Dear Dr. Britch,  
  
Your manuscript, JoVE58440 Visualizing efficacy of pesticides against disease vector mosquitoes in the field, has been editorially and peer reviewed, and the following comments need to be addressed. Note that editorial comments address both requirements for video production and formatting of the article for publication. Please track the changes within the manuscript to identify all of the edits.  
  
After revising and uploading your submission, please also upload a separate rebuttal document that addresses each of the editorial and peer review comments individually. Please submit each figure as a vector image file to ensure high resolution throughout production: (.svg, .eps, .ai). If submitting as a .tif or .psd, please ensure that the image is 1920 pixels x 1080 pixels or 300 dpi.  
  
Your revision is due by **Jun 28, 2018**.  
  
To submit a revision, go to the [JoVE submission site](http://www.editorialmanager.com/jove) and log in as an author. You will find your submission under the heading "Submission Needing Revision".  
  
Best,  
  
Phillip Steindel, Ph.D.  
Review Editor  
[JoVE](http://www.jove.com/)  
617.674.1888  
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**Editorial comments:**  
*Changes to be made by the Author:*

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

**AUTHOR RESPONSE: thank you – we cross-checked throughout.**

1. Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.

**AUTHOR RESPONSE: thank you – we cross-checked throughout.**

1. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.)

**AUTHOR RESPONSE: thank you – we cleaned all such personal pronouns from the Protocol section by rewording.**

1. **2.3.2:** The Protocol should contain only action items that direct the reader to do something. Please move the discussion about the protocol to the Results or Discussion sections.

**AUTHOR RESPONSE: we believe that 2.3.2 only contains action items directing the reader to do something (i.e., adding 10% to the beginning and end of the spray line) with minimal supporting information (i.e., to compensate for vagaries of the weather) that would be awkward to include elsewhere.**

1. **Lines 387-397:** Please consider moving this Note about the protocol to the discussion section.

**AUTHOR RESPONSE: we believe that this Note should remain with section 2 because it is most relevant to section 2. In our view, building local collaborators is key to successful field work and is as much a part of the protocol as laying out a grid of points.**

1. **Lines 413-415 and 5.2:** Please write the text in the imperative tense.

**AUTHOR RESPONSE: thank you; good catch. We re-wrote in imperative tense.**

1. **3.4:** Please write all the text in the imperative tense and break up into sub-steps.

**AUTHOR RESPONSE: thank you; good catch. We re-wrote in imperative tense, moved sentences to the Notes in sections 3 and 5.**

1. **References:** Please do not abbreviate journal titles.

**AUTHOR RESPONSE: thank you – we have reverted all journal abbreviations to their full titles.**

1. *There is a 2.75-page limit for filmable content.* Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.  
   **AUTHOR RESPONSE: we highlighted ~2.75 pp of filmable content per guidelines.**

**Reviewers' comments:**  
  
**Reviewer #1:**  
**Manuscript Summary:**The manuscript is well prepared and includes most of the details related to the experiments. However, I want to suggest few points which can make it more suitable to many people may not be well aware about the field properly.

**AUTHOR RESPONSE: thank you; and we appreciate the opportunity to improve the ms.**

**Specific comments:**Introduction line 85-97 more suited to Materials & Methods and should be removed from Introduction or modify.

**AUTHOR RESPONSE: We would like to retain lines 85-97 in the Introduction otherwise the rest of the Introduction will be, in part, without context.**

Protocol: Heading 1. Sentinel Insects should be "Insect rearing and sentinel cage preparations".

**AUTHOR RESPONSE: Thank you – we changed the heading to “Sentinel insect rearing and sentinel cage preparations”**  
  
Section 1.2. Include the details of rearing of the species used for the test.  
**AUTHOR RESPONSE: Details of rearing mosquitoes are specific to the species used and it would be burdensome to include rearing protocols for even the few prominent sentinel mosquito species. Specifics of insect rearing is outside the scope of this paper.**

Table 2. The data should be analyzed and compared for differences among the cages and the treatments. The individual data point table for an example is already given in Table 1.  
**AUTHOR RESPONSE: These two tables have different purposes in this manuscript. Table 1 is an example data form and we believe it would be confusing to populate it with mock data and it should stand alone. The point of Table 2 was only to show the limitations of presenting mortality data in tabular form compared with mapped and interpolated data as shown in Figure 6; analyzing Table 2 data is outside the scope of representative results.**

Fig. 6 & 7 quality is not good. Kindly use better resolution images.  
**AUTHOR RESPONSE: We submitted hi resolution version of these images and it is possible that the review copies of these images were of lower quality.**

Representative results: I believe it's the true results belong to the current manuscript. Authors should remove the word "unpublished" for the main data.

**AUTHOR RESPONSE: All data we have included in representative results are ‘true’ data from actual field trials. We indicated “unpublished” because they have not yet been written up in manuscripts and this is the proper way to cite data such as these per JoVE guidelines. We specifically chose these data to present because they are highly illustrative of the points we make in the ms.**  
  
The discussion part is lacking present study trials data interpretations.  
**AUTHOR RESPONSE: The JoVE guidelines specifically indicate that representative results should be discussed in the Representative Results section, and the Discussion section should “not depict on experimental results” – so we will leave these sections as they are.**

**General comments:**There should be a summary table for required items and manpower and time duration from mosquito rearing to final observations of the results for the trial  
**AUTHOR RESPONSE: Table 3 already presents the required items. A summary table of manpower and time duration for mosquito rearing would only be relevant for one mosquito species and it is cumbersome and outside the scope of this protocol to include.**

Units should be in cm, kg that is most widely accepted and can be useful for others.  
**AUTHOR RESPONSE: We have retained feet and inches, and changed any meter measurements to feet. These units make the most sense for the materials we use for these protocols. It is awkward to translate ¼-in into cm, for instance, and actually makes it harder to find and purchase materials.**

Given instruments and accessories details are highly specific and the same product may not available in different parts of the word. Kindly provide more details so other researchers can use this protocol to repeat the study.  
**AUTHOR RESPONSE: We are not sure which instruments and accessories you are concerned with; to the extent possible we have presented a materials table that can be interpreted generically where limitations exist.**

Authors mentioned Unpublished data many places, but I think they should be included in the present manuscript.  
**AUTHOR RESPONSE: Please refer to our earlier response regarding Unpublished data.**

Authors claimed the studies in different environment condition such as hot and dried etc…but was not supported by the data. If authors can provide the data will be helpful to others to understand the test conditions and modify their trials.  
**AUTHOR RESPONSE: We are not sure where we are missing information in this regard and we have provided several key references to the literature. The point of us discussing the flexibility across multiple environments was simply to communicate that the protocol is not limited to one environment and thus comparative studies *with essentially the same protocols* can be conducted across multiple environments.**

The authors should include a section about interpretations of the results and statistical analysis which is very critical portion of any study.  
**AUTHOR RESPONSE: Please refer to our earlier response regarding the specific journal guidelines from JoVE.**

**Reviewer #2:  
Manuscript Summary:**This paper presents a protocol to evaluate diverse combinations of pesticides targeting disease-vector insects (e.g. mosquitoes and sand flies) and equipment across multiple environments. In this study authors use cages of colony-reared sentinel mosquitoes (adults and immatures) or sand flies (adults) strategically arranged in natural habitat and exposed to pesticide spray. The mortality of these insects is recorded and visualized in a geographic information system.  
  
I find the manuscript interesting and potentially important. It provides useful information and a clear guideline to study the efficacy of pesticides targeting important insects such as disease-vector mosquitoes and sand flies. The title and abstract seem to be appropriate and the steps listed in the procedure are clearly explained. Overall, I think it is scientifically sound and well presented.  
There are some minor details missing or unclear, which need to be addressed.  
**AUTHOR RESPONSE: thank you; and we appreciate the opportunity to improve the ms to be more accessible and clear.**  
  
  
**Minor Concerns:**  
Table 1 and table 2 as well as the table listing all the materials and equipment are not cited in the text. Please, add citations.

**AUTHOR RESPONSE: Table 1 was cited at line 512 in the original ms; Table 2 was cited at line 609 in the original ms. We have retained these citations in the revised ms. Table 3 is named Table 3 per JoVE guidelines and will be a standalone table in the final published copy.**  
  
Figures 1, 2, 3 4 and 5 are not cited in the text. Please, add them.

**AUTHOR RESPONSE: As with the Tables in previous comment, all Figures were cited in text properly in the original ms. It is possible the review copy of the ms did not display these properly. We have retained all Figure citations in the revised ms.**  
  
Legend in Figure 6 is missing. Fig 6C is not completely clear. The type of diluent is missing. Please add this information.

**AUTHOR RESPONSE: Legends were included for all figures in our original submission; as with response to previous comment, these may not have translated properly in the review version of the ms. Also, we submitted hi resolution versions of all figure images and it is possible that the review copies of these images were of lower quality**  
  
You report that experiment considers also immature stages of mosquitoes, but a guideline of this stages is not present and you report some considerations only in the discussion section. Why? Moreover, in the introduction you mentioned that you will test the efficacy of pesticides studying the percent adult development using sentinel cups. There is no explanation, not even on the discussion section, of how to take or evaluate these development measure. Please add this information.

**AUTHOR RESPONSE: We did include detailed information on adjusting the protocol to study efficacy on immature stages – please see Discussion section lines 764-770 in the original ms. In-text citations (7, 23-25) at line 766 are included that have very detailed information on the sentinel cup protocol for measuring efficacy on immature mosquitoes.**