FINAL PROJECT REPORT (2007-2012)

S-1030: Flies impacting livestock, poultry and food safety

ACCOMPLISHMENTS OVER ALL PROJECT YEARS:

<u>Objective 1:</u> Characterize dispersal and population biology of stable flies and house flies, and develop monitoring methods for use in indoor and outdoor environments.

Subobjective 1: Characterize stable fly origins and dispersal

a. Larval habitats of stable flies.

Sites where large round bales of hay were provided as winter feed for cattle were identified as primary sources of stable flies (Stomoxys calcitrans) in pastures. In eastern Nebraska, stable flies began depositing eggs in these sites in early April and the first adults emerged in early May (≈235 accumulated Day-Degrees 10° C [DD_{10}]). Emergence peaked in late June and early July (400-900 DD_{10}), dropped to very low levels in late July (>900 DD10), and remained low for the remainder of the season. This seasonal pattern differed from that observed for adult collections on sticky traps which increased in the spring before significant emergence from the hay feeding sites was observed, dipped in midsummer soon after hay feeding sites became nonproductive, and then rebounded in the late summer when emergence from the hay feeding sites was very low. Larvae were primarily in the top 5 cm layer and no larvae or pupae were found > 10 cm below the surface. An average of ≈1,600 flies emerged per square-meter from these sites. Emergence density within sites was positively correlated with moisture, inorganic nitrogen, carbon, pH, and microbial respiration of the substrate. Each of those parameters was positively correlated with electrical conductivity which provided a single, easily measured metric for predicting emergence levels. No house flies (Musca domestica) were collected emerging from hay ring sites. These data have allowed us to quantify the roll of winter hay feeding sites as sources of stable fly infestations. Understanding the seasonality of these sites permits producers to schedule fly remediation measures when they will be most effective. The differences in phenology between adult collections on sticky traps and emergence from hay feeding sites indicates that late season stable flies are coming from other sources that have yet to be identified. (D. Taylor)

Dewatered biosolids (biosolid cake) stored at a wastewater treatment facility supported larval development of numerous Diptera, in particular stable flies (*Stomoxys calcitrans*) (80.2% of emerging flies), house flies (*Musca domestica*) (18.0%) and calliphorid flies (*Lucilia* spp.) (2.6%). Captures of stable flies and house flies peaked around mid-July each year and a second, smaller peak was observed among stable flies 5-8 weeks later. Total emergence was estimated at 551,404 flies/yr for stable flies and 108,188 flies/yr for house flies; overall fly production in biosolids was estimated at 670 stable flies/m^2 and 143 house flies/m^2. This study provides valuable insights into the utility of biosolid cake as a larval development substrate for stable flies and house flies. (L. Zurek)

The structure of the horse manure bacterial community changes over time and likely plays an important role in the oviposition behavior of stable flies. A series of two-choice bioassays were conducted using two-week old horse manure (standard) and aging horse manure (fresh to five weeks old and tested on weekly basis) to evaluate the effect of manure age on stable fly oviposition. The microbial community structure of all manure samples (fresh to five weeks old) were analyzed by 16S rDNA PCR with universal primers followed by 454 pyrosequencing. Preliminary analysis of comparing ~5,000 good quality sequences from each manure type revealed great differences in the microbial community structure with a major shift from strict anaerobes (*Clostridium, Eubacterium, Bacteroides, Ruminococcus, Prevotella* spp) in fresh manure to facultative anaerobes or strict aerobes (*Bacillus, Stenotrophomonas, Brevundimonas, Sphingomonas,* and *Pseudomonas,* spp.) in 1 to 4 week old manure. Overall diversity of the bacterial community was very high in fresh manure (OTU [3%] =1,458; H'=6.2) and greatly declined in aged manure (OTU [3%] = 796; H'=4.6). Assessment of the effect of the microbial community structure on SF behavior and further steps to establish a platform for a paratransgenic approach for management of SF are in progress. (L. Zurek)

b. Climatic factors affecting stable fly populations.

The seasonal dynamics of a stable fly population in eastern Nebraska was monitored for 5 yr. Stable flies appeared in eastern Nebraska in late March to early April, and populations increased to a peak during the last week of June and first week of July. In most years, the population decreases in midsummer, and then it increases to a second peak in mid-September. Temperature 0 to 2 wk before collection accounted for 63% of the variance in population levels and precipitation 3 to 6 wk before accounted for 11%. Reduced precipitation explained the lower stable fly populations observed midsummer. Changes in stable fly population levels were positively correlated with precipitation 1 to 2 wk prior and temperature the week of the change. Population change was negatively correlated with precipitation 6-8wk prior and temperature 6-15 wk prior. Low temperatures during October through January were correlated with higher populations the following June and July. Results of these studies make it possible to predict trends in stable fly populations based upon weather patterns. This will allow producers to make better decisions relative to the institution of control measures as fly populations approach economic impact levels. (D. Taylor)

A multistate field study involved 19 participants in 17 states and Canadian provinces to measure field development times of stable flies from egg to adult in summer and autumn, 2009, and spring of 2010. Development times of 45 cohorts ranged from 2 to 8 weeks, and could be predicted (R^2 = 0.6) from matching NOAA min-max temperature series, using a degree-day model with thermal constants of 369 degree-days between a temperature base of 3.7 and ceiling of 32 degrees C. The development model was implemented in NAPPFAST, the APHIS-NC State pest forecasting system, to predict and map average numbers of generations (egg to egg) per year. With addition of 131 degree-days for egg development, projected numbers of generations per year given 2000-2010 weather records ranged from 2–4 in southern Canada to 14–15 in southern Florida. If stable flies overwinter locally, then time from first emergence to pupation by F1 offspring would require 310 DDs after 1 January. Dates and locations of a projected northward moving pupation front ranged from early February in Florida to early-mid June in

southern Canada. These dates could be used to schedule regional debris cleanup programs. (R. Moon)

We analyzed a 16-year data set from a beef facility in Iowa to see if density dependence and weather could account for variation in population growth during the breeding season. Within-season population growth rates depended on antecedent density and on temperature and precipitation. Growth rates increased 1.05-fold per degree C while offspring were pupae, and decreased by the same amount while offspring were nulliparous adults. In contrast, growth rates increased 2.4-fold with increasing amounts of precipitation while offspring were larvae. Responses to density, temperature and precipitation were linear. These results indicated populations at Ames tended toward an equilibrium density of approximately 3 flies per trap day in summer, and that abundance was greater after periods when daily precipitation exceeded 0.35 cm (0.15 in). (R. Moon)

c. Dispersal of Stable Flies.

Seven mark-recapture studies were conducted over 3 yr to assess dispersal of newly emerging adult stable flies from larval development sites in a mixed agricultural environment in northeastern Nebraska. Infested hay debris piles were marked by dusting their surfaces with fluorescent pigments, adults were captured with surrounding grids of Alsynite traps, and specimens were dissected to determine feeding histories and reproductive age. Distances and directions of 3,889 marked specimens indicated males and females dispersed equally and in all directions. Midguts of males and females were equally likely to contain blood meal remnants. Percentage with blood remnants and percentage of females with yolk increased with distance from mark origin, indicating survival and spread were positively associated with host finding success. A time-integrated diffusion model fit to results from the seven studies indicated 50% of stable fly adults had dispersed beyond 1.6 km of their natal site, but only 5% had dispersed beyond 5.1 km. These results indicate that stable fly adults on cattle in a given area are most likely to have originated from larval development sites within a ~5 km radius of the subject cattle. (D. Taylor)

Stable flies were collected between April 13 and October 24, 2008 from four different locations in and around the Medicine Lake National Wildlife Refuge in northeast Montana including areas where pelicans have been significantly impacted and from nearby cattle ranches. Stable flies were abundant from mid-July through August. The range of stable flies collected on August 14, the peak of stable fly abundance, was from 10 to 1276 flies per card, with the latter collected on cards from the Schmidt Farm. More males were captured than females with a male/female ratio of 7:1 early in the season and 2:1 mid to late season. Less mature flies (physiological age <1.0) were collected from cattle sites and older flies (physiological age $\geq$2.0) were captured from a pelican site. Cattle leg counts ranged from 0 to 68 flies per animal. (G. Johnson)

A species-specific multiplex polymerase chain reaction targeting the cytochrome b gene of cattle, horses, humans, and dogs was developed to determine the blood meal sources of stable flies, collected from Florida equine facilities. Of 595 presumptive blood-fed stable flies analyzed, successful host amplification was obtained in 350, for a field host-detection efficiency of 58.8%. The majority of analyzed stable flies had fed on cattle (64.6%), followed by horses

(24.3%), humans (9.5%), and dogs (1.6%). A survey of animal-enclosed pastures occurring within 3 km of stable fly collection sites revealed that the nearest cattle were between 0.8 and 1.5 km from the four horse farm sampling sites. Cattle-feeding frequencies were greater on farms where cattle were located at distances of 0.8 km, suggesting that between farm differences in host-feeding frequency is related to the number of and distance from a particular host type. Time course evaluations of previously laboratory-fed stable flies demonstrated that host-detection efficiency with this system was 100, 50, and 0% when flies were evaluated at 16, 24, and 48 h post-blood feeding, respectively. The results of this study suggest short-term stable fly dispersal of up to 1.5 km in a 48-h time period. (P. Kaufman)

A series of studies was conducted to determine appropriate targets and target placement for achieving stable fly control with treated targets. An electric grid study was also conducted to compare the attraction of solid blue and solid black cloth targets to our standard blue/black target. Overall, the mean number of flies collected per hour for black and blue/black were not different. Electric grid studies were also used to determine if the height that targets were placed above ground influenced the number of flies collected, revealing that targets need to be placed at ground level. The number of targets per acre that would be required to kill stable flies closely associated with cattle was assessed with optimal target density determined to be two targets per acre. A preliminary study to protect cattle from stable fly attack using treated targets was performed, and results showed that the number of flies per animal and the number of stomps per group were lower for herds with treated targets. (L. Foil)

In 2010, a multi-year study was initiated to determine if fly population densities differ between pasture locations in West Central Nebraska. Four pastures were located near North Platte, NE were identified for this study. All four pastures were within a 5 mile-radius of North Platte and had similar topographic conditions; native vegetation, flat landscape, and some trees. Pasture size, stocking rates and cattle did vary between the 4 sites. Site A had 21cow/calf pairs with one bull, Site B had 65 cows, Site C had 42 cow/pairs and Site D had 32 yearling heifers with two bulls. Fly counts were initiated 9 June 2010, and recorded between 08:00 and 11:30 A.M. Horn flies numbers were enumerated using digital photography. One side of each of 20 animals was photographed using a Nikon D70 camera equipped with an 18-70 mm telephoto lens. Images recorded during each the fly counting session were later scanned using a computer imaging program GIMP 2. Horn fly numbers counted from each digital photograph were then doubled to express the total number of horn flies per animal. Stable fly numbers were visually recorded by counting the total number of flies per four legs and belly of 20 animals and expressed as total number of stable flies per animal. Face fly numbers were recorded by counting the total number of flies per face of 20 animals. To monitor stable fly populations a double sided stable fly trap (North Platte) was located near the water source for each pasture and monitored weekly. Environmental conditions were recorded for each site during each fly counting session. Horn fly numbers were significantly different for each week, but not between sites, with the exception of week 4, 6 and 8 ($P<.001$). Horn fly numbers at pasture B and D were not significantly different in population numbers. Stable fly numbers were significantly different for each week, but only different between sited during week 7 ($P<.001$). Face fly numbers were sporadic and were lacking in sufficient numbers for analysis. The initial study design was modified in 2011 due to flooding issues impacting two pastures used in 2010. Data collected in 2011 is awaiting analysis. This study will continue in 2013. (D. Boxler)

d. Overwintering dynamics of stable fly throughout the USA.

Two proposed hypotheses may explain the appearance of stable flies in early spring in the Midwest: overwintering of immatures in silage/manure mounds or the migration of adults riding southerly winds ahead of approaching cold fronts. To evaluate the possible dynamics of overwintering in silage/manure mounds, a study was initiated by building mounds of silage, manure, and manure mixed with two levels of hay. Temperatures were monitored at various depths within the mounded materials and 1st instars introduced, with plans for adult emergence recording. Temperature at the different depths differed significantly, with silage offering the most insulation whereas pure manure offered the least insulation. By the mid-winter temperatures of manure, and manure/hay mixtures had reached freezing and offered little insulation value to larvae. (A. Broce)

In separate work, the16-year data set from Iowa (described under objective 1[1]B) was examined for dates of first stable fly appearance each year, and for relations with antecedent temperatures and wind movement patterns. Evidence for southern immigration was stronger than for local overwintering. First capture dates ranged from 4 April (1986) to 25 May (1995), and averaged 25 April (SD = 14 d). Initial numbers were always low. Average ∑DD from 1 Jan to first date was 98 (SD = 56). Frequency of days with southerly wind events increased as date of first appearance neared. Mean number of southerly wind events up to first date was 3.6 (SD = 2.6). First dates were mildly correlated with dates when DDs reached 98.4, but were more strongly correlated with interpolated dates when 3-4 wind events occurred. (R. Moon)

Subobjective 2: Improve understanding of house fly dispersal and behavior, and develop methods for monitoring them in indoor and outdoor environments.

a. Trapping and Monitoring Methods.

Digital photography was utilized to assess pest fly abundance in Washington cow-calf herds during the summers of 2008 to 2011. Counting face flies on cattle face photos proved to be easier and less time-consuming than counting horn flies on side views of cattle. Visual grids were overlaid on side view photos in PowerPoint to facilitate counting. Adjustments to settings on the camera (e.g., increased resolution and aperture priority options) helped to mitigate the poor light conditions sometimes encountered during the photo shoots. Data on seasonal abundance of horn fly and face fly serve to fill in the gaps of knowledge for researchers and producers. These data will prove useful when developing site-specific IPM plans for pasture/rangeland fly pests. (H. Ferguson)

Relative house fly, *Musca domestica* L., activity at three large dairies in central California was monitored during the peak seasonal fly activity period (June to August) using spot cards, fly tapes, bait traps, and Alsynite traps. Counts for all monitoring methods were significantly related at two of three dairies; with spot card counts significantly related to fly tape counts recorded the same week, and both spot card counts and fly tape counts significantly related to bait trap counts 1-2 wk later. Estimate precision was determined by the coefficient of variability (CV) (or

SE/mean). Using a CV = 0.15 as a desired level of estimate precision and assuming an integrated pest management (IPM) action threshold near the peak house fly activity measured by each monitoring method, house fly monitoring at a large dairy would require 12 spot cards placed in mid afternoon shaded fly resting sites near cattle or seven bait traps placed in open areas near cattle. (A. Gerry)

Software (FlySpotter; http://ucanr.org/sites/FlySpotter/download/) using computer vision technology was developed to count fly spots on a scanned image of a spot card to dramatically reduce time invested in monitoring house flies. Counts provided by the FlySpotter software were highly correlated to visual counts. The increased efficiency provided by the automation of the spot counting process will make this monitoring tool more acceptable to animal facility operators. FlySpotter has been copyrighted by the University of California at Riverside, and we are currently looking for a commercial partner to sell this software. The use of spot cards for monitoring house flies is recommended for animal agriculture IPM programs. (A. Gerry)

Nine different house fly traps were compared in 2006 with the Terminator™ proving to be the superior trap. In 2007, the Terminator trap was compared to a Flies-be-gone™ trap with each trap having various attractant substances, including the attractants provided with each trap. Each trap worked best with the attractant that was provided with the trap, while overall the Terminator was the better trap design and collected the greatest number of flies. Molasses was found to be a good fly attractant and a modified bottle trap using molasses has piqued the interest of the military. Trap height was assessed on the capture of house flies using a Terminator jug trap, with traps closest to the ground catching the greatest number of house flies. House fly attraction to fermented molasses was evaluated given past results showing positive response to unfermented molasses. However, relative to unfermented molasses, the number of house flies captured using fermented molasses was reduced. (C. Geden)

b. Dispersal and Behavior.

Immuno-marking techniques were developed for marking dung-breeding flies to track their dispersal. Dung pats were sprayed with egg whites, and flies emerging from these pats were captured on alsynite-style sticky traps. Recaptured flies were analyzed for the presence of egg protein with an Enzyme-Linked ImmunoSorbant Assay (ELISA). Marker degradation was followed by analyzing marked dung pat crust samples collected over time for the marker. The marker persisted on pats for about 11 days and then degraded rapidly. In laboratory studies, flies also failed to pick up marker on pats older than 11 days. In an enclosed single pat arena, face flies emerging from the dung pat were tested for their ability to pick up marker, and about 77% of emerging flies acquired marker. A small-scale mark/recapture experiment was conducted in a rancher-cooperator's pasture. We sprayed fresh dung pats with egg white marker and recaptured flies with sticky traps. We captured 384 flies over three months; two were positive for the marker. In the same pasture, with the herd removed, large, replicated treated and control plots were set up and dung pats of all ages were sprayed. A total of 12 flies were captured; one was positive for marker. The low marking rate in both field experiments was probably due to marker degradation. This novel marking system can be used to follow flies directly affecting cattle. Our

results suggest that rotation paddocks would need to be at least 1 to 2 km apart in order for rotational grazing to have an impact on fly management. (H. Ferguson)

House flies were recognized in the field to be strongly associated with homopteran infested trees and plants in an urban environment. Flies readily consumed honeydew under laboratory conditions with increased survival and egg-laying relative to no food or sugar alone, respectively. Under laboratory conditions, flies were offered numerous food choices and were shown to be strongly attracted to insect honeydew. This attraction is likely to be responsible for the large number of filth flies found in association with urban trees and agricultural crops infested with homopteran pests. (A. Gerry)

House flies in three states (California, Georgia, and Minnesota) were captured at resting, feeding, and oviposition sites on dairies to determine age and sex specific behavior of the flies. House flies were shown to spend pre-dawn hours primarily in easily identified overnight resting sites. During the day, males and females were equally abundant at feeding sites, while females were more abundant than males at immature development sites (especially in late afternoon). Age structure of flies was compared to control efficacy. Control was achieved in California and younger flies were collected during that period of control. Mating pairs of house flies were also collected at all three states. The median age of mating females ranged from 2.5-4 days, while mating male flies were estimated to be much younger (< 2 days old). Also, 99.2% of female flies were mating for the first time as evidenced by analysis of their ovaries and spermathecae. The total hydrocarbon profile of mating flies differed regionally, with flies in California having the greatest concentration of hydrocarbons despite being the smallest flies. Of particular note, the concentration of the female house fly "sex pheromone" (Z-9-tricosene) found on an individual fly varied considerably even within a site. The California collection sites had the greatest proportion of flies with detectable levels of Z-9-tricosene. However, there were mating female flies at all locations lacking detectable levels of this pheromone. This might have implications for control as this hydrocarbon is routinely added to fly baits to increase attraction. (S. Butler, R. Moon, N. Hinkle, B. Mullens)

The potential of house flies to disperse from rural to urban areas and distribute antibiotic-resistant bacteria was examined by: (i) quantification of the dispersal rate of house flies from farms (rural areas) into a city (urban area) using multilocus DNA fingerprinting and (ii) profiling of the antibiotic resistance patterns of enterococci harbored by house flies collected in rural and urban environments. The population genetic analysis indicated that there was considerable dispersal between rural and urban habitats. Although there was a significant difference in allele frequency between the urban and rural samples, genetic divergence was low (mean FST 5 0.07) and migration rate relatively high (Nm 5 3 individuals per generation). Almost 95% of the genetic diversity occurred within populations, suggesting a nearly panmictic population. Profiling of antibiotic resistance of enterococci isolated from house fly guts showed that house flies collected in all five urban sites carried substantial numbers of antibiotic-resistant enterococci, supporting the results of the population genetic analyses. The results of this study imply that house flies, because of their dispersal behavior and capacity to transport antibiotic-resistant bacteria, pose a serious threat to public health. (L. Zurek)

A demonstration of the air curtain system for preventing flies and mosquitoes from entering commercial aircraft was conducted at the Accra (Ghana) International Airport in cooperation with Delta Airlines and the US Department of Transportation. The positive aspects of the demonstration were that the air curtain system could be installed on the truck-mounted stairs used at many airports and the net doors could be easily mounted on the doors used by catering and cleaning crews. An unexpected negative aspect was that the electrical systems on the truck-mounted stairs were not designed to handle the extra power required to operate the air curtain units. (J. Hogsette)

An 8 week study was performed to evaluate the movement of flies among facilities on a diversified farm housing dairy cattle, swine and beef cattle. The distance from the dairy to the swine facility was about 700m, and the distance from the swine unit to the beef facility was 500m. Fly traps equipped with house fly pheromone lures were placed at each location. One additional fly trap was placed half the distance between the dairy and swine unit and another half way to the beef unit. Laboratory reared house flies, 1000 each, were marked with day glow colored powders, green (dairy), orange (swine), pink (beef). Marked flies were released weekly at designated locations. Collections were made 24 and 72 hours post-release. About 1-2% of the released flies were captured in the traps. The trap at the beef facility recovered the most marked house flies, although the dairy produced the most unmarked flies. Fly movement from the beef to the swine unit was evident, few flies moved from the swine unit to the beef unit. No flies moved from the dairy to the swine or beef units. (W. Watson)

<u>Objective 2:</u> Establish extent of fly-borne dispersal of human and animal pathogens

a. Human Pathogens.

Filth flies may be involved with the deposition of human pathogenic bacteria to leafy green vegetables. A high prevalence of E. coli O157 was noted on filth flies captured in lettuce fields at a California field site. Laboratory studies showed that flies exposed to E. coli O157 could inoculate spinach plants with the bacteria. (J. Talley, A. Wayadande, A. Gerry)

The fate of an attenuated strain of E. coli O157:H7 acquired by the house fly, Musca domestica, from contaminated manure and deposited on spinach via regurgitation spots was studied by molecular methods and scanning electron microscopy. Retention of bacteria on fly body parts was studied by relative quantitative PCR analysis of the eae gene, indicating increased bacteria through day 4 followed by decreasing counts in subsequent days. Manure-acquired E. coli O157:H7 was more capable of replication on the spinach surface than bacteria acquired from LB-ampicillin plates. Retention of bacteria on tarsi and labella of flies exposed to E. coli O157:H7 contaminated manure was determined by microbiological assays and confirmed by end point PCR, with detection of bacteria up to 13 days post-exposure. (A. Wayadande)

The main reservoir of *Escherichia coli* O157:H7 is the digestive tract of cattle; however, the ecology of this food-borne pathogen is poorly understood. House flies (*Musca domestica* L.) might play a role in dissemination of this pathogen in the cattle environment. Eight calves were individually exposed to house flies that were orally inoculated with a mixture of four strains of nalidixic acid-resistant *Escherichia coli* O157:H7 (Nal^REcO157) for 48 hours. Another eight

calves were individually exposed to uninoculated flies and served as the control. Fresh cattle feces (rectal sampling) and drinking water were periodically sampled and screened for *Nal*^{R}EcO157 up to 19 days after the exposure. At the end of the experiment, all calves were euthanized and the lumen contents of rumen, cecum, colon, and rectum as well as swab samples of gall-bladder mucosa and the recto-anal mucosa were screened for *Nal*^{R}EcO157. On day 1 after the exposure, fecal samples of all 8 calves and drinking-water samples of 5 of 8 calves exposed to inoculated flies tested positive for *Nal*^{R}EcO157. The concentration of *Nal*^{R}EcO157 in feces ranged over time from detectable only by enrichment (< 10^{2}) to up to 1.1 x 10^{6} CFU per gram. Feces of all calves remained positive for *Nal*^{R}EcO157 up to 11 days after the exposure and 62% were positive until the end of experiment. Contamination of drinking water was more variable and all samples were negative on day 19. At necropsy, the highest prevalence of *Nal*^{R}EcO157 was in the recto-anal mucosa region, followed by rectal and colonic contents. House flies are likely to play a role in the ecology of this organism in the cattle environment. (L. Zurek)

Recent studies strongly indicate that house flies carry a large population of antibiotic resistant enterococci in the agricultural as well as residential environment and therefore may play a major role in the ecology of antibiotic resistant strains and resistance genes. Laboratory bioassays showed that a small number of house flies can greatly contaminate ready-to-eat food with enterococci within a short period of time. In this study, the potential of field collected house flies to contaminate ready-to-eat-food (RTEF) with enterococci was assessed by laboratory bioassays. House flies were collected in a cattle feedlot and exposed to a beef patty for 0.5, 1.0, 3.0, and 24 hours. The exposure of RTEF to flies resulted in 100% contamination with enterococci in all bioassays regardless of the number of HF and the length of the exposure time. Even a short-time exposure (0.5 hour) with 5 HF resulted in heavy food contamination. In another study, three RTEFs were sampled from five fast-food restaurants five times in summer and winter. The prevalence of enterococci was significantly higher in summer (92.0% salad and 64.0% burger) when HF are commonly present than in winter (64.0% salad and 24.0% burger). In these studies, ready-to-eat food was frequently contaminated with antibiotic resistant and potentially virulent enterococci. House fly management should be integrated into pre-harvest as well as post-harvest food safety strategies. (L. Zurek)

Enterobacter sakazakii is an opportunistic food-borne pathogen causing meningitis, enterocolitis, and sepsis, primarily in immunocompromised infants. It has been suggested that stable flies, *Stomoxys calcitrans* L., are a vector/reservoir of this pathogen. Studies assessed the a) vector competence of adult stable flies for *E. sakazakii*, b) effect of *E. sakazakii* on stable fly development, and c) survival of *E. sakazakii* during stable fly development and colonization of the digestive tract of newly emerged flies. Results indicated that adult stable flies can maintain *E. sakazakii* for at least 20 days regardless of the food source (blood or sugar) and contaminate the food source. The concentration of the pathogen per individual stable fly ranged from 1.8 x 10^{5} to 6.4 x 10^{6} CFU. *E. sakazakii* supported development of immature stable fly in sterilized cattle manure and sterilized artificial medium (78.3 and 76.7% SF survival to adult stage, respectively). In addition, *E. sakazakii* survived during stable fly development and colonized the gut of emerging adult stable flies. This research also indicated that the vertical transfer of bacterial symbionts plays an important role in the oviposition behavior and new habitat selection for stable flies. (L. Zurek)

Biophotonics may be a useful real-time model to evaluate the ecology of bacteria in relation to flies as mechanical vectors. In one laboratory study, *E. coli* which had been transformed with the XEN-14 plasmid was inoculated into autoclaved manure to confirm that house fly larvae were ingesting and retaining *E. coli*. Ingestion of the bacteria was noted using the real time model of biophotonic's; which detects bacterial presence based upon the emission of photons from the transformed bacteria. Under laboratory conditions, the transfer of resistant genes from resistant to susceptible bacteria within the house fly was evaluated. This transfer occurred frequently in flies infected with both bacteria. Recombination events may occur commonly between bacteria being harbored by house flies; perhaps this is one of the mechanisms for the rapid spread of antibiotic resistance genes in bacteria associated with animal agriculture. A related study showed that inoculation of house flies with pathogenic bacteria like *E. coli* is decreased when flies make contact with manure containing many natural bacteria relative to manure that has been sterilized and inoculated with the same amount of *E. coli*. Larval feeding on the *E. coli* results in contamination of the pupae, but the bacteria is lost during adult eclosion, so adults must contact infectious material to pick up new pathogenic bacteria. (D. Nayduch)

Extensive use of antibiotics as growth promoters in the livestock industry constitutes strong selection pressure for evolution and selection of antibiotic resistant bacterial strains. Insects such as house flies (*Musca domestica*) and German cockroaches (*Blattella germanica*) can move freely between animal waste and food and may play a significant role in the dissemination of antibiotic resistant bacteria within and between animal production farms and from farms to residential settings. Enterococci from the digestive tract of house flies (n = 162), and feces of German cockroaches (n = 83) and pigs (n = 119), collected from two commercial swine farms were isolated, quantified, identified, and screened for antibiotic resistance and virulence. The majority of samples (93.7%) were positive for enterococci with concentrations $4.2 \pm 0.7 \times 10^4$ CFU/house fly, $5.5 \pm 1.1 \times 10^6$ CFU/g of cockroach feces, and $3.2 \pm 0.8 \times 10^5$ CFU/g of pig feces. Among all the identified isolates (n=639) *Enterococcus faecalis* was the most common (55.5%), followed by *E. hirae* (24.9%), *E. faecium* (12.8%), and *E. casseliflavus* (6.7%). *E. faecalis* was most prevalent in house flies and cockroaches, and *E. hirae* was most common in pig feces. Our data showed that multi-drug (mainly tetracycline and erythromycin) resistant enterococci were common from all three sources and frequently carried antibiotic resistance genes including *tet*(M) and *erm*(B) and Tn*916/1545* transposon family. *E. faecalis* frequently harbored virulence factors *gelE, esp,* and *asa1*. PFGE analysis of selected *E. faecalis* and *E. faecium* isolates demonstrated that cockroaches and house flies shared some of the same enterococcal clones that were detected in the swine manure indicating that insects acquired enterococci from swine manure. (L. Zurek)

b. Animal Pathogens.

Flies were collected by sweep net from the vicinity of two small groups of "backyard" poultry (10-20 chickens per group) that had been identified as infected with exotic Newcastle disease virus (ENDV) in Los Angeles County during the 2002-2003 END outbreak in California. Exotic Newcastle disease virus was isolated from pools of *Phaenicia cuprina* (Wiedemann), *Fannia canicularis* (L.), and *Musca domestica* L. and identified by hemagglutination inhibition (HI) with

Newcastle disease virus (NDV) antiserum. Viral concentration in positive pools was low (<1EID_{50}/fly). Isolated virus demonstrated identical monoclonal antibody binding profiles as well as 99% sequence homology in the 635 bp fusion gene sequence when compared to ENDV recovered from infected commercial egg layer poultry during the 2002 outbreak. House flies (Musca domestica) and little house flies (Fannia canicularis) were examined for their ability to take up and harbor a velogenic strain of exotic Newcastle disease virus (family Paramyxoviridae, genus Avulavirus, ENDV). Laboratory reared flies were allowed to feed on evaporated milk containing ENDV at a virus concentration of $10^{8.3}$ egg infectious dose $(EID)_{50}$/0.1 ml or poultry feces containing an ENDV titer of $10^{5.8}EID_{50}$/0.1 g . Flies exposed to either infectious food source for 24 h became transiently infected with virus. Virus persisted predominantly in the mid- and hindgut with relatively little virus isolated from the remainder of the fly body. Both fly species acquired viral titers greater than the infective dose for a susceptible chicken ($10^{3.0}EID_{50}$ – $10^{4.0}EID_{50}$) and flies fed evaporated milk containing a high titer of ENDV maintained viral titers above the infective dose for up to 4 days post-exposure to the infectious food source. Flies fed on infective feces retained a chicken infective dose for only one day. The decrease in viral titer over time was significantly explained by logistic regression for both fly species ($p<0.05$). (A. Gerry)

To assess the impact of a 2007 WNV epizootic in an American white pelican colony Medicine Lake NWR in northeast Montana, counts of dead pre-fledged pelicans were being made twice weekly from July through mid-August. During a mid-July assessment, flies were observed feeding on moribund pre-fledges and were collected from the birds and identified as stable flies. A total of 1,291 stable flies (83% male, 17% female) were collected. Eight percent of the flies were blood fed (i.e., had visible blood in their abdomen). Flies without visible blood were pooled (60 pools each containing up to 20 flies) and assayed for WNV. Eighteen of 60 pools were positive for WNV. This represents the first report of stable flies feeding on pelicans and first detection of WNV in stable flies. (G. Johnson)

Porcine Reproductive and Respiratory Syndrome (PRRS) is a globally significant swine disease, resulting in pneumonia and late-term abortions in sows. The link between outbreaks on farms within an area despite biosecurity measures remains unclear. Stable flies were collected around PRRS-negative boar stud barns in North Carolina and tested for presence of the virus. None of the flies collected were positive for PRRS virus. Pigs inoculated with Porcine Respiratory Virus (PRRSV) were capable of transmitting the virus to house flies in the same pig house. On some occasions, house flies were also found infected with PRRSV in an adjacent pig facility (120 m away), with pigs in the adjacent facility also becoming infected with PRRSV. This study provides additional evidence that flies are capable of harboring, dispersing, and transmitting pathogenic organisms under natural conditions. We investigated the vectorial potential role of stable flies in the transmission of PRRSV in the field and under laboratory conditions. Nucleic acid of PRRS virus was detected by RT-PCR in stable flies fed blood treated with live or chemically inactivated virus. Detectable virus declined over time suggesting that virus was not replicating in the fly. Active virus was detected up to 96h after a single feeding. Although live virus was isolated from insect mouthparts, stable flies did not transmit PRRS virus to pigs. These results suggest the mouthparts carried insufficient quantities of virus to cause an infection without an infusion of macrophage cells to the feeding area. (W. Watson)

House flies collected from each of 5 hog barns in North Carolina were examined for the presence of *Campylobacter coli* and *Salmonella.* Results indicate that house flies collected from swine barns carried *Campylobacter coli* and *Salmonella* was rarely collected. Although the prevalence of these bacteria was relatively low, these data illustrate that house flies may disseminate bacteria from within farm and likely are a source of contamination regardless of biosecurity efforts between farms. Furthermore these experiments suggest that house flies may function as a means to spread pathogenic bacteria between vertebrate hosts, i.e. swine to cattle or swine to poultry. This is particularly a concern for diversified farms that raise a variety of animals. (W. Watson)

The horn fly is closely linked to the harborage and transmission of *S. aureus*, a common cause of bovine mastitis in North Carolina. The prevalence of mastitis, both clinical and subclinical infections and the difficulty of eradication of *S. aureus* from dairy herds motivated this inquiry into the stable fly as an alternate vector of *S. aureus.* Stable flies were readily infected with *S. aureus* in the laboratory and the bacterium was recovered from stable flies up to 12 hours and occasionally at 24 hrs post exposure. In field studies no *S. aureus* was isolated from farm collected stable flies, yet *S. aureus* was isolated from horn flies collected the same day on the same farms. In contrast to the laboratory study, fly collections from local farms displayed a lack of persistence and harborage of *S. aureus* in the stable fly. (W. Watson)

Enterococcus faecalis is an important nosocomial pathogen and house flies have been implicated in the dissemination of this bacterium. In this study, GFP-expressing *E. faecalis*OG1RF:pMV158 was used to track the fate of the bacterium in the digestive tract of the house fly, *Musca domestica* (L.) to assess the vector potential of this insect for *E. faecalis*. Colony forming unit (CFU) counts were obtained from viable fluorescing *E. faecalis* recovered from mouthparts and digestive tract regions (labelum, foregut, midgut, and hindgut) at 1, 4, 8, 24, 48, 72, and 96 h after the bacterial exposure. Bacterial counts were signicantly highest in the midgut at 1 h and 4 h and declined during the first 24 h. In the labelum, *E. faecalis* concentrations were low within the first 24 h and then greatly increased. Bacterial counts and direct observations of the digestive tract under a dissecting microscope with ultra violet light revealed that *E. faecalis* peaked in the crop after 48 h and remained high until the end of the experiment. Concentrations of *E. faecalis* in the hindgut were low when compared with other parts of the digestive tract. Microscopy and CFU counts suggest that *E. faecalis* was digested in the midgut but proliferated in the crop. Both drinking water and feed (flaked corn) sampled at the end of the assay (96 h) were contaminated by fluorescing *E. faecalis*, demonstrating that the flies disseminated *E. faecalis*. Our data support the notion that house flies can act as a bioenhanced vector for bacteria. (L. Zurek)

<u>Objective 3.</u> Improve management tactics for stable flies and house flies.

a. Biological Control.

Parasitoids are an essential component of a successful dairy calf coverall house and stable fly IPM program. In the first year of this three-year study, individual species parasitoid releases were compared. During years 2 and 3, the best individual parasitoid from Year 1 (*M. raptorellus*) was compared to a 50:50 ratio of *M. raptor* and *M. raptorellus*. Overall successful parasitism averaged 3% on the no-release farms, 54% on *M. raptorellus* farms and 52% on *M.*

raptor/M. raptorellus farms during the release period. While total parasitism averaged 13% on no-release farms, 75% on *M. raptorellus*-release farms and 74% on *M. raptor/M. raptorellus*-release farms. Based on the results from this three year study, releases of *M. raptorellus* alone when compared to a 50:50 mix with *M. raptor* consistently provided better parasitism of house flies in dairy calf coveralls in New York State. In addition, producer costs for releasing only *M. raptorellus*, would be one-half that of a 50:50 mix with *M. raptor*. (D. Rutz)

A multi-state (Arkansas, Mississippi and North Carolina) southern region SARE project to evaluate commercial pteromalid wasp releases against filth flies was completed. Baseline data indicate that 12, 13 and 15 pteromalid wasp species occur naturally in Arkansas, Mississippi and North Carolina dairies, respectively. Natural populations of parasitoids were augmented with releases of commercially reared parasitoids. Although the emergence of commercial shipments of parasitoids were low initially (30% *Muscidifurax zaraptor* and *M. raptorellus*) an impact on parasitism rates was noted. Data from subsequent commercial parasitoid shipments (*M. zaraptor, M. raptorellus* and *Trichomalopsis sarcophagae*) indicated a significant increase in emerging parastioids (75%) resulting in the target release rate (200-250 per cow per week). Studies on the usefulness of freeze-killed house fly pupae to serve as effective, distance parasitoid sampling tool have been completed. (C. Geden)

The parasitoid species attacking house flies in Denmark were examined using sentinel bags of fly pupae placed at animal facilities throughout the country. Common parasitoids that emerged from the fly pupae were *Nasonia vitripennis* and *Aphaereta minuta* (a braconid). Neither *Tachinaephagus* nor *Trichopria* spp. were recovered from sentinel pupae. In contrast, the most common fly parasites in the USA are *Spalangia cameroni* and *Spalangia nigroaenea*. The parasitoid *Tachinaephagus zealandicus* was assumed to be an exotic species to the US and kept under quarantine. However it was determined that this species had entered the US from the southern hemisphere naturally. In 2008 a survey of *T. zealandicus* was undertaken to determine the range of dispersal. This parasitoid was found in three states in the US, Kansas, Missouri, Illinois and in Northern Europe (DN). (C. Geden)

Salivary gland hypertrophy virus (SGHV) of house flies is a nonoccluded, enveloped, rod-shaped double-stranded DNA virus, first discovered in fly populations in Florida. Infected flies regardless of sex display enlarged salivary glands and virus particles are thought to be deposited when infected flies feed. Healthy flies acquire the infection when feeding on contaminated substrates. Fly susceptibility to salivary gland hyperplasia virus (SGH) was determined for different fly species, with only house flies being fully susceptible. Stable fly mortality was high following infection. Surveys for this virus proved that SGH virus could be found worldwide. SGHV from Danish house fly populations were submitted for sequencing and virulence testing. (C. Geden)

The effect of *Musca domestica* salivary gland hypertrophy virus (MdSGHV) on selected fitness parameters of stable flies (*Stomoxys calcitrans* [L.]) was examined in the laboratory. Virus-injected stable flies of both genders suffered substantially higher mortality than control flies. Fecundity of control flies on days 6-9 was 49-54 eggs deposited per live female per day, whereas virus-injected flies produced 4-5 eggs per female on days 6-7 and <1 egg per female per day thereafter. Infected flies produced about 26% as many fecal spots as healthy flies. Virus-

injected stable flies did not develop symptoms of salivary gland hypertrophy. PCR demonstrated virus replication in injected stable flies. MdSGHV in stable flies displayed tissue tropism similar to that observed in house fly hosts, with higher viral copy numbers in fat body and salivary glands compared to ovaries. Virus titers were 100x higher in house fly than in stable fly hosts, and this difference was probably due to the absence of salivary gland hypertrophy in the latter species. (C. Geden)

Weekly stable fly surveillance at four equine facilities near Ocala, FL, was conducted using Alsynite sticky traps for adults and by searching immature developmental sites for pupae. Adult stable fly trap captures were highly variable throughout the year, ranging from 0 to 1,400 flies per trap per farm. The greatest adult stable fly activity was observed during the spring months of March and April, with weekly three-trap means of 121 and 136 flies per farm, respectively. The importance of cultural control measures was most apparent on the only farm with no reported insecticide use and the lowest stable fly trap captures, where an intense daily sanitation and composting program was conducted. A survey of on-site filth fly pupae revealed that 99.9% of all parasitoids recovered were *Spalangia* spp., consisting of *Spalangia cameroni* Perkins (56.5%), *Spalangia nigroaenea* Curtis (34.0%), *Spalangia endius* Walker (5.8%), and *Spalangia nigra* Latreille (3.7%). (P. Kaufman, C. Geden, J. Hogsette)

b. Chemical control.

The efficacy of a granular formulation of cyromazine (Neporex 2SG) to control immature stable flies developing in winter hay feeding sites was assessed. A single application of granular cyromazine in May provided 97% reduction in the number of adult stable flies emerging from sites. Stable fly control did not decline during the 12 wk season. A small decline in control was observed relative to anthomyiid, sarcophagid, and syrphid flies developing in the sites. However, none of those flies are considered to be pests and ≥ 50% control of those flies was maintained for 65 d after application. (D. Taylor)

Bird netting treated with insecticide was assessed as a barrier to fly dispersal. Treatments consisted of label-rate concentrations of four products (formulations of beta-cyfluthrin, bifenthrin, lambda-cyhalothrin, and pyrethrins) and sun versus shade exposures. Face flies and house flies were bioassayed on netting samples taken at up to 14 weeks after field deployment of treated netting. Treated bird netting left in direct sunlight retained toxic residues of insecticide against flies when treated with formulations of beta-cyfluthrin and lambda-cyhalothrin through 12 weeks of exposure, while netting treated with bifenthrin and pyrethrins formulations showed a more rapid loss of toxicity (within one to three weeks) against house flies and face flies. In a separate set of experiments, ear tag efficacy trials were performed in 2011 in Washington State, using one control and five treated herds. While up to three months of control for horn flies was achieved with all three tested ear tags, the same ear tags showed much lower efficacy against face flies. Field-collected horn flies and face flies were evaluated for insecticide resistance against synergized zeta-cypermethrin, synergized abamectin, and diazinon, using a petri dish/filter paper assay. In horn fly, a low level of resistance was found for synergized zeta-cypermethrin, while a moderate level of resistance was found for diazinon. No resistance factors could be calculated for face fly because there are no published LC_{50}s for susceptible face fly

populations. Based on the LC_{50}s determined for face fly, levels of resistance are presumed similar to what was found for the local horn fly populations. While synergized abamectin was efficacious against horn fly (LC_{50}=6.12 µg/cm^2), no mortality was seen for face flies even at the highest concentration tested (100 µg/cm^2). No resistance was detected for synergized abamectin for either fly species. (H. Ferguson)

Because the beta-cyfluthrin and lambda-cyhalothrin formulated products showed good resistance to sun degradation, bird netting treated with these formulations should prove to work well as a perimeter fly barrier in beef and dairy operations. The data are not clear enough to recommend the same for the bifenthrin formulation. We conclude that the pyrethrins formulation is clearly not a suitable candidate for this barrier technique because of its rapid photo-degradation. Results from ear tag efficacy trials and resistance screening bioassays indicated that it should prove worthwhile to conduct similar trials during the summer of 2012 but rotating to an ear tag with a different class of insecticide with each treated cattle herd. For researchers and beef producers, both the efficacy data and resistance screen data represent new, significant, and useful contributions to the knowledge base of cattle fly pest management in Washington State. (H. Ferguson)

House fly (*Musca domestica* L.) resistance to insecticides is a growing problem for animal agriculture in southern California. Previous studies have demonstrated fly resistance to imidacloprid under laboratory and field conditions and suggested that resistance was partially due to changes in behavior. Flies from insecticide-resistant and insecticide-susceptible laboratory colonies were placed into an enclosed arena with separate dishes containing either imidacloprid-treated or untreated sugar for food. House flies from both colonies similarly visited food dishes containing either untreated or imidacloprid-treated sugar, indicating that resistant flies were not detecting the presence of imidacloprid and avoiding contact. Following contact with the food dishes, resistant flies disengaged from an imidacloprid-treated sugar dish significantly more often than susceptible flies. Also, while susceptible flies consumed both treated and untreated sugar equally, resistant flies consumed significantly less treated than untreated sugar. Imidacloprid appeared to act as a contact irritant causing locomotion stimulation in both susceptible and imidacloprid-resistant house flies, but the stimulation was significantly stronger in the resistant flies. Imidacloprid may also have acted as a feeding deterrent in resistant flies, but it was difficult to separate this effect from the irritancy effect. (A. Gerry)

Targets consisting of insecticide treated blue and black fabrics were developed as a tool in the management of stable flies. Studies were conducted to compare attractiveness of 3 configurations of blue/black cloth targets for stable fly control. There was no significant difference in mean numbers of flies attracted to flat or cylindrical targets. Targets in the cylindrical conformation may prove to be better at withstanding higher wind conditions than targets in the flat configuration. A perimeter of imidacloprid-treated visual targets provided partial protection of a Florida calf barn from immigrating flies. Electrocution techniques were used to determine if stable flies would land on and remain on cloth targets for a long enough time to absorb a lethal dose from an insecticide impregnated surface. In a series of two experiments, a half blue and half black (UK) 1 m^2 target constructed of trigger cotton poplin in an electrocution device (UK grid) was determined to be acceptable for development studies. In the first experiment, an average of 350 stable flies per hr (maximum 794 flies in 1 hr) was collected using

the UK grid. A time-delayed circuit trial using untreated UK grids demonstrated that stable flies remained on the targets for at least 30 seconds. Two experiments were conducted with time-delayed circuits and UK grids treated with 0.1% lambda-cyhalothrin and showed that the treated targets (TT) were not repellent. The number of flies collected with UK grids was 6.1-fold higher than that for Alsynite trap (AT) in two experiments. Blue-black targets with Alsynite traps (UKAT) were placed around pastures of different sizes to determine if more flies could be attracted when cattle were present. No difference in the number of flies captured was observed when the cattle were present in 10 acre pastures, but capture increased in smaller 1 to 5 acre pastures when cattle were present. We intend to repeat these studies, but our data indicate that four TT could be used in 3-4 acre pastures to affect a high number of flies associated with the cattle. For now, a TT per acre would be a good starting place for demonstration studies. The distance between the cattle and the targets is likely the most important variable, and to be effective the targets should never be more than 100 meters from the cattle. A short trial evaluated treated targets (TT) to protect cattle from stable fly attack. Two groups of steers in an intensive grazing experiment being conducted by another researcher using 1.5 acre pastures were used. The average number of stable flies per leg pretreatment was 12 to 17, and both groups were exhibiting bunching behavior and not grazing. Four treated targets were deployed around one group and four untreated targets around the other. The fly populations were fluctuating due to the time of year, but the number of flies on the cattle with TT dropped to zero flies after 8 days and averaged less than one per leg for the next five days. This reduction was associated with a lack of bunching and defensive behavior. The average number of flies per leg on the control group was 6, 1, 7 and 10 on days 8, 10, 11 and 13, respectively, and there was continued defensive behavior and occasional bunching. (L. Foil)

The number and sex ratio of adult stable flies collected on electrocution grids placed near and away from cattle was determined. Two UK grids were set up in a 64 m x 316.2 m pasture; one grid was positioned in the center of the pasture; the second grid was placed along the fence line approximately 75 m away. The grids were run for a 30 min period in the morning and then again in the afternoon. Twenty cows were held near the grid placed in the center of the pasture in morning, and then near the grid at the fence line in the afternoon. The mean number of flies killed on grids near cattle (510.5 ± 16.5) versus on grids away from cattle (120.5± 23.5) was significantly different (paired t-test, $t = 54.86$, $P = 0.01$), and the ratio of females increased from 34% female: 66% male when cattle were absent to 44% female: 56% male when cattle were present. Stable flies were also collected at the same time with AT placed in pastures near cattle and without cattle and the age of 30 male and 30 female from each collection was determined. Pterin concentration in fly heads was measured using methods described by Butler et al. (2009). The mean age in days for females and males collected near cattle was 6.57 ± 0.85 and 6.39 ± 1.13, respectively; the age for females and males collected away from cattle was 1.98 ± 0.42 and 3.78 ± 1.03, respectively. These data suggest that there is a significant population of older flies in close association with livestock. There could be several explanations that would explain this phenomenon including: 1) older flies have obtained multiple bloodmeals and are more fit to find and attack cattle 2) the flies remain close to the cattle after they have had a successful bloodmeal 3) the flies leave after a bloodmeal but return to the same pastures later. (L. Foil)

An acidifier, sodium bisulfate (SBS), used to reduce odors associated with animal manures was evaluated as a means of chemical control of larval house flies. The addition of SBS to manure

resulted in a dramatic drop in manure pH, resulting in a reduction in available bacteria following by a reduction in the number of flies developing in the manure. (A. Gerry)

Three new-to-science chemistries were identified and patents were filed. These chemistries (beta-damascone, cyclemone A and melafleur) have been shown to be effective against house flies and stable flies. These chemistries are equally effective against pyrethroid-resistant strains of house flies. (P. Kaufman)

House fly immatures were found to be highly sensitive to the JH analogue pyriproxyfen. Laboratory tests demonstrated that adult flies could be used as autodissemination vehicles to transfer pyriproxyfen to oviposition sites. (C. Geden)

Ear tag efficacy trials were performed from June to October 2011 in Washington State, using one control herd and five treated herds. While up to three months of control for horn flies was achieved with all three tested ear tags, the same ear tag treatments showed much lower efficacy against face flies. Field-collected horn flies and face flies were evaluated for insecticide resistance against synergized zeta-cypermethrin, synergized abamectin, and diazinon, using a petri dish/filter paper assay. In horn fly, a low level of resistance was found for synergized zeta-cypermethrin, while a moderate level of resistance was found for diazinon. No resistance factors could be calculated for face fly because there are no published LC_{50}s for susceptible face fly populations. Based on the LC_{50}s determined for face fly, levels of resistance are presumed similar to what was found for the local horn fly populations. While synergized abamectin was efficacious against horn fly (LC_{50}=6.12 µg/cm^2), no mortality was seen for face flies even at the highest concentration tested (100 µg/cm^2). No resistance was detected for synergized abamectin for either fly species. (H. Fergusen)

Experiments were conducted to determine if the monoterpene geraniol was an effective contact repellent or spatial repellent for house flies and stable flies in a petri dish bioassay. Geraniol in water was applied to one half of a filter paper with 0, 1, 2, 3 and 4% solution. The opposing half of the filter paper was untreated. House and stable fly activity was restricted to the untreated portion of the filter paper. House flies were sensitive to 2 and 3% geraniol while stable flies were most sensitive to 1 and 2% geraniol. House flies and stable flies exposed to 4% and 3% geraniol were anesthetized, respectively. House flies (50%) became anesthetized after 160 minutes and 50% of the exposed stable flies were knocked down in 54 minutes. In a second choice experiment, female flies were allowed to select for oviposition substrates treated with or without geraniol at concentrations of 0, 1, 2, 3, and 4%. Flies were deterred from ovipositing on substrates treated with 4% geraniol. (J. Zhu)

A study was initiated to determine if LSU Treated Targets (TT) would suppress native stable fly numbers on grazing cattle in West Central Nebraska. Treated Targets (TT) were soaked to the point of saturation with a 0.1% Lambda-cyhalothrin. Two treatment (A&B) pastures and one untreated pasture (C) were identified for use in this study. Cattle numbers ranged from 25 cow/calf pairs to 53 cow/calf pairs. Each pasture received two targets which were located within five feet of a cattle water tank. Targets were protected from cattle by fencing .Stable fly target counts and stable fly animal counts were conducted on a weekly basis. Target counts were made

by counting the total number of stable flies that landed on each target during one minute. Stable fly animal counts were made by counting the total number of flies on all four legs and belly of 15 adult animals. Fly counts were made between 8 and 11 AM. Combined stable fly counts from the two targets at Site A, totaled 57 stable flies, with an average of 4 flies per target during the 7 week study. The total stable fly count from the two targets at Site B was 84 flies with an average of 5.28 flies per target during the study. Site C which had the untreated targets had a combined total of 108 stable flies with an average of 7.71 flies per target. Animal stable fly counts on cattle at Site A averaged 3.5 flies per animal, compared to 3.2 stable flies per animal on cattle at Site B, and 3.6 flies per animal on cattle from Site C. The average number of stable flies on cattle from the untreated group was 2.79 flies per animal. No significant differences were detected in animal fly numbers between the three pastures. (D. Boxler)

To determine if field exposed LSU Treated Targets (TT) have residual activity against laboratory reared stable flies. Treated Targets (TT) (0.1% lambda-cyhalothrin) and untreated targets were removed from the field after 110 days of exposure. Target material was harvested and placed in separate plastic bags and placed into a freezer for storage until residual studies were initiated. Stored Targets were removed from the freezer and allowed to warm to room temperature before studies were initiated. Several three inch by five inch pieces of the Target cloth were randomly removed from the treated and untreated Targets. Exposure cages of wood construction were designed to allow a 3" x 5" inch piece of Target cloth to be inserted within the cage for fly exposure. Each cage contained 15 laboratory reared stable flies with four replications. Stable flies were exposed to treated and untreated cloth at three different intervals 15 seconds, 30 seconds and 60 seconds. After the specific exposure intervals the cloth was promptly removed. Stable flies remained in their respective cages until mortality was assessed 2 hours post-exposure. The control cages had no mortality and there was 100% mortality for all three exposure intervals for the treated Target material. (D. Boxler)

A three year study to evaluate the efficacy of a mist blower sprayer for the reduction of horn flies and stable flies on pastured cattle was completed during the fall of 2010. The study utilized cow/calf pairs varying in herd size from 15 – 48 pairs with treatment groups ranging from two to four. Horn fly counts were made by counting the total number of flies on both sides of 15 randomly selected cows during study years 2008 and 2009. Stable fly counts were compiled using the same animals used for counting horn flies. Total number of stable flies per four legs and belly were recorded and that number expressed as number of stable flies per cow. An untreated group of cow/calf pairs served as a comparison. During the three year study two different classes of insecticides were evaluated (Synthetic Pyrethroids, and a Spinosad). Spray applications were made using a Model SD-RM20-H855 mist blower provided on loan to the university by A1 Mist Sprayers Resources Inc., Ponca, NE. Spray application rates ranged from 2.0 gallons for smaller groups to 4.5 gallons for the largest groups. Results from 2008 indicated horn fly numbers averaged 136 flies per cow on sprayed groups versus 360 flies on the untreated group for a 58% reduction in horn fly numbers. Stable fly numbers averaged 2.90 per animal on treated groups compared to 7.18 flies per animal on the untreated group resulting in a 70% reduction in stable fly numbers. Results from the 2009 study indicated horn fly numbers on sprayed groups averaged 128 flies per cow compared to 292 flies on the untreated group resulting in a 56% reduction in horn fly numbers. Stable fly numbers averaged 1.03 per cow on sprayed groups versus 7.18 flies per cow on the untreated group resulting in an 86% reduction in

stable fly numbers. Results from the 2010 study indicated horn fly numbers averaged 215 per cow on sprayed groups compared to 339 horn flies per cow on the untreated group resulting in a 41% reduction in horn fly numbers. Stable fly numbers averaged 3.26 flies be cow on sprayed groups versus 8.44 flies per cow on the untreated group resulting in a 61% reduction in stable fly numbers. Overall, for the three year study, horn fly numbers averaged 159 per cow on sprayed groups versus 339 flies per cow on untreated groups, resulting in a 53% reduction in horn fly numbers. Stable fly numbers averaged 2.4 flies per cow for sprayed groups compared to 8.46 flies per cow for the untreated groups, providing a 72% reduction in stable fly numbers. (D. Boxler)

Insecticide ear tag efficacy trials were conducted from June to September 2010 in West Central Nebraska, using one control herd and three treated herds. Cattle numbers ranged from 25 to 100 replacement heifers. Horn fly numbers on the control group averaged above the economic injury level (EIL) of 200 during all 16 weeks of the study. On insecticide ear tagged cattle, the EIL was exceeded during week 13 for Corathon,™ week 16 for CyGuard™ and for XP 820™ fly numbers did not exceed the EIL. (D. Boxler)

Insecticide ear tag efficacy studies were conducted from June to September 2011 in West Central Nebraska, using one control herd and six treated herds. Cattle numbers ranged from 25 cow/calf pairs to 45 cow/calf pairs. Horn fly numbers on the control group averaged above the economic injury level (EIL) of 200 during all 15 weeks of the study. On insecticide ear tagged cattle, the EIL was exceeded during week 7 for Warrior™ and week 8 for Double Barrel™ VP and PYthon MagnuM™ of the 15 week study period. For the remaining insecticide ear tag treatments, the EIL was exceeded the last two weeks of the study for cattle tagged with PYthon™ ear tags and with cattle tagged with XP 820™ tags, the EIL was exceeded during week 15, the last week of the study. (D. Boxler)

Initiated a two year study in 2011 with Bayer Animal Health to evaluate the impact of Corathon™ insecticide ear tags on pasture fly control and evaluate that added effect of fly control on calf and stocker weight gains. This study will conclude in the fall of 2012. (D. Boxler)

Preliminary work was initiated in June 2011 to design and evaluate a stable fly leg patch for the control of stable fly numbers on pastured cattle. Initial studies focused on patch size, shape and adherence properties. Further evaluation of this methodology will continue during 2012 and 2013, studies will concentrate on adhesives, patch size, shapes and ultimately efficacy. (D. Boxler)

A study was initiated to evaluate the lethal properties of a water-based formulation of an essential oil against laboratory reared stable flies. A 1% lemongrass water-based and mineral oil formulation were evaluated against stable flies as a knockdown and as a vapor. Replicated knockdown studies indicated the 1% water-based formulation provided only 4% mortality, 6 hours post-application compared to the 1% mineral oil formulation which provided 100% mortality in just 1hr post-application. To evaluate the effects of lemongrass vapor on stable fly mortality acrylic solid wall cages were used in replicated tests to compare the two formulations. The water-based formulation provided 20% stable fly mortality during a 7 hour exposure period while the mineral oil formulation provided 100 % stable fly mortality in just 2 hours of exposure.

Results from these studies indicate that a water-based formulation of lemongrass oil will provide significantly less stable fly mortality than a mineral oil formulation. (D. Boxler)

c. Insecticide Resistance Management.

A national survey of house fly resistance was completed, using standard procedures developed in New York. Resistance to methomyl, tetrachlorvinphos, cyfluthrin permethrin, pyrethrins + PBO and imidacloprid were evaluated in flies collected from dairies in nine states. Resistance varied considerably by location, indicating the need to monitor resistance locally. In addition, we examined the frequency of alleles of two genes (Vssc and CYP6D1) which confer resistance to pyrethroid insecticides. Again, the distribution of resistance alleles was variable between states. The super-kdr allele of Vssc confers the highest levels of pyrethroid resistance, but was found only in five states, and generally at low levels. Both the kdr and kdr-his alleles of Vssc were found, but their pattern of distribution varied considerably. CYP6D1v1 (resistance allele) was common and susceptible CYP6D1 alleles were rare in all states. These results indicate that the fitness cost of different resistance alleles varies by location. These results provide a framework for which we could develop improved resistance management strategies for house flies. However, stakeholders continue to use scheduled sprays in many locations. Their hesitation to implement IPM measures (in some states) suggests that changing their behaviors to implement resistance management plans will require a major educational effort. (J. Scott)

Five adult house fly strains from four Florida dairy farms were evaluated for resistance to four insecticides (beta-cyfluthrin, permethrin, imidacloprid and nithiazine). Significant levels of tolerance were found in most field strains to all insecticides, and in some cases substantial resistance was apparent. At the LC_{90} level, greater than 20-fold resistance was found in two of the fly strains for permethrin and one fly strain for imidacloprid. Beta-cyfluthrin LC_{90} resistance ratios exceeded tenfold resistance in three fly strains. The relatively underutilized insecticide nithiazine had the lowest resistance ratios; however, fourfold LC_{90} resistance was observed in one southern Florida fly strain. House fly resistance to pyrethroids is widespread in Florida. Imidacloprid resistance is emerging, and tolerance was observed to both imidacloprid and nithiazine. (P. Kaufman)

The FDm house fly strain was created by a 20% contribution from each of five colonies collected from dairies in Florida with known imidacloprid resistance. The FDm strain was used to evaluate the level of imidacloprid resistance after five selections near the LC_{70} value of each selected generation. Overall, the mean selection mortality was 72.7, with males being considerably more susceptible than females. The unselected (F0) FDm strain showed considerable susceptibility to imidacloprid after its creation, compared with the five parental strains. Between 9,500 and 14,000 virgin house flies were used in each selection. After the fifth and final selection, a 331-fold increase in imidacloprid resistance at the LC_{70} was observed over the parental FDm strain. In parallel studies, the FDm strain showed increasing tolerance of the commercial imidacloprid product QuickBayt. A wild-caught stable fly colony was selected for resistance to permethrin over 5 alternate generations reaching 15-fold resistance. This colony was subjected to genetic analysis and the resistance mechanism has been determined as a sodium channel mutation. (P. Kaufman, P. Olafson)

d. Economic Impact of Stable Flies.

An explicit and dynamic model for estimating the economic impact of stable flies on cattle production was developed. Based upon USDA-NAS data from January 2008, commodity prices from May 2008, and injury levels derived from the literature, we estimate the economic impact of stable flies on cattle production systems to be approximately $2 billion per year. (D. Taylor, R. Moon)

A survey of cattle producer pest and pesticide use assessment was conducted at the University of Florida. Despite the widespread distribution of surveys, very few were returned. Responses were obtained from beef cattle producers with operation sizes from 10 animals to 1,000 animals. Most producers (58%) applied pesticides once per season for flies on pastured animals. Approximately 78% of producers treated for cattle grubs and 70% treated for lice. Producers identified flies on pastured animals and fire ants (damaged equipment) as their major pests. (P. Kaufman)