

Submission ID #: 70440

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Title: Assessing the Repellency of an Insecticide-Fungicide Combination to Bees using Bioacoustics

Authors and Affiliations:

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

Videographer: Please film the computer screen for all shots labeled SCREEN

3. Filming location: Will the filming need to take place in multiple locations? **No**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 09

Number of Shots: 14

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Lillian Johnson:** This research examined the visitation patterns of bees on soybean flowers and investigated whether a tank-mix pesticide application could deter them.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Lillian Johnson:** Existing bee monitoring methods lack temporal precision and scalability; this bioacoustics protocol enables continuous, high-resolution monitoring across large geographic areas.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.3.1*

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Lillian Johnson:** The protocol allows researchers to measure bee activity more passively over time at specific points of interest.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Lillian Johnson:** A major challenge of this protocol is managing the large volume of audio files, requiring efficient data storage and organization systems.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Lillian Johnson:** Future studies can use this approach to investigate bee foraging preferences, flowering resource use, and temporal patterns of bee activity.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

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Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Acoustic Monitoring of Bee Activity Following Pesticide Application

Demonstrators: Lillian Johnson, Luke Hearon

- 2.1. To begin, prepare the field for soybean cultivation according to the treatment plan [1].
 - 2.1.1. Establishing shot of the talent with the prepared field. ***Videographer:** Please obtain CU shot as well for this shot.*
- 2.2. Set up the recorder by fastening handheld audio recorders to plastic step-in electric fence stakes using hook-and-loop tape [1]. Place open-cell foam over the microphone to serve as a windscreen [2].
 - 2.2.1. Talent fastening the handheld audio recorder to a plastic step-in electric fence stake using hook-and-loop tape.
 - 2.2.2. Talent placing open-cell foam over the microphone of the audio recorder.
- 2.3. Place the audio recorders at the center of the field approximately 24 hours before the first pesticide application and keep them running for at least 24 hours [1].
 - 2.3.1. Talent placing the audio recorder in the center of a soybean plot.
- 2.4. Use 12 audio recorders on each application day, with 4 recorders assigned to each time-of-day group across both R3 and R5 growth stages [1].
 - 2.4.1. Talent arranging 12 audio recorders.
- 2.5. Next, wear full personal protective equipment [1]. Using a custom-made handheld backpack sprayer, apply the previously prepared pesticide mixture to individual soybean fields within each block [2]. Apply the pesticide mixture at the top of the soybean canopy at approximately 3 miles per hour [3]. After starting the pesticide application process for a given treatment, allow approximately 10 minutes for all plots to receive the application [4].
 - 2.5.1. Shot of the talent wearing a PPE kit
 - 2.5.2. Talent applying the mixture to individual soybean plots within each block using a custom-made handheld backpack sprayer.
 - 2.5.3. Talent spraying the pesticide mixture at the top of the soybean canopy while walking through the plot.
 - 2.5.4. Talent continuing the pesticide application across all plots for a given treatment.

- 2.6. To process all audio recordings, use the machine learning tool BuzzDetect [1]. Use the program to distinguish insect buzzing noises from other environmental sounds, including cars, combines, and airplanes [2].

2.6.1. SCREEN: 70440_2.6.1-2.7.1.mp4: 00:05-00:10.

Videographer: Please film the computer screen for all shots labeled SCREEN

2.6.2. SCREEN: 70440_2.6.1-2.7.1.mp4: 00:29-00:35

- 2.7. Next, distinguish buzzing noises from some non-buzzing insect noises, while noting that certain sounds remain difficult to distinguish [2].

2.7.1. SCREEN: 70440_2.6.1-2.7.1.mp4: 00:49-01:01

- 2.8. After audio analysis, use R to calculate the detection rate within 1-hour and 8-hour time intervals [1].

2.8.1. SCREEN: 70440_2.8.1.mp4: 00:10-00:24

- 2.9. Finally, perform defoliation and yield assessment followed by statistical analysis [1].

2.9.1. Talent in front of the computer screen showing some defoliation-related data.

Results

3. Results

3.1. Average detection rates were significantly higher in the R3 stage than in the R5 stage for the 8 hours following pesticide application [1]. However, bee activity did not differ significantly when the pesticide tank mix was applied [2].

3.1.1. LAB MEDIA: Figures 1A–B.

3.1.2. LAB MEDIA: Figures 1A–B. *Video editor: Highlight the control and treatment boxplots within each time group*

3.2. In the hour following pesticide application, average detection rates were significantly higher during the R3 growth stage compared to the R5 growth stage [1]. Additionally, detection rates were notably greater in the hour after the mid-day spray than in the hour after the mid-morning spray. [2].

3.2.1. LAB MEDIA: Figures 2A–B.

3.2.2. LAB MEDIA: Figures 2A–B. *Video editor: Highlight the mid-day group and then the mid-morning group on the horizontal axis*

3.3. However, bee activity was not significantly different when the pesticide tank mix was applied [1].

3.3.1. LAB MEDIA: Figures 2A–B. *Video editor: Highlight the control and treatment boxplots within each time group*

3.4. Detection rates increased rapidly around 10:00 and declined sharply around 17:00 in both stages [1]. Peak detection rates at approximately 14:00 were substantially higher in R3 than in R5 [2].

3.4.1. LAB MEDIA: Figure 3.

3.4.2. LAB MEDIA: Figure 3. *Video editor: Highlight the peak near 14:00 for the light green R3 curve and compare it with the lower dark blue R5 curve*

3.5. Soybean yield was not significantly different across treatments [1].

3.5.1. LAB MEDIA: Figure 4.

3.6. Defoliation did not differ significantly based on the time of day of pesticide application [1]. Overall defoliation levels were low, remaining below 5% in most cases [2].

3.6.1. LAB MEDIA: Figure 5.

3.6.2. LAB MEDIA: Figure 5. *Video editor: Highlight the area below 5 percent on the vertical axis*

4. Soybean
Pronunciation link: <https://www.merriam-webster.com/dictionary/soybean>
IPA: /'sɔɪ.bi:n/
Phonetic Spelling: soy·been
5. Cultivation
Pronunciation link: <https://www.merriam-webster.com/dictionary/cultivation>
IPA: /ˌkʌl.tə'veɪ.ʃən/
Phonetic Spelling: kul·tuh·vay·shuhn
6. Microphone
Pronunciation link: <https://www.merriam-webster.com/dictionary/microphone>
IPA: /'maɪ.krəˈfoʊn/
Phonetic Spelling: my·kruh·fohn
7. Pesticide
Pronunciation link: <https://www.merriam-webster.com/dictionary/pesticide>
IPA: /'pes.təˌsaɪd/
Phonetic Spelling: pes·tuh·syde
8. Canopy
Pronunciation link: <https://www.merriam-webster.com/dictionary/canopy>
IPA: /'kæn.ə.pi/
Phonetic Spelling: kan·uh·pee
9. Machine learning
Pronunciation link: <https://www.merriam-webster.com/dictionary/machine%20learning>
IPA: /məˈʃiːn 'lɜː.nɪŋ/
Phonetic Spelling: muh·sheen ler·ning
10. BuzzDetect
Pronunciation link: No confirmed link found
IPA: No verified IPA available
Phonetic Spelling: buzz·dih·tekt
11. Environmental
Pronunciation link: <https://www.merriam-webster.com/dictionary/environmental>
IPA: /ɪnˌvaɪ.rən'men.təl/
Phonetic Spelling: en·vy·ruhn·men·tuhl
12. Detection
Pronunciation link: <https://www.merriam-webster.com/dictionary/detection>
IPA: /dɪ'tek.ʃən/
Phonetic Spelling: dih·tek·shuhn
13. Defoliation
Pronunciation link: <https://www.merriam-webster.com/dictionary/defoliation>
IPA: /ˌdiː.foʊ.li'eɪ.ʃən/
Phonetic Spelling: dee·foh·lee·ay·shuhn
14. Statistical
Pronunciation link: <https://www.merriam-webster.com/dictionary/statistical>
IPA: /stə'tɪs.tɪ.kəl/
Phonetic Spelling: stuh·tis·tih·kuhl

15. Significantly

Pronunciation link: <https://www.merriam-webster.com/dictionary/significantly>

IPA: /sɪɡˈnɪf.ə.kənt.li/

Phonetic Spelling: sig·nif·ih·kuhnt·lee

16. Boxplot

Pronunciation link: <https://www.merriam-webster.com/dictionary/boxplot>

IPA: /ˈbɑːks.plɑːt/

Phonetic Spelling: boks·plot

17. Substantially

Pronunciation link: <https://www.merriam-webster.com/dictionary/substantially>

IPA: /səbˈstæn.ʃə.li/

Phonetic Spelling: sub·stan·shuh·lee

18. Yield

Pronunciation link: <https://www.merriam-webster.com/dictionary/yield>

IPA: /jiːld/

Phonetic Spelling: yeeld