller. 1989. Effect of experimental bedding treatments on the density of immature *Musca domestica* and *Stomoxys calcitrans* (Diptera: Muscidae) in outdoor calf hutches. J. Econ. Entomol. 82: 1134-1139.

Schmidtmann, E.T. 1991. Suppressing immature house and stable flies in outdoor calf hutches with sand, gravel, and sawdust bedding. J. Dairy Sci. 74: 3956-3960

Schmidtmann, E.T. and P.A.W. Martin. 1992. Relationship between selected bacteria and the growth of immature house flies, *Musca domestica*, in an axenic test system. J. Med. Entomol. 29: 232-235.

Scott, J.G., D.A. Rutz, and J. Walcott. 1988. Comparative toxicity of seven insecticides to adult *Spalangia cameroni* Perkins. J. Agric. Entomol 5: 139-145.

Skovgard, H. and G. Nachman. 2004. Biological control of house flies *Musca domestica* and stable flies *Stomoxys calcitrans* (Diptera: Muscidae) by means of inundative releases of *Spalangia cameroni* (Hymenoptera: Pteromalidae). Bull. Entomol. Res. 94: 555-567.

Smith, L. and D. Rutz. 1985. The occurrence and biology of *Urolepis rufipes* (Hymenoptera: Pteromalidae), a parasitoid of house flies in New York dairies. Environ. Entomol. 14: 365-369.

Smith, J.P., R.D. Hall, and G.D. Thomas. 1987. Field parasitism of the stable fly (Diptera: Muscidae). Ann. Entomol. Soc. Am. 80(3): 391-397.

Smith, L. and D. Rutz. 1991. Relationship of microhabitat to incidence of house fly (Diptera: Muscidae) immatures and their parasitoids at dairy farms in central New York. Environ. Entomol. 20: 669-674.

Stafford III, K.C. 2008. Fly management handbook: A guide to biology, dispersal, and management of the house fly and related flies for farmers, municipalities, and public health officials. Conn. Agric. Exp. St, New Haven, CT. Bulletin 1013.

Talley, J.L., A.C. Wayadande, L.P. Wasala, A.C. Gerry, J. Flethcher, U. DeSilva, and S.E. Gilliland. 2009. Association of *Escherichia coli* O157:H7 with filth flies (Muscidae and Calliphoridae) captured in leafy greens fields and experimental transmission of E. coli O157:H7 to spinach leaves by house flies (Diptera: Muscidae). J. Food Prot. 72: 1547-52.

Ward, P.L., J.E. Wohlt, P.K. Zajac, K.R. Cooper. 2000. Chemical and physical properties of processed newpaper compared to wheat straw and wood shavings as animal bedding. J. Dairy Sci. 83: 359-367.

Ward, P.L., J.E. Wohlt, S.E. Katz. 2001. Chemical, physical, and environmental properties of pelleted newspaper compared to wheat straw and wood shavings as bedding for horses. J. Anim. Sci. 79: 1359-1369.

Wasala, L., J.L. Talley, U. DeSilva, J. Fletcher, and A. Wayadande. 2013. Transfer of *Escherichia coli* O157:H7 to spinach by house flies, *Musca domestica* (Diptera: Muscidae). Phytopathology 103: 373-380.

Wieman, G.A., J.B. Campbell, J.A. DeShazer, and I.L. Berry. 1992. Effects of stable flies (Diptera: Muscidae) and heat stress on weight gain and feed efficiency of feeder cattle. J. Econ. Entomol. 85: 1835-1842.

Appendices

Appendix A
Site descriptions

State	Date	Young calf housing	Loafing area	Lagoons	Barnyard lot	Rotten feed
WI	19 Aug 2010	Bottle calves were kept in indoor pens in groups of four to six, then moved to an open sided barn with pasture access as they grew. Both were kept on straw bedding.	Cows had access to an open sided loafing barn in the evening which had a sand/dirt floor and automatic water tanks.	Manure was stored in an outdoor lagoon with a crust.	There was no open barnyard holding lot for these cattle as they had access to a specific loafing barn.	Grain was stored in a silo leading into a small shed where there was some rotten grain on the ground, and there were the remains of winter hay bales near the loafing barn.
WI	23 Aug 2010	Bottle calves were kept in indoor pens in groups of two to three, then moved into one large group in an underground portion of a barn. All calves were bedded on straw.	Cows were kept on pasture at all times through the grazing season and were only allowed in the straw bedded loafing barn during the winter months.	Manure was piled on a concrete pad and spread every 2 to 4 days. We did not collect from this location, as there was no manure present on the day we were collecting fly pupae.	There was no open barnyard holding lot for these cattle.	There was no spilled or rotten grain or hay available to sample
WI	26 Aug 2010	Calves began in individual hutches and then were moved into group pens of three to four calves. All calves were bedded on wood chips.	Cows had access to both pasture and a sand bedded loafing area.	Manure was stored in an outdoor lagoon with no crust.	There was no open barnyard holding lot for these cattle as they had access to a specific loafing barn	The remains of winter hay bales were present near the loafing barn.
MN	16 Aug 2010	Bottle calves were kept in individual dirt floored hutches then moved to a small group pasture with a dirt floored	Adult cows were on pasture at all times.	Manure was stored in piles near the calf hutches and spread on fields once or twice a year	There was no open barnyard holding lot for these cattle.	Rotten grain spilled under the edges of winter feeding areas was sampled as well as covered silage piles kept

		shed.				on concrete pads
MN	30 Aug 2010	Bottle calves were kept in indoor straw bedded pens in groups of three to four, then moved out into small pastures when weaned.	Adult cows were kept on pasture in the summer but allowed access to a straw bedded loafing barn during the winter months, this barn was sampled as the winter bedding had not yet been removed.	Manure was stored in outdoor piles until field space was available. Manure had been spread prior to our arrival and there were no piles available to sample	Adult cows were brought onto concrete floored pens and held prior to milking	There was no spilled or rotten grain or hay available to sample
MN	2 Sept 2010	Bottle calves were kept in individual dome hutches and bedded with wood chips then transferred to groups of six to seven calves in straw bedded sheds with outdoor access.	Adult cows had a dirt/concrete floored loafing barn where they received supplemental feed.	Manure was stored in a pile on a concrete pad and spread when time permitted. Manure had just been hauled and spread prior to our arrival and no fly pupae were found.	There was no open barnyard holding lot for these cattle as they had access to a specific loafing barn.	Spilled and rotten grain under the feed bunks was sampled for fly pupae.