

Rebuttal Document for JoVE (manuscript #: JoVE58880)

Original* Title: Insight into *Colletotrichum fioriniae* biology via bioassays utilizing floral extracts from blueberry and cranberry

Modified Title:

Bioassays utilizing water- and chloroform-based floral extracts provide insight into *Colletotrichum fioriniae* biology on blueberry and cranberry

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Please note:

There was a numbering error early on in the original manuscript, a “Protocol # 3” was in place without any methods, skewing the numbering system. This has been corrected throughout the revised manuscript and the responses to the comments reflect this change. For example the original step 6 is now, appropriately, step 5.

An additional note refers to the original communications with Jaydev Upponi. Due to the ephemeral nature of flowers, some of the protocols were filmed prior to completing the editorial process of submission/review. Photos and videos of the pre-filmed steps have been included as supplemental photos and videos with associated captions in the manuscript text. Originally the plan was to include this footage in the main video production but due to the complexity of this protocol, we believe it better to leave as supplemental figures/photos and movies, so to include more filmable protocol steps.

In the revised submission all of the in-text modifications have been tracked. As per broad editorial and reviewer comments such as “add more detail to protocols, focus the summary/abstract/introduction more towards the protocols, and better tailor the discussion to JoVE requirements”, a large portion of the manuscript has been edited. Below, *italicized* text denotes reviewer comment, with responses to the comments shown as bullet points.

Rebuttal to editorial comments:

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

- The manuscript has been carefully read through and should not contain any spelling or grammatical errors.

2. Please rephrase the Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to ...”

- A new summary has been written to more adequately address the purpose of the protocol.
- New Summary: (highlighted in red in the manuscript)

- (50 words) Here, bioassays designed to monitor the development of a fungal pathogen, *Colletotrichum fioriniae*, in the presence of blueberry or cranberry floral extracts on glass coverslips are described. Water-, chloroform-, and field rainwater- based floral extractions techniques are detailed as well as insight into how this information can be applied.

3. Please spell out each abbreviation the first time it is used.

- This has been corrected throughout the manuscript, figures, and Table of Materials.

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

- This has been corrected throughout the manuscript, figures and Table of Materials.

5. Please include a space between all numerical values and their corresponding units: 15 mL, 37 °C; etc.

- This has been corrected throughout the manuscript, figures and tables.

6. Please revise the protocol to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.).

- This has been corrected throughout the manuscript, any non-imperative terms have been restricted to notes or other text areas.
- Safety procedures have been inserted at appropriate locations throughout the manuscript.

7. Please revise the Protocol steps so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary.

- In many cases steps have been either broken down, simplified, or joined with appropriate sub-steps/notes added.

8. Please add more details to your protocol steps.

- This has been carefully evaluated throughout the text and the tracked changes reflect the incorporation of more protocol detail. Almost the entire methods section has been updated as per editorial comment.

See examples below:

- Specific examples have been addressed (below) in addition to others that fall within the bounds of this editorial comment.

1.1: How large is the culture dish?

- Step 1.1 has been clarified to describe the diameter of the cell culture dish, (9 cm diameter). Additionally the size has been adjusted within the Table of Materials.

3.1: How many flowers are collected and placed into a plastic bag? Are blueberry and cranberry flowers placed into separate bags? Please specify.

- Step 2.1 has been clarified to denote when to separately pick each crop as well as how much to gather, “Place either blueberry or cranberry flowers into plastic bags (fill 100 mm X 150 mm bag) and immediately refrigerate at 4° C once flowers are collected”.

6.1: Please describe how to construct the collection device.

- The protocol for both blueberry and cranberry collection devices have been extensively modified.

9. Step 2: Please describe any actions being performed here. Citation only is not sufficient.

- Additional information has been added.

10. Line 101, 131, etc.: Please number the video files as Movie 1, Movie 2, etc., and reference them in the protocol. Please also name the different images mentioned as Figure 1, Figure 2, or supplemental figures, etc.

- All additional photos and videos have been noted as supplemental materials. Two extraction methods, *active*- extraction and *passive*- extraction as well as the cranberry rainwater collection device deployment have been noted as supplemental movies. Due to the length of this protocol the additional videos are favored as standalone supplemental resources. A caption has been added for all supplemental materials as well as in-text notation.

11. 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.

- Highlighted text (yellow) has been corrected to meet the requirements; at ~40 lines per page * 2.75 pages = ~110 lines, we have selected ~72 lines (to allow for spaces and headings) of filmable text. We have also included 3 supplemental videos, which should be left out of the main video production.

12. Figures, Table of Materials: Please use SI abbreviations for all units (mL, μ L, etc.) and include a space between all numerical values and their corresponding units (15 mL, 33 μ L, 24 h, etc.).

- Spaces have been added throughout the figures and within the Table of Materials. Abbreviations have also been announced if not commonly used.

13. Figures 3, 4, 6: Please include a space between the number and the units of the scale bar (40 μ m).

- A space has been added between the number and the units of the scale bar in figures 3, 4, and 6.

14. JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please revise the Discussion.

- The discussion has been significantly updated to be more aligned to the practices of a JoVE manuscript discussion section.

Rebuttal to Reviewer #1 comments:

Although the methods are the main focus, I suggest changing the title to give the method focus, e.g. Floral extract bioassays give insight to C.f. biology on blueberry and cranberry. Summary, abstract and introduction could also be rewritten to put more focus on the methods than on the pathogen.

- The title has been modified to address this concern and more accurately represent the body of work as described.
- New Title:
 - Bioassays utilizing water- and chloroform-based floral extracts provide insight into *Colletotrichum fioriniae* biology on blueberry and cranberry

Minor Concerns: *More focus on Methods in summary, abstract and introduction may improve the article.*

- This has been carefully evaluated throughout the text, the tracked changes reflect this.

Rebuttal to Reviewer #2 comments:

I am not sure that this will be very effective for use in disease forecasting.

- This suggestion has been removed until our research group can more substantially validate this claim.

Minor Concerns: *Comments on the pdf as sticky notes.*

- Sticky note comments have been addressed throughout the manuscript. Examples of word-choice suggestions are detailed below.
 - Replacing the word “inoculate” when describing the action of dispensing the treatment mixtures containing conidia on the glass coverslips has been modified.
 - Replacing the word “tissues” when describing the parts of the flower has been changed to “organs” and tissues has been omitted.
 - Numerous other editorial-based comments have been addressed as well throughout the manuscript.