Milestones:

Years 1-3:

1a. Identification of repellents most suitable for application to animals. Development of efficient repellent delivery systems. Determine distance limits for placement of insecticide treatments to achieve goals of the study.

1b. Evaluate recognition software for the identification of flies in the environment.

1c. Vestergaard-Frandsen fencing studies will begin in 2013 with sites secured and studies begun in Washington DC and Lincoln NE. Project near Gainesville already begun, and other two projects continued. Data for all three projects will be organized and a decision made on whether to continue or stop. If the former, then projects will continue through 2015. Identify potential bait formulations and perform laboratory studies. Evaluate promising baits under semi-field conditions.

1d. Small-scale laboratory experiments will be conducted to evaluate and identify the best bedding types and best bedding treatments that result in the greatest fly reduction to be used for the on-dairy experiments. Develop practical way to monitor flies affecting individual calves in a multi-hutch environment relative. Field trials will be conducted to determine proof-of-concept and comparative efficacy for fly-reducing bedding or bedding treatments. Develop and evaluate methods to fly populations and lying time. Identify study sites and place walk through fly traps for comparative studies.

2a. Determine the linkage of imidacloprid resistance in the house fly. Conduct permethrin resistance surveillance for stable flies in at least three states participating in this project. Complete evaluation of 2012 stable fly field collections originating from six states for prevalence of the stable fly *kdr-his* allele. Request stable fly field collections from collaborators to conduct a 2013 survey for prevalence of the stable fly *kdr-his* allele. Develop and test the insecticide resistance protocol and field-testing kits for horn flies in Florida. Save stable flies and horn flies (by freezing) for subsequent genetic profiling of resistance mechanisms. Determine the role of the *Rdl* mutation in resistance of horn fly populations to the cyclodiene endosulfan. Investigate the mechanisms of resistance to imidacloprid in the house fly. We will conduct permethrin resistance surveillance for stable flies in at least three additional states participating in this project. Genetic profiling of resistance mechanisms in stable flies from the resistance monitoring study will begin. Comparisons between stable flies that survive vs. die will be used to identify mutations associated with resistance such as *kdr*, *kdr-his* and *super-kdr*. We will provide horn fly insecticide resistance field-test kits to collaborators in at least 5 cooperating states. Horn flies from resistance monitoring tests will be evaluated for genetic resistance mechanisms. A discriminating dose diagnostic bioassay will be developed to screen populations of horn flies for *kdr* and *super-kdr* mutations.

2b. Make the house fly genome publically available. Identify collaborators to assist with assembly and annotation of the stable fly genome sequence data, to be provided by The Genome Institute at Washington University (St Louis, MO). Identify a mechanism to ensure proper dissemination of the data within the scientific community, but most importantly to the stable fly community, e.g. a hosted website that contains the database and is searchable by keyword or sequence similarity.

3a. Attractant and repellent compounds will be identified from larval stable fly development sites. The anterior, mid, and foregut of stable fly larvae will be characterized using histological and microscopic techniques.

3b. The temporal and spatial local expression of secreted effector molecules in the alimentary canal of house flies (e.g., lysozyme, AMPs) on both the transcriptional and peptide levels will be known. We will 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.

3c. Cattle fecal samples will be assess for the presence of shiga-toxin virulence genes to see if flies might serve as bio-indicators of the genetic diversity of bacteria. Infection rates of house flies with salivary gland hypertrophy virus will be determined from Massachusetts. TEM and SEM studies of the salivary and crop organ systems will be completed. Laboratory studies of horn fly response to essential oils will be completed.

4a. Complete Mark-Release-Recapture studies for confined animal facilities in Nebraska. Initiate Mark-Release-Recapture studies in Florida and Minnesota. Identify SNP markers**.** Complete population structure analysis.

4b. Complete study on early spring development of stable flies. Complete spatial variation of sticky trap collections study (study initiated under previous project).

4c. Complete facility surveys.

5a. Develop framework for online pesticide database suitable for the needs of our clientele. Contact industry representatives with a plan of action to facilitate the gathering of needed pesticide information. Launch database and advertise its presence to clientele.

5b. Determine a framework for collaboration among those developing extension material to ensure common access to this material. Develop an action plan to generate research and extension product (REP) database. Load REP with information and test with clientele groups.

5c. Generate annual report on REP accomplishments. Provide report in print and electronic versions to stakeholders identified in Sub-objective C. Present pest-specific extension Webinars or other non-site bound regional extension programs. Conduct commodity and pest specific conference calls with interested clientele, as outlined in Sub-objective C.

5d. Collaboratively submit grant proposals to appropriate funding agencies identified by project members to accomplish extension goals as outlined in this project. Encourage project participants to consider extension of project outcomes in individual or collaborative research projects in line with the other objectives outlined in this proposal.

Years 4-5:

1a. Conduct replicated field trials.

1b. Test software for known pitfalls, accuracy, color variance. Apply technology in field studies used by the members.

1c. Evaluations of newly registered products by the USDA Kerrville Lab are dependent on industry partners and mutual agreements. It is anticipated that Kerrville will be testing 2 or 3 new products for livestock each year 2015-2020. The USDA CMAVE lab in Florida will be conducting autodissemination studies from 2015-2018. Non-target effect studies will be conducted from 2017-2020. Mortality effects of essential oils and fatty acids will be conducted in Nebraska as a joint effort between the University of Nebraska and the USDA MLIL. These studies will be conducted from 2016-2020. Data organized, analyzed and published beginning in 2017. Evaluate final candidate baits in the field and write publications.

1d. Asses the impacts of fly populations on calves and calf behavior relative to variety, bedding and season interactions. Evaluate fly traps under field conditions for quantitative and qualitative efficacy for target insects. Collect data on the relative ease of different trap types and cost to operate. Technical reports will be published in newsletter articles and poster presentations from with an extension bulletin or a research publication will be developed. Prepare publications for extension and peer reviewed journals.

2a. Continue to screen field populations of house flies and stable flies on an annual basis for the presence of *kdr-his*; Expand this screening to include any additional mutations that are identified as a result of the national survey for insecticide resistance. Stable and horn fly populations in additional states or sampling of additional sites within already tested states will be conducted based on cooperator interest. Stable and horn flies that survive or die in testing will be submitted for genetic profiling, as in Years 1 and 2. Live-fly bioassay data (horn fly and stable fly) will be summarized and publications prepared for submission. Develop a multiplex PCR for *Rdl, kdr, super-kdr*, and G262A acetylcholinesterase mutations and use this assay to determine changes in the frequency of mutations in horn fly populations exposed to different insecticide control approaches. Horn fly resistance mechanism profile data will be summarized and a publication prepared for submission.

2b. Assemble and annotate the stable fly genome. Make the genome publically available. Organize a workshop to familiarize the house fly and stable fly communities with the results from the genome and to facilitate use of the sequence data in enhancing house fly and stable fly research programs. Use input from workshops as a basis for preparing a collaborative manuscript detailing the stable fly genome sequence. Identify the stable fly odorant receptor and gustatory receptor families from the sequence data and describe their spatial and temporal expression profiles as a means of identifying life stage-specific receptors that can be manipulated to develop novel control approaches.

3a. Attractant and repellent compounds identified from larval development sites will be evaluated in laboratory bioassays and under field conditions for stable fly response.

3b. We will evaluate expression of epithelial immunity molecules in response to ingestion of three different bacteria isolates, *E. coli, Citrobacteri freundii*, and *Serratia marcescens*. The impact of microbes on *Culicoides sonorensis* development and fitness will be determined.

3c. Essential oils will be evaluated under field conditions for reducing horn fly populations, with an expected reduction in teat damage and mastitis infection.

4a. Complete Mark-Release-Recapture studies in Florida and Minnesota. Complete dispersal analysis**.** Complete local adaptation analysis.

4b. Incorporate developmental data into population growth model using time-series datasets. Complete spatial variation of host infestation studies.

4c. Complete microbial and physical property analyses.

5a. Improve database based on feedback from all clientele (industry, end-users, regulators, etc.).

5b. Add new materials and continue advertising of REP database.

5c. Produce and update static collaborative extension products based on project accomplishments. Present pest-specific extension webinars or other non-site bound regional extension programs. Conduct commodity and pest specific conference calls with interested clientele, as outlined in Sub-objective C.