Objective 1: New technologies for management of biting and nuisance flies in organic and conventional systems

a. Novel push-pull strategies (PPS)

Used in integrated cropping systems, the PPS relies on the manipulation of the pest by applying pressures to induce behavioral changes that result in less damage to the crop (Pickett 1997). PPS uses repellents, oviposition deterrents, and antifeedants to push the pest away from the crop. PPS couples these agents with other agents such as attractants and traps to reduce pest populations to below threshold levels. Application of PPS has utility in the management of pests in animal agriculture by providing alternative pasture fly management technologies, reducing pesticide use and contributing to a more sustainable production system (Cook et al. 2007).

Insect repellents are commonly used to protect humans from disease vectors, biting flies, mosquitoes, and ticks. Efficacy varies widely with formulations. For example mosquito repellents containing DEET (23.8%) providing the most protection (301.5 minutes), followed by natural products 2% soy bean oil (94.6 minutes), 10% citronella (19.7 minutes), and citronella blends with other oils (ranging from about 10-18 minutes) (Fradin and Day 2002). These natural products are listed among 31 minimal risk active ingredients exempt from the registration requirements of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (CFR40). The application of these "generally regarded as safe" or GRAS repellents could become a great benefit to producers wishing to reduce or eliminate reliance on pesticides when treating their livestock. Plant derived insect repellents fall into 3 broad chemical catagories; Alkaloids, Phenols and Terpenoids (Moore et al. 2007). Terpenoid insect repellents are most common and include a variety of known materials; citronella, limonene, eugenol, neem, and thyme. These compounds are known for repellency against mosquitoes and ticks, and many are also active against biting flies for livestock. At issue is that a biting fly repelled from one animal becomes a problem for another unless the fly is removed from the system. As a result, the use of repellents to push pests away, coupled with a lure to attract and remove the pest is the primary goal of PPS.

The potential benefits of plant oils as insect repellents for livestock would provide needed relief to animals from the persistent attack of biting flies. This is particularly important for small farms. By developing a PPS the majority of the animals could be treated with a repellent and one or two "trap" animals could be treated with an insecticide. One approach may be to apply insecticides to inanimate objects or targets designed to attract flies from repellent treated livestock.

Early mark-and-recapture studies, using surrogate animals, were designed to determine the mechanisms behind host finding behaviors (Kinzer et al. 1978). Marked horn flies were attracted to dark artificial cow shapes, emanating heated water vapor and CO_2 in the absence of real cattle. In field trials stable flies were highly attractive to CO_2, and white targets (Zhu et al. unpublished data). It is clear that flies use both visual and chemical cues to identify hosts and resting sites. In Africa, field observations indicated that stable flies were attracted to the blue and black cloth used for the Nzi trap for the control of tsetse fly (Mihok et al. 1995). In the US blue and black insecticide treated cloth targets have been used to effectively reduce stable fly densities (Foil and Younger 2006). Flies visually attracted to the blue/black color were killed by the insecticide following a 30s exposure. Research is needed to determine the feasibility of using similar target

designs for other pasture flies, optimizing distances and the number of targets necessary to achieve control. We anticipate that using these technologies in concert will enhance the effectiveness of PPS in livestock systems.

Novel push-pull strategies on Cattle: Various plant-based repellents (e.g. geraniol, catnip oil, palmarosa oil and others) will be evaluated in a push-pull system to manage fly populations on pasture cattle in Nebraska and North Carolina. Initial studies will be conducted in North Carolina using young dairy cattle, steers and heifers under pasture conditions. Dairy pastures in NC range from 2-5 acres. Initial studies will use small treatment groups divided equally among calves on 1 acre fenced plots with cattle in visual sight. At this small scale push pull is expected to be effective because abandoning flies will have alternate hosts in close proximity. Mark and recapture studies will be necessary to establish spatial limits for success at a large scale, see below. In subsequent studies in Nebraska, testing will be done with groups of 10 to 20 yearling heifers or steers (226 to 272 kg) in field conditions similar to common producer practice in West-Central Nebraska. Treatments, assigned to pastures, will be combinations of push (cattle treated with a repellent) and pull treatments. The pull treatment will be cattle without repellent (naturally attractive) treated with a conventional insecticide (lure and kill). It is expected that flies moving from cattle with the repellent will be managed after alighting on insecticide treated cattle. Treatment combinations applied to pasture cattle include: Pasture 1 - half the cattle with push and half with the pull treatment, Pasture 2 - push treatment only, Pasture 3 - pull treatment only, and Pasture 4 - water application (negative control).

Mark and Recapture studies will examine dispersal distances of horn flies forced to abandon repellent treated cattle. Horn flies will be collected from cattle, marked with fluorescent powders and release in the vicinity of repellent treated cattle. Untreated herds located at distances of 1 to 3 miles will serve as recipient animals. Collected horn flies from the recipient animals will be examined for color markers to establish maximum repellency distances. Although complicated a similar analysis was applied to stable fly dispersal (Taylor et al. 2010) using the Turchin and Thoeny (1993) model. Fly captures from distances of 1, 2, and 3 miles will be calculated as radial distance from the release site and analyzed using an empirical regression model to examine rates of decline in the daily catch rate with days after release and distance. Slopes for distance by day will be compared using analysis of covariance (Taylor et al. 2010).

Encapsulated catnip oil will be applied to stable fly oviposition substrate in emergence cages set in field locations attractive to ovipositing flies to push flies away. Other cages (without a repellent) will be treated with an insect growth regulator (Neporex 2SG) as the pull (lure and kill) strategy. The objective is to test if limited use of an IGR in a push-pull system can reduce fly emergence. If initial testing with emergence cages show promise, testing will be expanded to larger field situations and other species such as the house fly.

b. Evaluation of improved monitoring systems

Pest fly populations must be quantified in some manner so we may evaluate the effects of experimental treatments. Furthermore, pest monitoring a key to the successful IPM program because observers must know when economic thresholds have been exceeded. As a result monitoring flies usually relies on visual observation of either the insects themselves or

quantifiable indicators that insects are present. To avoid subjectivity, or observer variance, quantitative monitoring are considered more reliable. House flies within barns can be effectively quantified by counting the fly fecal and vomit drops left on index cards placed inside livestock facilities (Lysyk and Axtell 1985). Hogsette et al. (1993) effectively quantified flies using sticky cards. More subjective counting methods are those that count insects on predilections sites; stable flies on the legs of cattle and horn flies on each side of animals. While researchers routinely use these monitoring methods, use by producers is frequently unreliable. User friendly methods for monitoring pest populations are needed to allow farmers to implement control measures at the proper time.

Development of visual recognition software (Flyspotter®software) to automate the counting of speck cards has significantly reduced the time required to establish population thresholds (Gerry et al. 2011). Development of additional recognition software technologies to quantify flies would be a great benefit to the industry. Currently horn flies are counted visually by a trained observer. Studies comparing counts of trained observers and photographs indicate that trained observers can consistently provide reproducible estimates of horn fly densities, and do so much less expensively (Castro et al. 2005). However, this work was done with relatively low fly populations (<200). Studies conducted in NC in the summer of 2010 indicated that the time required for a trained observer to estimate fly densities on animals was about 1 minute per animal (fly numbers >500). Digitally photographing animals required about 3 minutes per animal, and counting flies on the digital images required another 30 minutes per animal, for a total of about 300-fold more time than visual field observations alone. This is a clear example of the need for improved technology to facilitate quantification of horn fly populations when numbers are high.

Estimating horn fly numbers on cattle with digital photography may be feasible using recognition software provided there is sufficient contrast between the flies and their host. The following tutorial demonstrates a technique that was used to count flying birds against a light sky and can be accomplished by a variety of image processing software (http://massapoag.org/journal/files/1e6160219ffb644d38ff9ebb0740c9aa-27.html). The limitations would be having to eliminate any part of the picture that isn't part of the host or flies prior to counting (because the whole image of an animal will include background images as well) and if the animal is dark or shaded, the flies may not contrast well enough for the software to distinguish them from the host.

c. Novel toxicants and delivery systems

Resistance to currently available insecticides remains a major problem for the control of house fly and horn fly in the United States. Resistance to pyrethroid insecticides has been detected in stable fly populations in Florida in recent years as well (Pitzer et al. 2010; Olafson et al. 2011). New insecticides with novel modes of action can be very useful in managing resistance problems. Novel toxins and delivery systems will be tested for their utility in fly control. Historically, pest control for Public health and animal health benefited from insecticides developed by major chemical companies for control insect pests of crops. There are several novel insecticides that are in the process of EPA registration for crop pests, including a number of new molecules (such as chlorantraniliprole and cyantraniliprole) of the diamide chemical

class. These molecules are ryanodine receptor agonist, causing impairment of insect muscle function which results in rapid cessation of feeding (Annan et al. 2011). A new insecticide (SYP-9080) with similar mode of action has also been developed. IPP-10 and cycloxaprid are two new neonicotinoid. Another novel insecticide, isoxazoline, is a potent blocker of insect ligand-gated chloride channels (Ozoe et al. 2010). Samples of these novel insecticides will be obtained and tested on the three major fly species (the house fly, horn fly and stable fly) using established bioassay techniques. A recently published study found no cross resistance between these novel molecules and currently used insecticides in the whitefly (Li et al. 2012). This is encouraging in term of resistance management. Other novel insecticides that are currently being registered or have been registered in recent years for controlling crop pests, such as pyrifluquinazon, sulfoxaflor, novaluron, methoxyfenozide, clothianidin, and flonicamid, will also be evaluated for fly control when possible.

Biopesticides received more attention in recent years (Geden 2012). Essential oils have been evaluated as insecticides for the control of various insect pests (Regnault-Roger et al. 2012). However, relative few studies have been conducted on essential oils for fly control. Zhu et al (2010) reported repellency and toxicity of the catnip oil against the stable fly. Essential oils are effective against the myiasis-producing fly, *Lucilia sericata*, in Egypt (Khater et al. 2011). A study is in progress at the USDA-ARS Kerrville laboratory to evaluate toxicity of various essential oils and other natural products against biting flies affecting humans and livestock.

In the present project, we will continue this effort by acquiring and testing new materials from collaborators within USDA-ARS, universities and international collaborators. Enhanced control can also be achieved through development of new insecticide formulations and/or delivery systems. This has been demonstrated in a study in Argentina with a new spot-on formulation containing chlorpyrifos for controlling horn flies on cattle (Juan et al. 2010). Similarly, a new remote insecticide application technology (VetCap) for control horn flies on cattle has been evaluated and validated by a study by Li et al (2011). This new insecticide delivery system developed by SmartVet™ is now commercially available to ranchers in the United States. We will continue to work with industry partners to develop more efficient and safe insecticide delivery systems for cattle ranchers in the U.S.

Autodissemination of pyriproxyfen: Pyriproxyfen (PPF) is a juvenile hormone analog that inhibits pupal-adult metamorphosis when applied to larval habitats (Invest & Lucas 2008, Seng et al. 2008). It has high activity against immature dipterans including mosquitoes and some flies (Hatakoshi et al. 1987, Kawada et al. 1987, Bull & Meola 1994). Although PPF can be applied as a broadcast larvicide, such treatments are labor-intensive and can have unintended effects on non-target species. Recently it has been shown that pyriproxyfen can be disseminated to aquatic habitats of mosquitoes by the adult females themselves; both in the laboratory (Gaugler et al. 2011) and field (Devine et al. 2009). In this "autodissemination" approach, adult female mosquitoes pick up a dust payload of PPF at stations, and transport that payload to egg-laying sites where the PPF is deposited along with eggs. The result is pinpoint delivery of a larval control product at the point where is it needed, and the results have been impressive (Devine et al. 2009). Recently house flies have been found to be highly sensitive to PPF as well, and early testing has already proved the concept that adult house flies can be used as autodissemination vehicles to transport PPF to fly larval breeding sites (Geden & Devine 2012). But several

questions remain. The first has to do with formulation. The formulations of PPF that are presently available are not of sufficient potency to provide the desired degree of control in autodissemination delivery systems, and we propose to develop and test new, higher-potency formulations using technical PPF. The second is to determine whether attract-and treat stations can be improved using novel attractants to increase the proportion of wild flies that are treated with PPF in field situations. The third question is whether PPF use is compatible with natural enemies of flies (discussed in section d).

Although many essential oils and fatty acids are primarily considered natural repellents, some of these natural repellents cause mortalities in treated insect populations. Previous work has shown that C8910, a mixture of octanoic, nonanoic, and decanoic fatty acids, has both repellent and insecticidal activity to pasture flies. The components of C8910 are generally recognized as safe (GRAS) by the FDA and have been in commercial use as direct food additives for decades. Water-soluble formulations of C8910 will be tested at the University of Nebraska West Central Research and Extension Center Wind Tunnel Evaluation Center. The most suitable formulation will be used in field studies on cattle at the University of Nebraska-Lincoln West Central Research Extension Center. C8910 will be compared to permethrin and water (control) treatments. Applications and fly monitoring will be repeated weekly throughout the fly season.

House flies, stable flies and face flies are commonly seen resting on various surfaces following feeding. These resting behaviors provide an unconventional control opportunity using insecticide treated targets and resting sites. Such control strategies may be designed to prevent insecticide exposures for humans and animals. The USDA CMAVE laboratory in Gainesville, FL will evaluate the efficacy of Vestergaard-Frandsen (VF) treated fence for management of stable flies and other nuisance flies. In this study, animals and structures to be protected from flies will be surrounded by the VF treated fence. Efficacy will be estimated by having comparable untreated control situations with monitoring devices (e.g. traps) inside and outside of the enclosures. Potential study sites include the National Zoo in Washington, DC; a dairy farm near Lincoln, Nebraska in cooperation with Dave Taylor; and an Exotic animal rescue unit near Gainesville.

Evaluation of toxic and non-toxic sugar baits for management of stable flies: For these studies candidate sugar baits will be evaluated in the laboratory and under semi-field conditions. Promising bait combinations will be evaluated in the field. Evaluations will be based on increased attraction by the bait when compared with similar situations without the bait. Potential study sites: Initial site will be in Gainesville, with other candidate site selected during the course of the project.

d. Non-pesticide management options

(i) BIOLOGICAL

Insect Pathogens*:* Several strains of the entomopathogenic fungi, *Beauveria bassiana,* have been tested for control of filth flies in agricultural systems. However, balEnce™, the *B. bassiana* product that is commercially available and labeled for house fly control, has had mixed results in the field. Laboratory studies have demonstrated that although the balEnce strain of *B. bassiana* (HF23) is highly pathogenic against house flies, the formulated product contained few viable

conidia and the product failed to perform better than a control treatment. The same lab-based studies identified another strain (GHA) as highly pathogenic against house flies. The strain is available commercially in two formulations: Botaniguard ES and the Organic Materials Review Institute (OMRI) approved Mycotrol O. Another product, MET 52 EC, containing the *Metarhizium anisopliae* strain F52 was also found to be pathogenic (although not OMRI approved). Finally, a *B. bassiana* strain (EN1) was collected from a Florida horn fly and is currently maintained in the laboratory. In laboratory evaluations, this strain has been found pathogenic to horn flies. The strain represents the first U.S.-collected *B. bassiana* strain from horn flies and the second reported in the literature.

Formulated strains of entomopathogenic fungi that are effective against house flies, stable flies, horn flies and face flies will be determined in the laboratory, and then tested for their ability to control these flies in the field. Additionally, we will select for increased virulence and evaluate the efficacy of the enhanced EN1 strain against these flies using similar protocols.

Initial laboratory experiments will involve the testing of commercially available formulated strains of *B. bassiana* GHA, HF23 and *M. anisopliae* F52 and the EN1 strain against house flies, stable flies, horn flies and face flies to identify the most efficacious product for each system. Flies will be exposed through contact assays using treated filter paper and then moved to holding containers in their treatment groups. Every 24 h dead flies will be counted and removed from the containers. Dead flies will be isolated for determination of sporulation at day 10 post treatment. For each species of fly and fungi, a dose response curve will be completed to calculate the optimum dose for treatment. The percent sporulation will be compared between doses and treatments.

In further laboratory studies, the most pathogenic fungi formulation/strain for each fly species will be tested in a suitable fungal application system. For example, house flies will be exposed to bait treated with fungal formulations and mortality determined. Choice tests will be completed to compare the attractiveness of the treated bait with naturally-occurring food sources. Different doses will be tested to generate dose response curves. Sporulation of the flies also will be monitored. The effectiveness of the fungi against stable flies will be tested by treatment of bedding containing stable fly eggs with a dry formulation. The percent emergence of adults will be calculated and compared with control treatments. Emerging adults will be held to monitor mortality and sporulation. Dead larvae or pupae in the bedding will be extracted and held to check for sporulation. As both horn flies and face flies are most likely to be controlled through contact with treated animals, these strains will be tested by exposing flies to treated cattle hide or a cattle hide substitute. The duration of activity could be determined by exposing the cattle hide to sunlight and evaluating the effect on fungal pathogenicity with increased UV absorption.

Following on from laboratory trials, fungal products that achieved successful control of flies in bioassays will be evaluated in livestock operations with nuisance fly problems. The effectiveness of the fungi at reducing fly numbers will be evaluated through the use of a suitable monitoring method for each fly species, before, during and after the treatment. On cattle farms the efficacy of baits will be tested by monitoring the effect on the resident house fly population with Scudder fly grids and sticky ribbons, before, during and after the treatment. Where situations allow baited traps will also be deployed. Field trials on equine facilities will test the application of the treated

dust to bedding for stable flies. On equine farms, the emergence (%) of adult stable flies will be monitored with emergence traps from both treated and untreated bedding and feeding sites known to produce stable flies. The effect of the treatment on the population will be monitored by counting the number of stable flies landing on the lower legs of horses in both treated and untreated stalls. Samples of larvae, pupae collected from breeding sites and any adults that eclose from collected pupae will be monitored for mortality and sporulation. The application of fungal formulation as liquids or dusts to cattle will be evaluated for horn flies and face flies. Any field work on face flies will be completed in collaboration with a state where they are an economically important pest. On-animal sampling of both horn flies and face flies are conducted by counting the number of flies on animals. Samples of flies will also be regularly taken following treatment to monitor the number of flies infected with fungi. Flies will be taken to the laboratory where they will be allowed to die naturally and then observed for sporulation.

Host preferences and parasitoid selection for augmentative releases: Modern livestock production systems contain a myriad of substrates suitable for production of house flies and stable flies. Although the two fly species are sometimes superficially sympatric they exploit different habitats for larval development and have different phenologies (reviewed in Hogsette and Farkas 2000, Geden & Hogsette 2001). Surprisingly little is known about the preferences of parasitoids for these two important pest species. In no-choice assays with "naked" pupae (no media to search through) the two hosts are attacked equally by parasitoids and produce similar numbers of progeny, suggesting no inherent fitness advantage of one host over the other (Geden et al. 2006). The only other information on host preference comes inferentially from various field collections of both host species for parasitoid emergence (Skovgaard & Jespersen, 1999; Romero et al., 2010; Pitzer et al., 2011; Meyer et al., 1991; Jones & Weinzierl, 1997). Taken together, the above studies suggest several items: 1) that *S. cameroni* attacks both target species at comparable levels and occurs across a wide geographic range; 2) that *S. nigra* preferentially attacks stable fly; and 3) that *Muscidifurax* spp. preferentially attack house fly. But can we rely on these inferences for IPM decision-making? Olbrich and King (2003) warn that the different phenologies of house flies and stable flies can distort the conclusions from field studies. For example, stable fly populations in the American Midwest peak in May-June and then plummet in midsummer when house fly populations are high. This can make it difficult to collect adequate numbers of both host species throughout the fly season, and the intervals when both host species are available to parasitoids in comparable numbers are short. If a species of parasitoid has a seasonality that coincides more with that of one fly host than the other, then a season-long view of the data can create the impression that it "prefers" the species with which it coincides. In this way, early-season parasitoids can appear to prefer stable flies whereas late-season species prefer house flies. In an augmentative release program such phenological distinctions are less important than innate differences in searching behavior and host preferences. We propose to conduct laboratory and field studies in Florida and Nebraska to identify differences in the host preferences of candidate parasitoids to select appropriate species for augmentative releases.

Improved monitoring of parasitoids for biological control: Augmentative releases of pteromalids such as *Spalangia* spp. and *Muscidifurax* spp. have proven highly effective at suppressing flies under certain conditions (Morgan & Patterson 1990, Geden et al. 1992b, Petersen & Cawthra 1995, Crespo et al. 1998, 2002, Skovgaard & Nachman 2005, Geden & Hogsette 2006). In other instances, parasitoid releases have had little impact on fly populations

or parasitism levels (Meyer et al. 1990, Andress and Campbell 1994, Weinzierl and Jones 1998, McKay and Galloway 1999, Kaufman et al. 2001). Releases are most effective when the released species is selected after initial surveys to identify the dominant species present. In the US, survey data for parasitism in house flies and stable flies are available from several states and production systems (Legner & Olton 1971, Rutz & Axtell 1981, Greene et al. 1989, Meyer et al. 1990, 1991, Jones & Weinzierl 1997, Kaufman et al. 2001a, Romero et al. 2010). Outside the US, surveys have been conducted in Denmark (Skovgard & Jespersen 1999, 2000; Skovgard & Steenberg 2002), Hungary (Hogsette et al. 2001), Israel (Havron & Margolit 1991), South Korea (Rueda et al. 1997), Malaysia (Sulaiman et al. 1990), India (Srinivasan & Balakrishnan 1989), China (Guo et al. 1997), Brazil (Ferreira de Almeida & Pires do Prado 1999, Monteiro & Pires do Prado 2000), Canada (Floate et al. 1999, McKay & Galloway 1999), and elsewhere. Although over a dozen species are commonly found in these surveys, six species typically make up the vast majority of collections: *M. raptor*, *M. zaraptor*, *S. cameroni, S. nigroaenea, S. endius* and *S. nigra*. Reliable estimates of relative abundance of the species present are essential to identification of the best candidate for use in biocontrol programs. However, comparisons among studies are confounded by differences in sampling approaches. Parasitoids can be monitored by either collecting wild fly pupae to be held for parasitoid emergence, or by the placement and retrieval of lab-reared sentinel pupae. Both approaches have advantages and liabilities but can produce very different pictures of overall parasitism rates as well as relative abundance of the species present; moreover, distortions due to sampling method can vary depending on the production system (Rutz & Axtell 1980, Meyer & Petersen 1982, Petersen & Watson 1992). As a result, there is still little consensuses on which species are the best candidates for augmentative releases. Recently a new sampling method has been developed that bridges the differences between the two prevailing methods. In this approach, sentinel house fly and stable fly hosts are placed in the field as larvae in their respective rearing media and retrieved after they have pupated and been exposed to wild parasitoids. This approach amounts to the placement of fly-breeding hot spots with hosts that have pupated in-situ with their attendant host and habitat kairomones. The results are impressive; the technique provides a highly sensitive method for detecting otherwise-rare species and consistently delivers higher rates of parasitism than either of the traditional methods. We propose to use this "improved sentinel method" to determine relative parasitoid species abundance in Florida and Nebraska) to narrow the range of species for consideration as augmentative biocontrol agents.

An experimental design to delineate phenological and host preferences was developed around a new sentinel sampling system. Pans containing house fly and stable fly larvae in rearing media were placed in a protective enclosure to prevent vertebrate molestation but allow access by parasitoids. These larval containers serve as fly breeding hot spots. On the medium surface of each pan is a screened satchel containing 100 pupae of the respective larval species in the pan. Another satchel containing house fly pupae without larval substrate is placed 1-2 meters distance and serves as the positive control. All items are recovered after 4 days, taken to the laboratory and sorted. Pupae found in the larval pans are gel caped for parasitoid emergence, pupae recovered from the sentinel bags are gel caped and held for parasitoid emergence.

Treatments are summarized in 5 classes: 1) House fly in substrate, 2) Stable fly in substrate, 3) House fly in satchel on substrate, 4) Stable fly in satchel on substrate, and 5) House fly in satchel 1-2 meters from main setup (control). Preliminary data suggests that this method corrects for the

seasonal phenological differences described above, provides parasitoids with choices to identify host preferences if any and to improve on the traditional sentinel sampling method used in livestock and poultry pest management.

(ii) CULTURAL

Animal bedding: The calf hutch environment is traditionally a problem area for dairy producers. Calf bedding soiled with manure and urine provides an ideal breeding site for stable flies and house flies (Schmidtmann et al. 1989). Various animal bedding and bedding treatments will be tested for fly control in dairy calf hutches. Companion small-scale studies in the laboratory will be conducted prior to on-dairy research to determine which bedding type and which bedding treatment results in the greatest reduction in fly larvae. Bedding types to be examined include wheat straw, sawdust from hybrid poplars, and pine wood shavings. Calf bedding will be treated with amendments including sodium bisulfate, sugar, kaolin, and diatomaceous earth. Fly numbers, pH, and moisture content of bedding samples will be monitored during the treatment period.

Bedding samples in calf hutches will yield seasonal abundance data for fly larval populations. At the same time, adult fly population abundance will be monitored with various devices, including sticky traps, white index cards, digital imaging, etc. Correlation analysis will be used to compare adult and larval counts which will help determine the best monitoring system for flies on dairies.

There is a growing body of evidence indicating the importance of lying for the health and productivity of lactating dairy cows. The relationship among lying behavior, health, productivity, welfare, and management is less understood for dairy calves. While dairy cows will typically spend 10 to 14 hr per day resting, dairy calves may spend 18 hr per day lying down and reduced lying times may result in reduced growth rates. Lying time has also been used to assess the adaptation of dairy calves to novel housing environments. Therefore, it is likely that management factors limiting this behavior may reduce the well-being of dairy calves. The objectives of this research are to a) develop a practical way to monitor flies affecting individual calves in their hutches and b) to assess the impact of fly populations on overall calf comfort (measured by behavioral and physiological differences).

Effects of winter bedding choices for dairy cows on subsequent stable fly populations the following spring and summer. Four herds of organic dairy cows will be housed at Morris, MN, in two replicate out-wintering lots with straw bedding packs and two more sawdust compost bedding barns in winters of 2013 and 2014. The main study will determine how the two winter housing systems affect dairy cow health and productivity. After cows are moved to pastures in spring, replicate conical fly emergence traps will be installed over each kind of bedding substrate to quantify stable fly emergence per unit area. Traps will be repositioned weekly on each pile until emergence ceases, and numbers emerged will be estimated by extrapolation from trap to pile area. Expectations are straw bedding packs will yield thousands of flies per week into August, but the compost bedding packs will yield none.

(iii) MECHANICAL

Bruce (1940) was the first to publish plans for a passive horn fly trap that consisted of a screen covered wooden frame sufficient in size to allow cattle to pass through. Curtains suspended from one end brushed flies from the animals as they passed through. Fleeing flies were captured in the screened hollow walls (Bruce 1940, Hall and Doisy 1989). Tozer and Sutherst (1996) modified the trap design with a translucent top to increase efficacy by increasing ambient light within the trap. This Australian Fly trap was more efficient than the Bruce trap. Similar fly traps continue to be used by producers with mixed results stemming from altered fly behaviors. Moreland et al. (1995) patented a modified Bruce fly-trap by adding a rigid canopy and black lighted electrified grids on the ceiling and side-walls. A centrally suspended curtain brushed flies from the surface of the animal as it passed through. For a time, disturbed horn flies, attracted toward the black lights, were killed in the electrocution grids (Watson et al. 2002). Although the trap significantly reduced horn fly densities, the cost (>$10,000) was unacceptable to producers (Surgeoner et al. 1998, Watson et al. 2002).

S-1030 researchers at NCSU have developed a unique vacuum pressure walkthrough fly-trap that physically removes flies from the cattle and the air surrounding the cattle as they pass through. Using this device, horn fly densities were kept below threshold levels for 14 weeks during peak horn fly season without the use of insecticides. Studies in North Carolina have demonstrated horn fly control with traps. Mean horn fly densities were above 700 per cow when the study was began. The fly vacuum was started on May 29, 2007. Within one week of operation the device removed 410,000 horn flies from the cattle passing through twice daily. By Sept. 26, 2007 over 2.4 million flies had been removed from 180 cows. These cattle have been insecticide free for 6 years.

Further study is needed to explore the efficacy of this trap for other species, particularly the face fly and stable fly. Regional efficacy studies demonstrating pasture fly control for milking herds for all three pasture flies are needed. Economic analyses are needed was well as comparative studies with similar devices.

Participating farms will be selected in winter 2014, based on presence of predominant fly problems and similar herd management. Surveillance of the existing fly problems will be initiated until cold weather terminates fly activity. Environmental data and phenological models to predict stable fly activity have been developed by S-1030 participants. Similarly these models will be used to predict the likely date that horn flies and face flies will break diapause (Lysyk 1999, Krafsur and Moon 1997), and those dates will be used to predict fly activity in each state.

Comparative study of the CowVac, Bruce and Australian walk through fly traps for the control of pasture flies. Our goal is to determine the efficacy of each system for horn fly, face fly and stable fly, and weigh the benefits against the cost of each trap and its upkeep. CowVac traps will be purchased for research from Spalding Labs, Reno, NV. Construction of the Bruce and Australian fly traps will be performed by the participating state following design schematics and assembled on site. Our goal is to demonstrate to the producers that these traps will significantly reduce fly densities to an acceptable level and maintain densities below economic thresholds without the use of insecticides. We will gain essential information on the efficacy of each trap

design for each pest species, horn fly, stable fly and face fly. In addition to monitoring the different flies on the animals, we will quantify the number of flies captured by the traps each week by removing the collection container and cold chilling the flies to immobilize. If fly densities are high containers may fill up, requiring twice weekly replacement. Flies will be transferred to plastic bags and frozen. Thawed flies will be air-dried and weighed. The total number of flies captured per week will be calculated by extrapolation from subsamples sorted to species.

Limited effective insecticides and/or repellents for management of horn flies in organic dairies necessitates the use of the Bruce (1940) trap in some organic dairies. The modified Bruce (Hall and Doisy, 1989) trap provides limited efficacy of 50-70%. Another trap, the Australian trap (Sutherst and Tozer 1995, US Patent 1993,), has been used more extensively in Australia and demonstrated 90-96% horn fly control in a Florida study (Tozer and Sutherest 1996). The Australian trap is simpler in design than the Bruce trap and possibly a more economical option for producers. This trap does not rely on trapping elements to catch the flies (as does the Bruce trap) but instead, dislodged flies will congregate on the translucent sides and top of the trap and die from desiccation. Although the Australian trap has been evaluated in a Florida study, direction comparisons with the Bruce trap and other "organic" methods are needed.

Comparative studies on the efficacy of the CowVac systems will be conducted on six organic dairy farms. Three farms will be receiving the original trapping system and three additional farms will receive the Spalding CowVac™. Studies are designed to compare trap efficiency for the number of flies captured and the range of species collected.

Additional studies will further develop and demonstrate walk-thru traps for summer horn fly control. A study at Morris, MN, will measure fly removal rates using different walk-thru fly traps in 2013 and 2014, and then demonstrate leading designs through on-farm studies in 2015. A modifiable Bruce trap with opaque or transparent roof and a modifiable Spalding CowVac™ running at half or full vacuum power will be installed on opposite sides of the dairy parlor entryway. Known numbers of horn flies marked with fluorescent dusts will be released onto four cow subherds that will be walked through the four kinds of traps to estimate percent flies removed per passage. Captured flies will be released back onto the cows after milking, a different color of dust will be used each week, and flies captured in subsequent weeks will be used to estimate natural disappearance. Fly reproduction in pastures will be measured by sampling cow dung pats to quantify new fly emergence (Moon et al. 1993). Efficacy of different traps with varying removal rates will then be modeled in a spreadsheet, and results will be used to determine for a hypothetical herd, which (if any) of the four trap designs would be able to keep horn flies under control. Expectations are a Bruce trap with transparent roof and CowVac™ at full power will be equivalent, but the other two designs will be inferior.

In 2015, six cooperating organic dairy farms will be enlisted to evaluate traps. Treatments will be no trap (untreated control), a Bruce trap, or a CowVac. The three treatments will be run in a cyclic crossover design among farms and months (June, July and August), such that each farm will receive all three treatments within the same year. Flies will be counted on the milk cows at the six farms twice per week from mid-May through late August, and monthly fly population growth rates (changes in numbers in log scale) will be compared among farms when the different

traps were in operation.

Objective 2: Insecticide resistance detection and management

Over the last five years several groups have documented issues associated with insecticide resistance in house fly, stable fly and horn fly (Barros et al. 1999; Byford et al. 1999; Foil et al. 2005; Foil et al. 2010; Li et al. 2009; Olafson et al. 2011; Pitzer et al. 2010; Rinkevich et al. 2012; Sabatini et al. 2009; Temeyer et al. 2008). Especially notable were documented cases of resistance to some of the first neonicotinoid insecticides (Gerry and Zhang 2009; Kaufman et al. 2007; Kaufman et al. 2010), and the first national survey of insecticide resistance in house flies. Organophosphate, carbamate, pyrethroid and neonicotinoid insecticides are currently the major classes of insecticide used for house fly control in the US. Specific examples of previous and ongoing work are given below.

House flies evolve resistance to pyrethroid insecticides due to mutations in the voltage sensitive sodium channel. Three *Vssc* alleles are known to confer resistance to pyrethroid insecticides: *kdr*, *kdr-his* and *super-kdr*. There have been multiple evolutionary origins of each of these alleles (Rinkevich et al. 2012). However, the level of resistance conferred by these alleles (*super-kdr* > *kdr* > *kdr-his*) is not consistent with the frequency of these alleles at many locations in the USA (Rinkevich et al. 2007; Rinkevich et al. 2006).

Selection of field collected house flies with imidacloprid resulted in a strain with >1000-fold resistance. The resistance has a significant fitness cost under laboratory conditions. Studies on the linkage, inheritance and mechanisms underlying this resistance are underway.

Stable fly susceptibility to a commonly used pyrethroid (permethrin) was determined in Florida to assess the possibility of resistance development. Diagnostic concentration evaluations of three stable fly field strains demonstrated a maximum of 57 and 21% survival to permethrin residues of 3X and 10X the LC_{99} of a susceptible strain, respectively (Pitzer et al. 2010). Stable flies from an equine facility with no reported insecticide use demonstrated approximately 20% survival with a 3X diagnostic concentration. Despite a distance of 91-km between field collection sites, survival profiles of field collected stable fly strains were similar. Although an established stable fly colony collected from a local dairy previously expressed low level resistance to permethrin residues, five generations of laboratory permethrin selection increased resistance 15-fold. Surprisingly, the resistance mechanism appears to be the *kdr-his* mutation in the voltage sensitive sodium channel (*Vssc*) (Olafson et al. 2011), rather than the more common *kdr* mutation. The *kdr-his* mutation is common in house fly (and stable fly), but rare in other insects.

Recent studies indicate that cyromazine is an effective agent for controlling stable flies developing in winter hay feeding sites (Taylor et al. 2012) and laboratory studies indicate that novaluron may be effective as well (Lohmeyer and Pound 2012). Both compounds are considered insect growth regulators, disrupting molting by interfering with chitin synthesis and deposition (Doucet and Retnakaran 2012). However, they belong to distinct chemical classes (cyromazine is a triazine derivative whereas novaluron is a benzylphenyl urea) and have different mechanisms of action (Doucet and Retnakaran 2012). Rotation of these compounds may be an effective method for delaying the development of resistance in stable fly populations.

Understanding mechanisms of resistance to these compounds is necessary before a rotation program can be developed. Previous studies on cross-resistance between benzylphenyl urea and triazine compounds have had conflicting results. Genes responsible for resistance to these two classes of compounds were found to be at the same locus (or closely linked loci) in house fly (Shen and Plapp 1990). In contrast, cross-resistance was found to be asymmetrical dependent upon which class of compounds was used for selection in laboratory studies with *Lucilia cuprina* (Levot and Sales 2004), and although resistance was found to both benzylphenyl urea and triazine compounds in house fly populations from Denmark, there was no correlation between the two (Kristensen and Jespersen 2003). Resistance to cyromazine has been detected in the US (Iseki and Georghiou 1986; Scott et al. 2000) and resistance was readily selectable from field collected house flies (Bloomcamp et al. 1987).

A multiplex polymerase chain reaction assay was developed to detect *kdr* and a recently reported G262A mutation in the horn fly acetylcholinesterase. Horn fly populations from Texas, Louisiana, Washington, Georgia, Mexico, and Brazil, were found to have *kdr* and this allele was more prevalent in females than males. The G262A acetylcholinesterase mutation was found in Texas, Louisiana, Washington, Georgia, and Mexico, but not Brazil. There was no correlation between the occurrence of the *kdr* and the G262A mutations. The lack of correlation between organophosphate resistance levels and the frequency of the G262A mutation suggests it is likely there is an additional resistance mechanism in organophosphate-resistant horn fly populations.

a. Assessment of insecticide resistance

Resistance monitoring efforts will again be carried out in many states with an effort to document resistance levels, reversion of resistance and the evolution of resistance to new insecticides that become available for fly control. In addition to using bioassay methods, molecular techniques (sequencing of PCR products, multiplex PCR, etc.) will be used to evaluate the frequency of important resistance alleles in house fly, stable fly and horn fly (Foil et al. 2010; Kozaki et al. 2009; Rinkevich et al. 2007). This two-pronged approach helps to not only document the level of resistance found, but also the underlying causes. In addition, studies will be carried out to determine mechanisms of resistance to neonicotinoid insecticides, mechanisms of resistance to pyrethroids and organophosphates, and patterns of cross-resistance between insect growth regulators. Specific examples are given below.

Recently a strain of house fly was collected from a Florida dairy and selected with the neonicotinoid insecticide imidacloprid. High levels of resistance (>1000-fold) were found in this strain. Work is underway to characterize the stability of the resistance, the number of genes involved in the resistance and to identify the mutation(s) that confer the resistance.

A nationwide survey for stable fly resistance to permethrin will be conducted. Treated jars will be shipped to collaborating scientists and results will be compiled by the University of Florida. Flies that survive the bioassay will be frozen and archived for evaluation of *Vssc* mutations using molecular techniques. Although a mutation in the stable fly *Vssc* has been described, its absence from field collections is probable and would support the presence of other *Vssc* mutations or other mechanisms of resistance unrelated to the target site. These specimens would enable additional screening. There is a disconnect between the levels of resistance conferred to

pyrethroids (at 25 ºC) by the three different *Vssc* alleles and their relative frequency at several sites. We will evaluate the level of protection these alleles confer at 20, 25 and 30ºC in side-by side experiments. Such information will be valuable in understanding the role of temperature in fitness of these alleles.

Resistance to benzylphenyl urea and triazine compounds has not been reported for stable fly. Therefore, we will use house fly as a model to evaluate cross-resistance and synergy between these compounds. Cyromazine resistant house fly colonies (3-4) will be established by collecting house flies from poultry operations with a history of using Larvadex (Bloomcamp et al. 1987). Susceptible colonies will be acquired from institutions maintaining susceptible house fly colonies. Bioassays will be conducted to establish LC_{50} values for cyromazine and novaluron for each colony. If novaluron resistance is observed in cyromazine resistant colonies, they will be crossed with susceptible colonies and progeny independently selected for cyromazine and novaluron resistance. Lines will be evaluated for susceptibility to both insecticides after selection. Synergistic effects of cyromazine, novaluron, and pyriproxyfen on stable fly and house fly will be evaluated as well.

Assessment of insecticide resistance in horn flies collected from beef and dairy herds and stable flies from dairy, beef and equine farms will include field surveys using multiple existing registered chemistries. Up to 10 farms will be surveyed and their resistance to insecticides in the pyrethroid (Type I and II) and organophosphate classes will be determined, with other classes added as available. Genetic profiling of for acetylcholinesterase (*Ace*) and *kdr-type* mutations (*Vssc*) as well as other biochemical assays will be carried out on these same fly populations. This study provides an opportunity to identify stable fly populations that may exhibit OP-resistance, facilitating identification of mutations occurring within the stable fly acetylcholinesterase gene (*ScAChE*) that associate with the OP-resistant phenotype.

b. Leveraging the *Stomoxys* and *Musca* genomes for novel control measures

The house fly genome was recently sequenced, is currently being annotated and will be publically available in 2013. Generation of an inbred stable fly strain for the genome sequencing was completed and genomic DNA was provided to the sequencing center with a goal of having the genome sequenced in 2013. Having these genomes will allow new insight into the biology of these important pests and may offer novel methods for control. This could be achieved in many different ways: developing inhibitors of key enzymes, new methods for sterile male production and release, or RNA interference (RNAi) to silence individual genes (novel control strategy). Relative to the house fly, groups are planning to exploit the genome for better understanding of pathogen defense xenobiotic defense, sex determination and potential control strategies.

Sequencing of the house fly and stable fly genomes also offers the potential for rapid identification of the mutations responsible for resistance. Identification of the alleles responsible for resistance allows for detailed studies of the evolution of resistance that are not possible with bioassays. We propose to use high-throughput Illumina sequencing of transcripts between resistant and susceptible house fly strains to rapidly identify the basis of resistance. This would be done for both spinosad and imidacloprid resistant strains.

Given the importance of olfaction to stable fly development (host location, oviposition), a more complete understanding of the genes involved in the olfactory pathway provides a means to rapidly screen attractant/repellent compounds *in vitro* to evaluate possible use in field settings. Transcriptome data have provided insight into stable fly olfactory genes (Olafson et al. 2011; Olafson et al., 2013); however, availability of a genome sequence would greatly strengthen this base, especially with respect to identifying the repertoire of ligand-selective odorant receptors that are known to be highly divergent within insects. Once identified, these receptors will be isolated and used *in vitro* for screening compounds of interest.

Objective 3. Investigation of the microbial ecology, epithelial immunity, and vector competence of biting and nuisance flies

a. Identification of the key bacterial strains and their metabolites playing a major role in oviposition and larval development of stable flies

Chemical ecology stable flies: Despite the progress in development of IPM strategies, the management of stable flies still relies heavily on use of chemical insecticides which results in development and selection of insecticide resistant pest populations, potential contamination of the environment and food products, and killing of non-target insects. In this project, attractant and repellent compounds emitted from larval developmental substrates (aged and fresh cattle and horse manure) will be identified by GC-MS-EAG analysis and tested in oviposition assays in the laboratory. As part of the push pull strategy, the attractant lures will be developed in combining with the trapping systems (alsynite trap and ovi-trap) to improve stable fly trap catch efficacy in the field, reducing stable fly attacks on cattle and horses and further infestation. We will also study potential unfavorable factors (oviposition deterrent and larvicidal active components) for deterring stable fly oviposition and larval development. Push-pull strategy has been mostly applied in agricultural crop pest management, which relies on the manipulation of the pest by inducing behavioral changes that result in less damage to the crop. Such a strategy can be developed for animal protection. The majority of the animals could be treated with a repellent and a few "trap" animals treated with an insecticide. In addition, the application of oviposition deterrents in areas/media where female flies may lay eggs can further reduce their further damages. Application of Push-Pull strategy in managing pests in animal agriculture can provide alternative pasture fly management technologies, therefore reducing pesticide use that will contribute to a more sustainable production system.

Gut morphology of stable fly larvae and identification of a potential physiological gradient along the digestive tract: Although it has been documented that stable fly larvae require microorganisms for development, little research has been conducted examining this microbial-larval relationship. Even less is known concerning larval digestion and physiology. Understanding the physical and chemical environment that ingested organisms are subject to will stimulate focused areas for research on microbial-larval interactions. In this study, a combination of histological and microscopic techniques will be used to delineate and characterize the anterior, mid, and foreguts of first, second, and third instar stable fly larvae. Additionally, a series of pH indicators will be fed to larvae removed from development media for 8 and 15 h so that different physiological regions of the digestive tract may be identified.

Inter-kingdom communication via quorum sensing - a mechanism for regulating blow fly behavior: We will focus on the interkingdom signaling between bacteria and blow flies. As a model, we will use *Proteus mirabilis* (known to use quorum sensing for swarming over resources) and the blow fly (*Lucilia sericata*) since we have identified via pyrosequencing a *P. mirabilis* bacterial strain harbored in the *L. sericata* salivary glands and since *Proteus* sp. are known to attract blow flies. *The transformative aspects of this research* are that we will (i) discover the mechanisms of interkingdom communication between the insects and bacteria so that we may (ii) disrupt this signaling process to increase food safety and biosecurity as well as (iii) to control biofilm formation and thereby revolutionize treatments for nearly all bacterial infections. Many groups focus on either signaling in insects or bacteria but rarely are the two kingdoms studied together as a system as we propose to do here. Our specific aims are to (i) Determine the chemical cues of bacterium *P. mirabilis* that attract the fly *L. sericata*, (ii) Determine the compounds of the fly *L. sericata* that inhibit the biofilm formation of many strains, (iii) Characterize the molecular profiles associated with the microbial community structure (species), function (metabolism of different carbon sources), and chemical signaling on carcasses in the natural environment in order to identify new interkingdom signals, and (iv) Develop systems simulations of biofilm formation/swarming on carcasses and its impact on blow fly distributions in population centers. Therefore, we will utilize engineering/biological approaches to discern fundamental (mechanistic) aspects of signaling between the multi-cellular fly and the bacterium behaving as a primitive tissue (swarming mass) which will lead to our ability to control fly behavior as well as to control biofilm formation for food, medicine, and engineering applications.

By discovering the interkingdom relationships between insects and bacteria, we will determine methods to control (i) these insect pests and (ii) biofilm formation as well as other QS related phenotypes. These results will have a transformative impact on national needs in terms of food safety, biosecurity, and medicine. In regard to food safety and health, blow flies are a serious detriment to livestock, poultry, and surrounding communities due to their development on decomposing materials (Graczyk et al. 2001) which leads to the transmission of over 100 pathogens (Greenberg 1973) Flies feed and defecate on food resources which likely contributes to food-related illness that results in 325,000 hospitalizations and 5,000 deaths annually in the U.S. alone (WHO 2009) with estimates of economic cost of all interactions between $152 (Scharff 2010) and $1,426 billion (Roberts 2007). In regard to biofilm formation, 80% of human bacterial chronic inflammatory and infectious diseases involve biofilms (Barraud 2009); hence, a detailed understanding of the genetic basis of biofilm formation is necessary to determine effective cures and prevent biofilm infections. Biofilms related to chronic wounds alone cost $25 billion each year in the U.S. (Petera 2010).

Fly behavioral responses to microbes: We have published data demonstrating the black soldier fly, *Hermetia illucens* (L.) larvae, which is another colonizer of decomposing carcasses (Erickson 2004), reared in dairy manure for 72 h, reduced *E. coli* by eight orders of magnitude (Liu 2009), and it is hypothesized that the bacteria serve as nutrients for larval development. Therefore, our data show flies alter bacterial populations. Furthermore, we have conducted a series of studies elucidating the relationship between microbes on decomposing animal tissue and their role as cues for resource location by blow flies. *C. macellaria*, which is similar in nature to *L. sericata*, prefers fresh liver ($t = -2.87$; $df = 26$; $P < 0.05$), while *C. rufifacies* prefers

aged liver ($t = 3.89$; $df = 26$; $P < 0.05$) based on residence time after a five minute exposure period in a Y-tube olfactometer. These data explain previous reports that *C. macellaria* arrive on fresh carrion and *C. rufifacies* prefer carrion that has decomposed for three or more days. Using similar methods, we determined that microbes cultured from the liver, and not the liver itself, release volatiles that attract specific blow fly species seeking resources for their offspring. Both *C. rufifacies* ($t = -2.47$; $df = 58$; $P < 0.05$) and *C. macellaria* ($t = -2.66$; $df = 58$; $P < 0.05$) preferred bacteria grown on agar over agar without bacteria. Further, we determined that the arrival sequence of adults along with state of decomposition of the remains play a role in the attraction of *C. macellaria* and *C. rufifacies*. Eggs, less than three hours after oviposition by *C. macellaria* ($\chi2 = 10.32$; $df = 25$; $P = 0.001$) and *C. rufifacies* ($\chi2 = 6.267$; $df = 25$; $P = 0.012$), attract intraspecific adults. These data have been used to refine the behavioral assays previously described.

Microbial ecology and excretome of blow flies: Blow flies are known mechanical vectors of human and animal pathogenic bacteria. Contamination of food, feed, and animals can occur when flies regurgitate, defecate or manually contact surfaces and deposit pathogens. We know that bacteria are integral to filth fly development and behavior and are just beginning to understand the importance that bacteria and other microbes play in the ecology of flies and the pathogens they transmit. In our effort to understand pathogen transmission processes, we are studying the microbial communities in the excreta (regurgitant + defecant) deposited by blow flies. Using massive parallel sequencing (454 pyrosequencing), we have identified over 600 bacterial taxa, 22 viral taxa, and 5 fungal taxa associated with the excretome of the black blow fly, *Phormia regina*. During the next five years, we plan to obtain and analyze the excretomes of four to five other blow fly species of medical and veterinary importance. To accomplish this, we will confine flies to a small area with clean glass plates, allowing them to regurgitate and defecate on the surface. Fly spots will be scraped off and the DNA and RNA extracted. Using standard methods, methods, the DNA and RNA will be subjected to next generation sequencing (454 pyrosequencing) both massively parallel sequencing and 16SrDNA sequencing. After annotation, data sets will be analyzed using Megan4 software, which will compare sequences to the NCBI database, identify taxa at various levels, group sequences into metabolic functional groups, and identify metabolic pathways.

We will generate searchable databases that will enable researchers to understand microbial ecology of these flies, the possible impact of bacteria and bacterial metabolites on fly behavior, and gene exchange between species and populations.

We expect to identify unique pathogenicity islands or gene clusters that evolve through association with the fly gut or crop. The data we obtain will be used to generate federal funding through USDA and NIH to study impact of flies on human/animal health.

b. Investigation of the midgut epithelial immunity of house flies, stable flies and biting midges

***Epithelial immunity of house flies*:** Several studies have shown that bacteria acquired by flies have varying fates in the alimentary canal, where some species may persist for days within flies (e.g. *Salmonella* spp., Greenberg et al., 1970; Chifanzwa and Nayduch, unpublished) and others

are just transient residents subject to lysis and/or excretion via peristalsis (e.g. *Streptococcus pyogenes*; Chifanzwa and Nayduch, unpublished). We hypothesize that one mechanism impacting the fate of bacteria in the fly midgut involves mediation by secreted effector molecules. Ingested bacteria face an onslaught of defense mechanisms in the fly midgut including physical barriers (peritrophic matrix), digestive enzymes (Terra et al., 1988) and secreted humoral defenses such as lysozyme (Cancado et al., 2008) and antimicrobial peptides (AMPs; Lemaitre and Hoffmann, 2007). Since these effector molecules directly lyse bacteria, assessing their temporal and spatial expression in the alimentary canal could provide insight into their role in antibacterial epithelial defenses and subsequently bacterial fate. Preliminary studies in my laboratory have shown that the alimentary canal of the house fly expresses the AMPs *defensin*, *cecropin* and *diptericin* and the digestive enzyme *lysozyme* on an mRNA level in response to bacterial feeding, irrespective of bacterial species. However, the temporal pattern and intensity of upregulation appears to be both species- and dose- dependent. Analysis of protein expression is ongoing, but recent data have shown that AMP protein spatial and temporal expression patterns of these effector molecules do not entirely correlate with mRNA expression. Interestingly, we have found that AMP protein is expressed only proximal to bacterial presence in the gut, and thus is either temporary (for some species of bacteria that progress rapidly through flies) or sustained (for species that persist in the gut). Interestingly, all three AMPs and lysozyme do not show the typical IMD or Toll specificity for peptidoglycan type (i.e., DAP-type or LYS-type, respectively) which differs from these microbial class-specific responses reported for *Drosophila melanogaster* (Lemaitre et al. 1997; Lemaitre and Hoffmann, 2007).

The proposed study will further examine the temporal and spatial local expression of these molecules (lysozyme, AMPs) on both the transcriptional and peptide levels, using qRT-PCR and immunoflourescence microscopy, respectively. The opportunity to investigate the expression of other components of the epithelial immune response may exist during the course of this project, as the sequencing and annotation of the house fly genome and several different transcriptomes is now under way (Jeff Scott, pers. comm.). This may include additional AMP effector molecules, transmembrane or secreted molecules used to detect bacterial components, or second messenger components. Primer validation, standard curve generation, and qRTPCR optimizations have already been performed for the genes *cecropin*, *defensin*, *diptericin*, *lysozyme* and the calibrator *rpS18*. Likewise, custom polyclonal antibodies have been generated for the protein products of these AMPs and lysozyme, and protocols for immunofluorescence on sectioned alimentary canals have been optimized and validated.

The investigations of immune effector expression (described above) will be examined along with concurrent assessment of bacterial location (via microscopy of the alimentary canal) and persistence/fate (via culture-recovery on selective media). This is facilitated by using GFP-transformed, antibiotic-resistant strains of these pathogens as previously described (McGaughey and Nayduch, 2007; Doud and Zurek, 2012).

Bacterial species used in this study will include both Gram negative and Gram positive human and animal pathogens such as *E. coli* O157:H7, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. Previous studies have demonstrated that flies are suitable vectors for these species of microbes, and information exists on their proliferation and persistence potential within the alimentary canal (Greenberg et al. 1970; Kobayashi et al. 1999;

Rahuma et al. 2005; Doud and Zurek, 2012). The innovative approach of the proposed study is to simultaneously examine the house fly-bacteria interaction from both the perspective of the microbe (i.e., location and survival within the alimentary canal, gleaned from previous or concurrent studies involving microscopy and culture) and the house fly (i.e., expression of antibacterial responses when bacteria are in these locations, both on the mRNA and protein level). The interplay between the timing of house fly defenses and concurrent location/status of bacteria underlies bacterial fate and ultimately transmission potential. While providing insight into the biology of house fly-microbe interactions, these studies may also reveal information that could possibly lead to novel targets for controlling the dissemination of bacterial diseases by flies.

Epithelial immunity of stable flies: The stable fly produces three known AMPs that are specifically expressed in the anterior midgut. Two defensin-like molecules, *Smd1* and *Smd2* (Lehane et al., 1997; Munks et al., 2001), as well as a unique molecule, *stomoxyn*, which demonstrates antimicrobial, antifungal, and anti-trypanolytic activity (Boulanger et al., 2002), are all expressed constitutively, and this expression is restricted to adult flies. *Smd1* and *Smd2* display increased expression in response to ingestion of a bacterial-spiked meal (Munks et al., 2001), while levels of *stomoxyn* remain unchanged in response to bacterial assault (Boulanger et al., 2002). In addition, a stable fly defensin sequence annotated as fat body-specific has also been deposited in GenBank. A transcriptome database representing stable fly genes expressed throughout development (Olafson et al., 2010) revealed the presence of additional genes with putative roles in epithelial innate immunity, including a fourth defensin-like protein (*Scal-defensin*) and at least four unique lysozyme-like sequences (*Scal-Lys1, -Lys2, -Lys3,* and *–Lys4*) that are currently being characterized. Numerous pattern recognition receptor-like transcripts were also isolated, including those encoding peptidoglycan recognition-like (PGRP-like) and gram-negative binding-like (GNBP-like) proteins, which are pathogen sensors that recognize bacteria, fungi and viruses. One transcript in particular appears to be expressed only during the immature stages, suggesting the presence of life stage-specific immune response transcripts. Given that stable flies require a microbial enriched environment for oviposition and larval development (Lysyk et al. 1999; Romero et al. 2006; Castro et al. 2007; Talley et al. 2009; Castro et al. 2010), we are interested in understanding the pathway of genes that are critical in the stable fly's response to bacterial isolates that appear to be either required or not for development. We hypothesize that immune response transcripts are differentially expressed in immature and adult stages relative to the importance of a bacterial isolate for oviposition and/or larval development. Results from this study will provide insight into stable fly genes that may be critical for its survival in a microbe-rich environment, providing viable targets for development of control alternatives.

We propose to evaluate the temporal and spatial expression pattern of *Scal-defensin* and the four *Scal-Lys* transcripts to describe the developmental stages and tissues in which the transcripts are expressed. Next, we will evaluate expression of these molecules in response to ingestion of three different bacteria isolates, *E. coli*, *Citrobacteri freundii* (effective at promoting stable fly oviposition; Romero et al. 2006), and *Serratia marcescens* (a poor ovipositional substrate; Romero et al. 2006). To facilitate monitoring location of these bacterial isolates in immature and adult stages of the stable fly, an existing GFP-expressing *E. coli* strain will be utilized (provided by D. Nayduch) and GFP-expressing *C. freundii* and *S. marcescens* strains will be produced in

order to visualize location of the bacteria over time using epifluorescent microscopy. Natural infection of adult flies with these bacterial isolates will rely on oral ingestion delivered in a droplet as described for house flies in McGaughey and Nayduch (2007), but modified for biting fly feeding behavior. The response to oral ingestion of bacteria will be evaluated in newly eclosed adults starved for 24 hours. Starved flies (N=30) will be individually fed a known quantity of the bacterial isolate diluted in an artificial bloodmeal and evaluated at 0h, 2h, 4h, 6h, 12h and 24h post-ingestion. At each timepoint, five flies will be anesthetized and the digestive tract (proventriculus, crop, midgut, hindgut, rectum) and fat bodies dissected and stored separately in RNALater at -80C until ready to process. The entire feeding experiment will be repeated twice for a total of three biological replicates. Total RNAs will be isolated from tissues at each timepoint post-ingestion using a modified TriZol (Sigma)/RNEasy Mini (Qiagen) protocol and subsequently treated with DNAse to remove contaminating genomic DNA. Complementary DNA (cDNA) synthesis will be primed using oligo-dT_{20}VN and reverse transcribed from an equivalent quantity of total RNA using SuperScript III Reverse Transcriptase (Invitrogen). The cDNA will be used as template to evaluate expression of the fourth *Scal-defensin* and the four *Scal-Lys* transcripts using relative real-time reverse transcription PCR (RT-qPCR). RT-qPCR will be optimized using iTaq SYBR Green Supermix with ROX (BioRad) and data collected and analyzed on an ABI7000 Sequence Detection System equipped with SDS software, v.1.2.3 (Applied Biosystems).

Natural infection of larvae will be conducted in plates on egg yolk medium inoculated with the three selected isolates, essentially as described in Watson et al (1993) and Lysyk et al (1999). For each bacterial isolate, 40 surface sterilized eggs will be plated on inoculated media and five each of embryo and surviving first, second and third instar larvae will be snap frozen and stored at -80C until ready to process. The entire feeding experiment will be repeated twice for a total of three biological replicates. As a control, replicate samples of immatures reared on a laboratory diet will be collected and stored in the same manner. This will enable us to evaluate the larval immune response to individual isolates relative to an enriched community available via diet. Total RNA isolation and cDNA synthesis from all larvae will be conducted as described for adult flies, and RT-qPCR will be used to evaluate differences in level of transcript expression, if any, as a result of exposure to individual isolates. ANOVA statistics will be used to test whether differences in transcript expression post-ingestion, if any, is significant. Existing information regarding suitability of a bacterial isolate for either adult oviposition or larval development will be used as a covariate in statistical analyses to identify any influence of the parameter on gene expression results.

Microbial ecology and vector capacity of Culicoides sonorensis for BTV and EHDV: The biting midge, *Culicoides sonorensis,* is an important vector of orbiviruses, including bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV), which are significant pathogens of domestic and wild ruminants. No effective mitigation strategies exist for controlling *Culicoides* biting midges, and little is known about factors contributing to their vector competence for orbiviruses. Larval *Culicoides* develop in microbe-rich habitats and the adult gut is colonized with bacteria. However, how the microbial communities in the natural developmental substrate and in the digestive tract of *C. sonorensis* impact midge development, fitness, and ultimately vector competence for orbiviruses is unknown. Our project goals are to: (1) evaluate microbe-midge interactions in relation to midge life history and fitness and (2)

assess the impact of the gut microbiota on the vector competence of *C. sonorensis* for BTV and EHDV. Our approach will be to characterize the microbial community of natural larval habitats, and gut of larvae, newly-emerged and wild-trapped adults using culture dependent and culture-independent (16S rDNA pyrosequencing) approaches. In the laboratory, *C. sonorensis* will be reared on individual and combinations of identified bacterial strains and the impact of microbes on midge development and fitness will be determined. Additionally, we will assess the effect of diet on the gut microbial community of adult females and evaluate the effect of the gut microbiota on vector competence for BTV-17 and EHDV-2. A better understanding of midge-bacterial interactions will result in the establishment of a new platform for the development of alternative strategies for managing *C. sonorensis* and BTV and EHDV.

c. Animal and human pathogen acquisition, dispersal, and deposition by house flies

***Assessment of house flies serve as a potential sensitive bio-indicator of genotypic diversity of Escherichia coli O157:H7 and other Shiga-toxigenic E. coli* (STEC):** *The detection and culturing of STEC from cattle feces typically relies on the enrichment and immunomagnetic separation (IMS) techniques that likely lead to selection of only some STEC genotypes (Bach et al., 2002). In our studies with house flies, we use direct plating of the fly homogenate without the need of enrichment and IMS (Alam and Zurek, 2004; Sanderson et al., 2006; Ahmad et al., 2007). House flies (HF) commonly build up very large populations on cattle farms and other animal facilities. We* reported t*he E. coli O157:H7 prevalence of 2.9% and 1.4% in HF collected in a cattle feedlot from feed bunks and cattle-feed storage, respectively* (Alam and Zurek, 2004). *E. coli O157:H7 counts ranged from* 3.0×10^{1} to 1.5×10^{5} *CFU per fly. The majority (>90%) E. coli* O157:H7 isolates (n=125) possessed the virulence genes *stx1, stx2*, and *eaeA* (Alam and Zurek, 2004). In another study, we have shown that house flies can transmit *E. coli* O157 to cattle and likely play a role in the ecology of STEC in the cattle environment (Ahmad et al., 2007).

In the study of Sanderson et al. (2004), we reported the prevalence and longitudinal distribution of *E. coli* O157 in feedlot cattle and the feedlot environment, including house flies. A single PFGE type predominated in all the cattle samples collected. The same PFGE type accounted for many of the house fly isolates, however, certain PFGE types were only found in isolates from house flies. These data suggest that house flies serve as a potential sensitive bio-indicator of genotypic diversity of *E. coli* O157:H7 and other STEC.

The fresh cattle fecal samples from two dairy and two feedlot cattle farms (n=120 each farm) will be sampled and screened by culturing (enrichment and IMS) for STEC over three summer months as described elsewhere in this proposal. At the same period of time, house flies from those four facilities (n=120 each) will be collected and screened for STEC as described previously by direct plating (Alam and Zurek, 2004). Up to ten selected isolates per each positive sample for STEC will be assessed for virulence genes including Shiga-toxins (*stx1, stx2, eaeA*) by PCR and genotyped by pulsed field gel electrophoresis (PFGE) using *Xba*I restriction enzyme. The genotypic diversity will be compared between STEC isolates originating from cattle manure and flies. Cluster analyzes will be performed with BioNumeric software using the Dice correlation coefficient and the unweighted-pair group mathematical average algorithm (UPGMA) (Sanderson et al., 2006).

Role of salivary glands and crop of flies in vector capacity for pathogens: The overall objective of this project is to better understand how the salivary glands and the diverticulated crop of flies (i.e., house fly, stable fly and face fly) involved in vectoring pathogens to human food and, also how these two glands are involved in acquiring and disseminating pathogens of humans and their domestic livestock, which include poultry, dairy cattle, and pigs.

House fly is a major vector of numerous food pathogens (e.g., *E. coli*); and, it has already been suggested that the fly crop is the major reservoir for the pathogen and also that this is where horizontal transmission of antibiotic resistance occurs. The salivary glands of most flies involved in vectoring pathogens are also involved in pathogen transmission and the flies nutrient and pathogen uptake while feeding. We know very little about those factors involved in the regulation of both crop filling and emptying in house fly, stable fly and face fly. At the same time, we know even less about the effect of various pathogens on salivary gland regulation/functioning. By better understanding how these two essential organ systems are regulated, we will obtain a better picture to explore how control strategies can be directed at interfering with the normal regulation of these two organ systems. Ultimately, non-traditional control strategies will be developed that rely on interfering with the function of these two systems, which are so essential to the fly. Thus, compromised longevity, pathogen vectoring, and/or reproductive development of the flies can be interfered with resulting in death, abnormal flight ability, and/or reduced fecundity.

A survey of poultry, dairy and pig farms in Massachusetts will be conducted to compare infection rates of house fly by the salivary gland hypertrophy virus. Once completed, the data should give us some information about the types of foods adult house flies are feeding on and why any differences in infection rates are observed. Adult flies will be sampled during the summer months, quickly frozen, returned to the laboratory where they will be kept frozen until dissected and examined for the salivary gland hypertrophy virus. This study may be also conducted by using PCR on large samples of flies from each type of animal facility. The virus will be maintained at low temperature and removed and used to infect non-infected flies maintained in the laboratory fly room. Both salivary glands and crops of the 3 fly species will be used to test various pharmacological agents to study their effect on crop contractions, thus regurgitation and/or passing of the crop contents, which include various pathogens. These effects will be video recorded and contraction rates determined for comparative effects of the agents. Both TEM and SEM studies will be conducted on both the salivary and crop organ systems to help better understand the effect of the pathogenic salivary gland virus on these structures and also to help elucidate the involvement of either nervous or exogenous neurohormonal/chemical control.

Essential oil - Horn fly repellency and antimicrobial activity: Insect repellents to protect humans from disease vectors, biting flies, mosquitoes and ticks are relatively common (Moore and Debboun, 2007). Repellent efficacy on cattle is largely untested; however there is sufficient evidence that natural plant repellents including geraniol, citronellol, eugenol, linalool, and citral among others may be useful in keeping insects off livestock (Moore et al. 2007). Also many of these compounds have antimicrobial qualities against *E. coli*, *E. faecalis*, *S. aureus* and others (Hammer et al. 1999) Lemongrass, oregano, bay, thyme and vetiver oils were most effective. Rosewood, coriander, palmarosa, tea tree, niaouli, the mints, and marjoram inhibited all but *P.*

aeruginosa. Many natural products are listed among 31 minimal risk active ingredients exempt from the registration requirements of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (CFR40). As part of this project we have proposed that the application of these "generally regarded as safe" or GRAS repellents could be a great benefit to conventional and organic dairy producers wishing to reduce or eliminate reliance on pesticides and antibiotics. Furthermore, we expect that these products will have antimicrobial activities and their use will mitigate bacterial infections that occur commonly on farms.

Laboratory studies will focus screening concentrations of essential oils for activity against common pathogenic bacteria associated with bovine mastitis using inhibition tests. Bacterial cultures will be obtained from ATCC and from farm isolations in reserve at the NCSU Mastitis Lab at the College of Veterinary Medicine. Bacteria will be cultured in the laboratory using standard microbiological techniques to produce a lawn culture. Essential oil treated disks and concentrations will be placed on the surface of the bacterial lawn and plates will be incubated for 24-48 hr. Zones of inhibition will be measured to quantify activity against bacterial species and strains. Antibiotic resistance levels will be evaluated.

Essential oils with documented antimicrobial activity will be further evaluated in field studies on heifer calves for the mediation of teat damage, an indicator of impending mastitis infection. Heifers will be evaluated for teat damage using the scoring systems similar to those of the national mastitis council. Calves will be scored for teat damage on a 0-5 scale with 5 representing the most serious damage. Calves will be treated with essential oils at concentrations observed to be most efficacious in the laboratory. Calves will be treated twice each week to determine the length of time required for the condition to resolve itself. Results will be compared to groups of untreated control animals.

Essential oils will be further evaluated for activity against horn flies, the putative vector of summer mastitis in heifer calves. Horn flies will be counted on the treated and untreated animals to estimate parasite load relative to teat damage.

Identification of putative repellents for study will be conducted in the winter of 2015. Laboratory studies focused on inhibition studies will be conducted in 2015-2016. Putative repellents and antimicrobial agents will be tested on cattle in 2016-17 and again in 2017-18. Results of the study will be presented at national livestock and dairy meetings, 2019-2020.

This study will identify the levels of antibiotic resistance in common causative agents of bovine mastitis. We will have also established the range of activities against horn flies and mastitis causing bacteria using essential oils that are commonly promoted for use.

Objective 4: Characterize population biology of biting and nuisance flies

Dispersal, especially long range dispersal, can be difficult to document or quantify (Nathan 2001), but is exceedingly important for developing management plans. Multiple methods can be used to evaluate dispersal (Nathan et al. 2003). The two most appropriate for characterizing stable fly dispersal are Eulerian (mark recapture) and genetic structure. Both methods have been

applied to stable flies; however, data on dispersal distances, phenology, and ubiquity of dispersal remain elusive.

Laboratory studies with flight treadmills indicate stable flies are capable of flying up to 29 km in 24 h (Bailey et al. 1973). Stable flies were observed to disperse 8 km in <2 h in south-central Oregon (Eddy et al. 1962) and up to 225 km over several days in the Florida panhandle (Hogsette and Ruff 1985). Gersabeck and Merritt (1985) found that 50% of flies released on Mackinac Island, MI, were recaptured within 0.45 km, and 90% were recaptured within 1.65 km. Flies released close to horses dispersed less than those released further away, and none of the released flies were collected on the Michigan mainland, 11 km away. Todd (1964) found that dairies adjacent to fly development sites in New Zealand were heavily infested, whereas stable flies were "no problem" within 1.6 km from developmental sites. More recently, studies at a mixed agricultural site in southeastern Nebraska observed that 50% of stable flies dispersed more than 1.6 km from their larval developmental sites and 5% dispersed more than 5.1 km (Taylor et al. 2010). In Florida, stable flies were observed moving at least 1.5 km within 48 hours from blood feeding sites to resting and / or oviposition sites (Pitzer et al. 2011).

Population genetics has been used as an indirect measure of stable fly dispersal. Allozyme studies in northern Florida implicated inland livestock facilities as sources of stable flies appearing on coastal beaches (Jones et al. 1991). Several studies using allozyme, AFLP, microsatellite, and mitochondrial markers found stable fly populations exhibited low levels of differentiation indicative of high levels of gene flow / dispersal (Gilles et al. 2007, Marquez et al. 2007, Dsouli Aymes et al. 2009, and Tainchum et al. 2010). Physical markers such as blood meals (Pitzer et al. 2011), and pollen (Jarzen et al. 2008) have been used to document stable fly movement as well.

Together, these studies indicate that stable flies can readily disperse long distances, but appear to do so only when resources, either hosts or oviposition sites, are inadequate. Relationships between weather phenomena, landscape features, and phenology on dispersal remain unknown as do the cues, mechanisms, and extent of long range dispersal. Genetic studies have been limited by the number of insects and variable loci available for analysis. Genomic and high throughput technologies have reduced costs and increased access to variable genetic loci. Application of these technologies may increase the resolution of genetic analyses.

***Climatic factors affecting stable fly populations* (*dispersal & phenology*):** Weather parameters, primarily temperature and precipitation, are important determinants of seasonal dynamics of stable fly populations. As part of the S-1030 project, relationships between weather variables and stable fly population levels were characterized in California, Nebraska, and Florida (Mullens and Peterson 2005, Taylor et al. 2007, Pitzer et al. 2011). Similar studies were performed in Canada, Denmark, Mexico, and Reunion Island (Cruz-Vazquez et al. 2004, Gilles et al. 2005, Beresford and Sutcliffe 2009, Skovgrad and Nachman 2012). Weather phenomena may be associated with stable fly dispersal as well (Jones et al. 1999). Data on weather / fly interactions are needed from additional locations in order to develop general models applicable to stable fly populations in different climatic regions of the United States. Studies are also needed to better understand mechanisms of temperature and precipitation effects on stable fly population dynamics. Time

series data from 15 geographic locations representing 2-16 consecutive years have been gathered during preceding projects and are awaiting analysis.

Temperature dependent growth tables for stable fly were developed during previous projects (Lysyk 1998, Gilles et al. 2005). Interactions between temperature, moisture, and substrate, including microbial associates, need to be addressed.

Larval habitats of stables flies: Stable fly larvae develop in a wide variety of substrates associated with decomposing vegetative materials (Skoda et al. 1991). Studies conducted in association with previous regional projects demonstrated that these substrates are suitable for stable fly development for only a short period of time as their physical and biological characteristics change rapidly under the influence of active microbial communities (Broce and Haas 1999, Romero et al. 2006, Talley et al. 2009, Taylor and Berkebile 2011). Identification and characterization of primary larval habitats contributing to stable fly populations in the United States remains incomplete as well as elucidation of the phenology of larval development in those habitats. A few habitats such as winter hay feeding sites in the central US (Broce et al. 2005, Talley et al. 2009, Taylor and Berkebile 2011) and calf hutches in dairies have been identified as primary sources of stable flies in selected regions of the country or animal management systems. However, studies in Nebraska observed discordance between adult population levels and emergence from characterized larval developmental habitats (Taylor et al. 2007, Taylor and Berkebile 2011) indicating an incomplete knowledge of population dynamics, even at one of the most thoroughly studied sites. Additional work is needed to characterize larval developmental habitats and elucidate the role of microbial communities in stable fly development and larval habitat succession.

a. Characterize effects of climate and landscape features on dispersal

Mark recapture studies to evaluate the effects of landscape features on stable fly dispersal will be conducted. Initial studies will evaluate effects of confined animal facilities. A primary goal will be to determine the "zone of influence" of confined animal facilities relative to stable flies; at what distance from the facility do flies begin to orient towards the facility. Later studies will evaluate the effects of other landscape features, tree lines, agricultural fields, etc. on the zone of influence of confined animal facilities. Standard Mark-Recapture methods will be used for these studies (see Taylor et al. 2010). Flies will be released at various distances from confined animal facilities and the directionality of their orientation will be evaluated. Once the facility horizon has been characterized, additional releases oriented such that test features are located between release points and confined animal facilities will be conducted. Initially, these studies will be conducted at the Agricultural Research and Development Center, Ithaca, NE. Studies will be repeated in Florida and Minnesota to determine regional variation.

Single Nucleotide Polymorphisms (SNP) will be identified in stable fly using genomic tools. Once an adequate number of SNP have been characterized, high through put methods will be used to score SNP genotypes of stable flies from 60 wide spread populations within the United States and among samples collected world-wide to evaluate population structure. Temporally repeated collections (5 / yr) from 9 sites representing north-south (Minnesota-Texas) and east-west (North Carolina-Oklahoma) transects will be analyzed to evaluate dispersal among

populations. Stable flies from 5 populations will be classified into phenotypic classes (host preference and larval developmental sites). Genetic analysis of flies relative to class using efficient mixed-model association will be used to evaluate local adaptation / differentiation of stable flies relative to hosts and larval developmental habitats.

b. Phenological and environmental effects on biting and nuisance fly populations

Influence of weather in late winter and spring on stable fly appearance and population growth will be studied at multiple sites throughout the United States. Cooperating sites will range from TX, LA and FL in the south to WA, MN and Ontario in the north, and available states in between. At each site, cohorts of stable fly eggs shipped from ARS colonies in Lincoln, NE, will be planted into standardized containers of artificial medium for rearing in the field. Plantings will be done at weekly intervals straddling the anticipated date when weather first becomes permissive for egg-adult survival. Adults that develop from each cohort will be counted and used to calculate survival rates and egg-adult development times. Patterns will be analyzed in relation to matching temperature records to develop a model to predict when and how fast field populations could begin developing at different locations throughout the country.

Contributors to S-1030 have amassed 57 sets of time-series measures of stable fly abundance in a total of 15 separate geographic locations over 2-16 consecutive years, depending on location. Patterns among the site-years vary substantially. Most are sharply to broadly unimodal, but some exhibit two peaks, one in late spring and a second in early fall. These data sets await formal analysis in conjunction with matching weather data. Methods for statistical analysis of time-series data have advanced substantially beyond correlation analysis. Mixed models can discern relations among population growth (change in numbers), time-lagged density dependence, and phenologically appropriate measures of relevant weather variables (e.g., Goulson et al. 2005). Preliminary models developed for individual sites have found temperature during the previous winter to be negatively correlated with populations (Broce et al. 2005, Taylor unpubl. data). Temperature 1 to 2 weeks prior and rainfall 3 to 5 weeks prior are also significant contributors to stable fly populations (Taylor et al. 2007). These results agree in general with those of previous studies (Greene 1989, Mullens and Peterson 2005).

Physiologically based demographic models (PBDM) will be developed to simulate stable fly population dynamics. Temperature and density dependent developmental rates, fecundity, and mortality for each life stage will be determined under laboratory conditions in representative substrates. Some of these data are currently available in the literature. Gaps will be filled by additional studies. The PBDM will be evaluated by comparing its predictions to the time-series data sets with corresponding weather variables and refined accordingly. The PBDM will be used to evaluate the effects of current climatic conditions and those predicted by climate change models on the seasonal and geographic dynamics of stable fly populations. As possible, the effects of pathogens, predators, and parasites relative to climatic variables will be incorporated into the PBDM model.

Spatial variation among stable fly populations within regions will be evaluated with adult trapping and fly counts on host animals. Sticky traps arranged in grids with varying trap densities will be used to evaluate spatial and temporal variation in trap catches. Effects of trapping site

features on absolute and seasonal trap catches will be evaluated. Similar studies will be conducted by evaluating infestation levels of cattle among pastures within regions.

c. Larval developmental habitat source identification

Whole facility surveys will be conducted to identify substrates suitable for stable fly and house fly larval development. Microbial communities associated with developmental substrates will be characterized using metagenomic and functional analyses. Temporal variation in microbial communities, as well as physical and chemical properties associated with substrate decay relative to suitability for fly development will be evaluated. Parameters defining a substrates as suitable for house fly development, stable fly development, or unsuitable for fly development will be emphasized.

Objective 5: Community and stakeholder engagement

a. Compile database of registered pesticides

With industry help and support we will compile a database for all pesticides registered in the US for use in animal agriculture. We each will be responsible for modifying this database to fit our own state's regulations and registrations. We will bridge the gap between industry and extension by providing industry with a mechanism to self-report their current and new products, including their state-by-state registrations. To accomplish this, we will develop and maintain a national product database that industry personnel could open and append with new products (and to remove products) that would include a state selection box indicating which states the product is currently registered in. Shifting the responsibility of tracking pesticide registrations from our extension personnel to industry (those individuals who know the registration history and have a company interest in making sure that we all know about their products) would be extremely helpful.

b. Maximize the exposure of our livestock entomology research and extension information to our stakeholders through electronic and print communication.

We will link currently available livestock entomology research and extension information from our respective programs across the US. Initially, this will be accomplished by providing web links to other institutions on all veterinary entomology extension websites hosted by our participating stations. During the first 1-2 years of this project, we will identify a more specific framework for collaborative extension of already developed information. As part of these collaborative extension efforts, we will also develop a national repository of extension products (REP) to be shared among our members for extension to all of our constituents. To accomplish this we will identify websites, archived webinars, etc. and all other pertinent existing electronic and print extension sources and then determine how we can compile these sources for general use with our stakeholders.

c. Educate our stakeholders and funding decision-makers.

Extremely valuable livestock entomology research continues to be published and presented at

scientific meetings, conferences, etc. It is essential that this research is converted into a form that can be used by extension personnel, veterinarians and policy makers across the US for immediate benefit to our stakeholders. To accomplish this, as stated in **(b)** above, we will establish within the REP a section for research results from our national livestock entomology group. Knowledge gained from these results/studies will then be written up by project participants and organized by objective leaders annually in a "public-ready" format for distribution to our stakeholders, including but not limited to conventional and organic livestock and poultry producers, veterinarians, commodity and industry organizations (e.g. Farm Bureau), state and federal legislators and regulators, newspapers, trade journals and magazines, TV, radio, etc. For decision-makers to fully understand the far-reaching, beneficial impact to animal agriculture that our current science offers, it is extremely important that our administrators, industry leaders, regulators, legislators, etc., at the state and national levels are made aware of the large number and extensive breadth of livestock entomology studies and extension programs currently being conducted by researchers at land grant universities and the USDA-ARS and by extension personnel across the US.

To garner the support of our livestock and poultry industries, we will prepare displays and written materials and exhibit/distribute these at state, regional and national meetings/conferences to increase stakeholder awareness of our livestock entomology research and extension efforts and its overall importance to their industries. We will collaborate on developing state, regional and national updates for user groups (extension agents, producer groups, veterinarians, etc.) through conference calls or on-line conferencing utilities. We will also conduct pest specific webinars for farmers/stakeholders (conventional and organic), private practice and state veterinarians and others and then widely advertise their availability. We will also develop a list of "available" speakers and their area(s) of expertise by region of the country. In the process, we will develop close partnerships with livestock and poultry producers, animal scientists, agricultural engineers, and others whose work has an impact on (or is impacted by) livestock pests; developing these connections with key stakeholders will help to ensure rapid and timely transmission of information among all stakeholders across the US.

d. Seek funding to support these extension/outreach efforts by developing proposals that will be submitted to various granting agencies including our Regional IPM Centers, etc.

We will submit a regional IPM grant proposal as a collaborative effort among researchers from all regions; up to $10,000 from each region. We will also seek funding as a component of research-oriented grant proposals developed by members of this multi-state project. It is expected that some funding for extension efforts would be suitable (and even desirable) for inclusion in proposal to USDA-NIFA grant programs.