Responses to Reviewers' Comments

Reviewer #1

Comment 1: I am particularly concerned that the introduction wisely emphasizes understanding biting and nuisance fly responses to climate change, but the objectives, methods and outcomes do not address climate change. A major objective should be projection of changes in geographic distribution and abundance as a result of climate change and how this will affect fly impacts. Furthermore, fly susceptibility to microbial or insecticidal treatments also may change with climate change. Some research should address this.

Response: We now specifically state that we will carry out measurements of insecticide resistance at different temperatures (Objective 2)

Comment 2: Paragraph 2 notes "these two flies"; the preceding sentence mentions "biting and nuisance flies" and the sentence after mentions house flies and stable flies. Which "two flies" does the second sentence mean?

Response: Corrected

Reviewer #2

Comment 1: The project is very optimistic.

Response: We agree, but we have an excellent team in place and are confident we can make significant progress on all of these goals.

Comment 2: The Methods sector is extensive and perhaps too detailed, yet the areas it addresses are all interesting and important.

Response: The level of detail provided was needed for a reasonable review of the project and they have not been changed.

Comment 3: The authors need to pay more attention to how the stations will collaborate.

Response: Many of the stations have collaborated in earlier projects, and this is expected to continue. Stations participating in each objective section are identified by state on the list of objectives near the top of the proposal. Funding to support objective goals often dictates specific collaborations so that anything more specific than what is provided is probably just wishful thinking at this point.

Comment 4: It appears from the Appendix E that some of the stations have not yet indicated their intention to participate. Seventeen stations in the previous project vs. 6 stations identified in this proposal.

Response: The remaining 11 stations will participate, but need an approved project before the station director can add the station to the project.

Comment 5: Very little attention is dedicated to how these proposals might obtain additional funding.

Response: Funding to accomplish project goals is pursued by individual or collaborative groups of researchers participating in this multi-state project. Typically, funding supporting the overall objectives is secured through competitive funding programs of the USDA and NSF. Animal commodity groups provide little or no funding to support ectoparasite-focused research, so commodity funding is not available as it is for food crop commodities. Extension and community engagement efforts are particularly difficult to fund as few programs earmark funding for this area. Additionally, the loss of some specialty funding programs at the USDA (e.g., USDA-RAMP, USDA-CAR) will make funding to support research on animal pests even more difficult than it has been in the past. So, little attention is dedicated to funding, because it is unclear to the group where project members are most likely to be successful.

Comment 6: Identify station collaborations as done in the previous proposal.

Response: Stations participating in each objective section are identified by state on the list of objectives near the top of the proposal. Funding to support objective goals often dictates specific collaborations so that anything more specific than what is provided is probably just wishful thinking at this point. Appendix E will be completed once the project is approved and Station Directors are then able to add their station to the project.

Reviewer #3

Comment 1. Methods, Objective 1a, Novel push-pull strategies: The idea of using this approach, adapted from field crop IPM, is appropriate and should reduce pesticides applied to cattle. My concern is the lack of detail about adjacency or proximity of the "push" cattle from the "pull" cattle. As written, it only says that (Pasture 1) half the cattle will be in each treatment, but nothing about spatial scale. The Mark-Recapture studies state that the powder-treated flies will be released "in the vicinity" of repellent-treated cattle, with recipient animals 1-3 miles away. Although the idea is reasonable and makes sense, data analysis is not so simple -- what does "not finding flies" at a certain distance truly mean?

Response: The spatial scale for these studies is now indicated. Additional discussion is also provided for mark-release-recapture analysis of dust marked flies.

Comment 2: Objective 1b, Improved monitoring: New methods to estimate fly numbers will likely ensure greater grower participation. Visual-recognition software can help reduce time necessary to count flies, with digital photos backing up the data. However, the digital imaging and analysis currently would require a significant amount of time -- whether that technology can be improved greatly enough to be beneficial, particularly because of not knowing whether the contrast is sufficient to separate flies from the animal coat.

Response: We agree that digital analysis of fly counts is currently very time consuming. If digital methods are to be utilized, software must be developed that automates the counting process. Efforts to

automate this process are currently being evaluated and if successful may be utilized in field research under this proposal.

Comment 3: Objective 1d, non-pesticide options, i. Biological control (pathogen): Dose-response curves for the pathogens make sense, but are all stages of the flies susceptible? And how comparative are the methods? For house flies, comparing baits with naturally occurring foods; for stable flies, treated bedding; horn and face flies, through treated hides or substitutes. Tough to evaluate or to know the investigators will set this up as a true comparison of substrates. UV absorption and the effects of UV on the efficacy of the pathogens will be critical -- just as the difficulty of implementing use of pathogens against field crops.

Response: The exposure methods used for the four fly species are not meant to be comparative between the different flies. Exposure methods incorporated into proposed studies target each flies biology and thus perceived vulnerability to *B. bassiana* exposure, whether this be adult feeding behavior (house fly), targeting of immature developmental sites, such as hay rings (stable fly) or adult resting sites on cattle (face fly and horn fly). Therefore, it is impractical to utilize a single exposure technique for post-Petri-dish assays (dose response studies). Comparisons identified in the proposal are done within a fly species, i.e. house fly comparisons are with the developed bait product with and without *B. bassiana* added as well as selected food sources found in livestock operations (manure, cattle feed, oviposition sites, etc.). Comparisons for other fly species will not be as diverse and will focus on treated and untreated substrates perhaps with multiple doses and the UV considerations described below. Essentially, the Petri dish assays will determine the LC value needed for desired mortality, while the simulated field and true field assays are used to evaluate if these LC values are sufficient in obtaining the same mortality under field conditions.

As the reviewer points out, UV degradation of fungi is an important consideration. We have been in contact with companies that have developed methodologies to protect the fungi from UV degradation and we would include this in the evaluations (treatments with protection and treatments without protection).

Comment 4: Objective 1d, non-pesticide options, i. Biological control (parasitoid): Host preference and parasitoid selection is treated far too superficially -- as stated, host phenology will affect the outcome, and will do so far more than "host preference" would. Avoiding phenological bottlenecks through augmentation is fine, but doesn't really address larger issues. Monitoring using the "improved sentinel method" is fine, but nothing about retrieval rates? Retrieval will be necessary to know how efficient the approach is -- how many larvae are lost? This entire section needs some more thought put into it.

Response: Information specific to the experimental design was added to the text to help clarify how the described study would separate phenology from preference by using the improved sentinel method and a control.

Comment 5: Objective 1d, non-pesticide options, iii. Mechanical: Why bother with the Bruce trap (50-70% effective) when the Australian trap is far more effective (90-96% effective, simpler and more economical)? And why use the Bruce trap for comparisons with the Cow-Vac? Economic comparisons

would be important to demonstrate to growers. The methods for assessing fly capture and disappearance seem pretty superficial, and can efficacy really be evaluated by sampling cow dung pats for fly emergence?

Response: The Bruce trap may be less efficient at sampling horn flies, but there is a lack of published data on efficacy of this trap or the Australian trap for face flies and stable flies. The three-way comparison is desired to evaluate the efficacy of each trap type for each of the fly species. We will also evaluate the economic aspects of each trap type including upkeep costs. Sampling of dung pats to demonstrate changes in the fly population by quantifying fly emergence from pats sampled over time has previously been successful (e.g. see Moon et al. 1993, cited in the proposal). Dung pats are often sampled as a means to quantify pest numbers, and in many cases this is probably a more accurate measure of management success than measures directed at enumerating adult flies which may well have immigrated from off site locations.

Comment 6: The outputs from Objective 1 would have the greatest likelihood of implementation in the near term, and so need some priority.

Response: We agree that objective 1 outputs are most likely to result in near-term implementation. Several members of the multi-state program have proposals already submitted or in preparation to fund research related to this objective.

Comment 7: Objective 2 -- Insecticide Resistance. Insecticide resistance has been documented, and rotation of compounds is mentioned, but how much rotation really occurs? How many chemical classes are currently used and how can those be shepherded better? This is a perfect opportunity for extension. Leveraging the genomes appears to be an excellent direction and should yield novel approaches for control.

Response: The classes of insecticides used are now specified in Objective 2. Patterns of resistance vary between states and resistance management tactics will need to be implemented on a regional basis. We agree this is a perfect opportunity for extension.

Comment 8: Objective 3 -- Microbial Ecology, Epithelial Immunity and Vector Competence. Very important component of the project, and likely to yield valuable outputs. In Section 3a (Chemical Ecology) -- why not apply the push-pull approach here? This seems to be a perfect opportunity to take findings from Objective 1 and integrate in this study. The inter-kingdom communication seems a little of a long shot, but this is the kind of project to look for novel, transformative results. I do, however, question the economic figure cited for the annual economic costs of biofilms -- $1,426 billion. Really? This is 10% of the US GDP. The studies and directions appear appropriate and important tot he project outcomes.

Response: We have clarified that attractive lures could be utilized in a push-pull strategy and that use of these strategies is one of the long term directions for IPM of animal ectoparasites and an area of research for many of the participants in this multi-state proposal. We have also clarified that the

reported economic costs are for ALL food related illnesses. There is a considerable range in these published estimates.

Comment 9: Objective 4. Population Biology. This seemed to be the weakest of the objectives, although results could help clarify the dispersal ability in both theory and practice. The majority of references indicate stable flies have minimal dispersal, but one reference listed 225 km dispersal in days. Although it may be possible to correlate climate factors with phenology and weather factors with dispersal, it will be a challenge to know with any certainty those factors affecting populations. Section 4b pointed to the large dataset accumulated through the previous project, yielding predictors for rain and temperature. Are these factors used in any way currently? The methods proposed for examining spatial variation among stable fly populations were not convincing -- after just mentioning the known effects of rain and temperature, there was no mention of controlling for those factors (or if it was even possible to do).

Response: The proposed research is meant to address the inconsistencies in dispersal identified by the reviewer. We intend to evaluate dispersal parameters to assess whether the choice of parameters measured is resulting in some of this inconsistency. Data from the previous project are currently being used to refine dispersal models in the mid-West and for comparison by researchers in the southeast, as well as to form the foundation for the work proposed in this project; however the general applicability of this data across larger geographic ranges is unclear and needs further examination. This proposal will examine spatial variability under natural conditions (including rain and temperature variation). Efforts will not be made to control for these conditions, but rather to account for their effect on spatial variation.

Comment 10: Objective 5. Engagement. There are far more approaches available and likely to engage the stakeholders than were provided here. Getting growers to use the information already developed would be a huge step forward (use of traps, delaying resistance through rotation of insecticides, alternatives to insecticides), but that is the weak link. Compiling a database of pesticides is nice, and having state-specific modification is necessary, but how can this information be translated into strategies for rotating pesticides on a local and larger scale? Linking information currently available -- sounds great. How does this really get compiled into something usable by growers? As stated, it is essential that research results are converted into a form usable by decision makers. Some of the methods proposed are reasonable, but the approaches will need to be adaptable to different constituent groups and end-users. Hiring a popular-science writer? Funded how? Seeking funding for these efforts will be critical, but this is treated as an afterthought. Seeking funding from MUVE (not defined)?

Response: The number of approaches to engage stakeholders is essentially limitless. As individuals, many of our project members engage stakeholders on a regular basis. For this project however, we hope to develop means of engagement that can be developed, managed, reviewed, and maintained collaboratively. This will not be an easy task! The ideas presented in the proposal are essentially a springboard to generate group movement toward collaborative engagement.

A database of registered pesticides for animal commodities is one need identified by stakeholders in several states that we believe can be accomplished and which will provide an important connection to stakeholders nationally. Rotation of pesticides to reduce resistance development can only occur if alternative chemistries are available and suitable products are known to producers. We hope to simplify the process for producers to identify products to rotate and perhaps to identify for our industry stakeholders gaps or needs in what is available for resistance management.

We have modified the proposal to remove the idea to hire a science writer as this is currently an unfunded idea. We have clarified how we intend to link currently available extension material, at least in the short term. Development of a long-term framework to link all of this information is a goal of the project and will be addressed by the group as we move forward. Funding will be a significant issue to accomplish these extension goals, as funding for extension only projects is very limited. Currently it is not clear to the group where funding for engagement goals will come from. It is expected that by including engagement as a specific objective of the project, participants and collaborators will build into their research–specific proposal some request for funds to support extension of their research outcomes through the national framework that we develop.

Comment 11: Engagement and implementation of the research results is probably the most important part of the overall project, yet it is not given the attention it is due. Many results and outputs from the previous project have not yet been implemented or remain in the category of research outputs, and have not yet been turned into outcomes. The Expected Outcomes or Project Impacts will not occur without greater attention given to transfer of the knowledge to end-users than is outlined here.

Response: We have found with earlier projects that while considerable research goals were met, this information was not sufficiently extended to growers or others who might make best use of it. We intend to focus on engagement as part of this project precisely to respond to the critique of this reviewer (which we also recognize and agree with). It was for this reason that we highlighted community engagement as a specific objective. We recognize that to achieve our engagement goals will require a more defined framework than we currently provide. However, that framework must be developed through collaborative discussion and trial; a process that we will undertake over the next 5 years.

Comment 12: Specific outputs: " Methods of evaluation of biological control agents and recommendations for alternative bedding -- not really what the methods stated. " Produce extension publications describing the mechanisms of insecticide resistance -- will these actually be beneficial to the end-users? They need to know how to minimize resistance, much less the mechanisms.

Response: Output 1d was modified to better align with the body of the research proposal. A database of resistance mechanisms will be useful to other researchers and to industry; this may also be used by extension agents and commodity groups to support area-wide pesticide rotation or replacement efforts.

Comment 13: Outcomes list "increased awareness" for producers or "increased adoption" but the methods used to disseminate the information to increase awareness and adoption are not convincing. And some of the outcomes listed were outputs, not outcomes.

Response: The recognized need to improve dissemination of information is addressed with objective 5. We expect to disseminate information through a more collaborative framework than in past proposals. We scrubbed the outcomes list to remove or alter outcomes there were actually outputs.