Submission ID #: 61500
Scriptwriter Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=18757548

Title: A 3D Printed Pollen Trap for Bumble Bee (Bombus) Hive Entrances

Authors and Affiliations:

Houston J. Judd [1,2], Craig Huntzinger[2], Ricardo Ramirez[1], James P. Strange [2,3]

[1]Department of Biology, Utah State University, Logan, UT, USA
[2]USDA-ARS Pollinating Insect Research Unit, Logan, UT, USA
[3]Department of Entomology, The Ohio State University, Columbus, OH, USA

Corresponding Authors:

James P. Strange
Email Address: strange.54@osu.edu
Tel: (614) 292-9325

Email Addresses for All Authors:

houstonjudd@gmail.com
craig.huntzinger@ars.usda.gov
ricardo.ramirez@usu.edu
strange.54@osu.edu

Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **No**

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

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

Current Protocol Length

Number of Steps: 17
Number of Shots: 32

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **James P. Strange:** This method allows researchers to collect pollen from bumble bee colonies with minimal disturbance to the bees and low risk of bee stings to the researcher.

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

1.2. **James P. Strange:** Using this protocol, pollen can be collected from multiple colonies simultaneously, permitting researchers to use more colonies with less labor and expense than hand collecting pollen.

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

OPTIONAL:

1.3. **James P. Strange:** This method will benefit research in pollination and bee health, pesticide science, and environmental contamination and can be adapted to multiple species of bumble bees.

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

Videographer NOTE: There were slight changes to shot numbers when it made sense to combine two shots and split up another into multiple shots. Those changes are reflected in the labeling of the files and were noted on the video's audio file

Protocol

2. Pollen Trap Assembly

2.1. Begin by removing the support structures printed with the trap body and catch basin, including those of the sieve structure of the trap body **[1]**. *Videographer: This step is important!*

2.1.1. Talent removing the support structures.

2.2. Clear any plastic strands crossing the raised edges of the pollen filter with a hand drill **[1-TXT]**, then use a box cutting razor blade and sandpaper to even out any bumps or raised edges on the flat side of the filter **[2]**. *Videographer: This step is important!*

2.2.1. Talent clearing the plastic strands with the drill. **TEXT: Use a 3/16-inch drill bit**

2.2.2. Talent evening out bumps with the blade or sandpaper.

2.3. Gently push the plastic pollen filter through one side of the trap body. The filter will only fit in one way, as the left side of the trap has a larger opening to accommodate passage of the raised filter cones **[1]**. *Videographer: This step is important!*

2.3.1. Talent pushing the pollen filter through the side of the trap body.

2.4. If the side slits are too small for the pollen filter to slide through smoothly, use a razor to scrape enough plastic away from the slit in the trap body **[1]**. Ensure that the filter fits securely in place with no more than a 2-millimeter gap between it and the trap body **[2]**. *Videographer: This step is important!*

2.4.1. Talent scraping the plastic away from the slit.

2.4.2. Talent making sure that the filter is secure.

2.5. Slide the raised edges on the bottom of the trap body into the groove on top of the catch basin. The catch basin should be positioned directly underneath the sieve region of the trap body **[1]**. *Videographer: This step is important!*

2.5.1. Talent sliding the raised edges into the groove of the catch basin.

2.6. Cut or sand the plastic to allow smooth placement and removal of the catch basin. Placement of the catch basin will secure the pollen filter into place **[1]**. *Videographer: This step is important!*

2.6.1. Talent cutting or sanding the plastic.

3. Bumble Bee Colony Preparation

3.1. Stop pollen feeding 24 to 48 hours before deploying colonies, which will cause workers to use up any stored pollen and stimulate them to leave the nest **[1]**.

3.1.1. Bee colony.

3.2. Prepare the hive trap body for installation by placing a trap closure insert into the filter slot to prevent bees from escaping **[1]**. Working under red light, lift the plastic nest box out of the cardboard outer box **[2]** and locate the entrance at the front of the nest **[3]**.

3.2.1. Talent placing the trap closure insert.

3.2.2. Talent lifting the plastic nest out of the cardboard.

3.2.3. Plastic nest entrance.

3.3. For Koppert-style hive entrances, pull up on the entrance tab until both entrance holes are open **[1]**, then insert the two tubes of the pollen trap into the entrance holes, ensuring that the sieve of the pollen trap is on the bottom **[2]**. Gently push down on the plastic entrance tab to secure the pollen trap in place **[3]**. *Videographer: This step is difficult and important!*

3.3.1. Talent pulling up on the entrance tab.

3.3.2. Talent inserting the pollen trap into the entrance holes.

3.3.3. Talent pushing down on the entrance tab.

3.4. To install the pollen trap into Biobest-style hive entrances, use a flat head screwdriver to gently pry the plastic entrance device from the nest box **[1]**. Insert the pollen trap into the nest entrance holes until it is placed firmly against the nest **[2]**, then secure it to the nest with tape or quick dry glue where the trap contacts the nest box **[3]**.

3.4.1. Talent prying the entrance device from the nest box.

3.4.2. Talent inserting the pollen trap.

3.4.3. Talent securing the connection with tape or glue.

4. Deployment of Nests and Pollen Collection

4.1. Place the nest boxes into the study area, providing cover from precipitation and anchorage for wind as these can adversely affect the quality and quantity of the collected pollen **[1]**. Provide the hives with adequate sun cover to prevent overheating **[2]**.

4.1.1. Talent placing the nest boxes in the study area, covering them, and providing anchorage.

4.1.2. Talent covering the hives from the sun.

4.2. Remove the trap closure insert from the trap body to allow bees to forage freely so that they can orient themselves with the surrounding area and the location of their

nest **[1]**. Orientation flight time should be complete in 24 hours under normal conditions **[2]**.

4.2.1. Talent removing the trap closure.

4.2.2. Bees foraging.

4.3. To engage the trap, slide the pollen filter into the filter slot, ensuring that it is securely in place **[1]**. Install the catch basin by sliding it onto the trap body from the front until it is fully closed **[2]**. If the catch basin is loose or falls off the trap body, use a rubber band to secure it **[3]**.

4.3.1. Talent sliding the pollen filter into the slot.

4.3.2. Talent installing the catch basin.

4.3.3. Talent securing the catch basin with a rubber band.

4.4. Observe bees entering and exiting the pollen trap at first deployment to ensure the pollen filter holes are large enough **[1]**. If workers are unable to pass through the pollen filter, remove the filter and use the hand drill to enlarge the holes **[2]**. Increase the diameter sequentially, as holes that are too large will not collect any pollen **[3-TXT]**. *Videographer: This step is important!*

4.4.1. Bees entering and exiting the trap.

4.4.2. Talent removing the filter.

4.4.3. Talent enlarging the hole. **TEXT: 1/32 inch at a time**

4.5. Once bees are able to pass through the filter, continue observing the entrance to ensure pollen is being removed upon re-entry **[1]**.

4.5.1. Talent observing the bees.

4.6. After the designated period of pollen collection, remove the catch basin from the trap body and process the pollen loads according to the experimental design **[1]**. Remove the pollen filter to allow workers to forage freely, leaving the trap body attached to the hive for the duration of the experiment **[2]**.

4.6.1. Talent removing the catch basin.

4.6.2. Talent removing the pollen filter.

Results

5. Results: Comparison of Pollen Filter Designs through Video Footage

5.1. Eight pollen filter designs were tested to determine their efficacy and efficiency at removing corbicular pollen loads from returning bumble bee workers **[1]**. All designs were successful at removing at least one load **[2]**, but some were found to slow workers from leaving or entering the hive or failed to remove pollen loads **[3]**.

5.1.1. LAB MEDIA: Video footage of bees entering/leaving a hive with the trap and/or Table 1. *Video Editor: Emphasize the Design ID and Entrance Shape columns.*

Authors: Please upload this to your project page: https://www.jove.com/account/file-uploader?src=18757548. Alternatively, we can show shot 4.4.1 here too.

5.1.2. LAB MEDIA: Table 1. *Video Editor: Emphasize the Corbicular Pollen Loads Collected column.*

5.1.3. LAB MEDIA: Table 1.

5.2. The filters were tested sequentially on 4 laboratory reared colonies of *B. huntii* Greene foraging on *Phacelia tanacetifolia* for a cumulative 138.5 hours and 229 collected corbicular pollen loads over 7 days. Fifty-two hours of video observation and 142 pollen loads were collected during the test period **[1]**.

5.2.1. LAB MEDIA: Table 1. *Video Editor: Emphasize the Total row.*

5.3. The number of corbicular pollen loads collected was divided by the number of observed pollen laden foragers that passed through a filter to calculate efficiency. Pollen filter design efficiencies ranged from 2 to 58.9%. Corbicular pollen loads were removed from the legs and fell as cohesive pellets of pollen into the catch basin **[1]**.

5.3.1. LAB MEDIA: Table 1.

5.4. Circular filter openings improved pollen collection and movement of workers into the nest environment. In addition, filter designs that had raised structures that extended away from the nest box also improved pollen removal from the hind legs of foragers **[1]**. Design number 8, a circular trap entrance with raised edges, was the final design **[2]**.

5.4.1. LAB MEDIA: Table 1. *Video Editor: Emphasize the rows with the circle entrance shape.*

5.4.2. *Shot 2.4.2 or any other shot of the filter from the protocol section (4.3.1, 3.3)* and LAB MEDIA: Table 1. *Video Editor: Emphasize the Design 8 row.*

Conclusion

6. Conclusion Interview Statements

6.1. **James P. Strange:** When affixing the pollen trap body to the bumble bee hive body, there is a risk of bees escaping and the researcher getting stung. It is important to place the trap closure into the trap before attempting this. Working under red light will further reduce the risk.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1 – 3.3.3.*

6.2. **James. P Strange:** Following pollen collection the researcher can compare foraging preference, pesticide exposure studies, or environmental contaminant exposure studies.

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

6.3. **James P. Strange:** This technique has allowed us to compare bumble bee pollen foraging to honey bee foraging behaviors and it has been used to look at pesticide exposure of bumble bees returning to the nest with contaminated pollen.

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