

Parasitoid Wasp (*Anisopteromalus calandrae*) Body Wash and Efficacy Against Bean Beetles

Introduction

In previous weeks, we have been introduced to cowpea weevils, parasitoids, as well as testing hypothesis regarding the use of essential oils as pest repellents. The first part of today's lab will show you how to proceed in producing body-washes of recently euthanized parasitoid wasps (*Anisopteromalus calandrae*).



As arthropods, wasps produce a hard exoskeleton. This exoskeleton can produce chemicals, which could serve as potential cues for the recent presence of wasps. Prior to following the protocol to produce an insect body wash, consult the following resource about cuticle chemicals:

[AMER. ZOOL., 38:394-405 \(1998\) Roles of Hydrocarbons in the Recognition Systems of Insects.](#)

Based on what we have learned regarding cowpea weevils, their avoidance behavior to essential oils, how do you think weevils will respond (if at all) to the cuticle chemicals from *A. calandrae*?

Do you think we may witness differences between male and female responses? Why or why not?

Materials:

3 Small Choice-chambers
Whatman Filter Paper
Micropipetors
1 mL Transfer Pipettes
Capped Micro-test tube of Freshly Euthanized Parasitoid Wasps (20 individuals)
Acetone
Funnel
Hexane
Brushes
Beakers and vials
Graduated Cylinders
Disposable Spatulas
45 Male and 45 female Bean Beetles in Two Weeks
2 Petri Dishes with lids
Labeling tape & sharpie

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Parasitoid Body Wash

Open vial of euthanized wasps and add 1.5 mL of a 1:1 ratio (acetone to hexane, 0.75 mL each) solution to the vial. Allow to sit at room temperature for 30 minutes, agitating every 5 minutes (flick with finger or use vortex mixer).

After 30 minutes, using a centrifuge, spin to collect wasp bodies as a pellet. Then using appropriate sized micropipette, remove the supernatant from the tube and place in vial (micro-test tube). Label your micro test tube with group number and initials. Place cap on tube. Place tube in rack as shown in right figure. It will be stored in freezer until the next lab (experiment 1) and allowed to evaporate down to concentrate chemicals.



Microtube will contain euthanized wasps

1.5mL of 2:1 Acetone:Hexane Solution Added to microtube (0.75mL each).

Aggitate every few minutes for 30 minutes

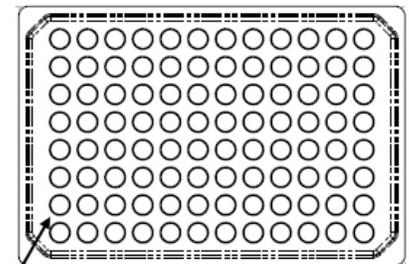
While you wait and once you have prepared the body wash, use the time to complete the genetics practice problems in your lab group.

Next Week:

Experiment 1

For each of your 3 small choice chambers, do the following:

- Cut Whatman filter paper to fit into each side of a choice chamber (Cut equal sections to place in chambers). Obtain 30 male and 30 female beetles and place into separate petri dishes.
- Sterilize your lab bench with appropriate cleaning solutions and let air dry. Place your filter papers on the newly, dried sterilized area. Using a sterile pipet, add one drop of the body-wash solution to one filter paper to be placed into a choice chamber, and using another sterile pipet, add one drop of acetone:hexane to the other filter paper to serve as a control. Allow the acetone time to evaporate (10 min). Then place each filter paper to it



Well #

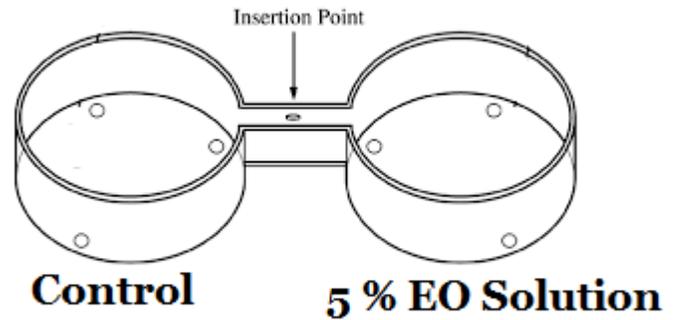
Frontside

The figure above illustrates the information you need to ID the placement your wasp body-wash sample correctly and to record that information. The well number you use will be located to the lower left-hand side of the well. Be sure to record this so you may retrieve your sample in the future.

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side of the chamber. ***Be sure to add oils outside of the chambers, as they dry, they will become glued to the dish.

- After 10 minutes, using your brush and disposable spatula, place 10 male beetles into the center of one choice chamber and place lids on each side. Do this for the remaining male beetles and female beetles. You should have 3 male choice chambers and 3 female choice chambers.
- Allow the beetles to move freely for 20 minutes undisturbed
- After 20 minutes, using the center of the chamber apparatus as the dividing point, count how many male or female beetles are on each side of your chamber (control vs. Parasitoid Body Wash Solution).
- Record your counts. Repeat this for your female bean beetles.
- After you have finished, clean up your lab table and submit your data for your group
- Experiment 1 Data can be added via this [Google Form](#)



While your experiments are underway, please answer the following questions:

What was your biological hypothesis for this experiment?

What are your null and alternate hypotheses for experiment?

How would you go about analyzing the data from each experiment? Would you use a similar analysis to the paired t-test used for our essential oils experiment? Why or why not?

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How would the “Ecology of Fear” we discussed in previous labs and in your pre-lab handout apply to a host-parasitoid system, similar to what is seen in Predator-Prey Systems as you saw in the introduction to parasitoids lab? (*Hint: Google Ecology of Fear, Wolves and Yellowstone Park*)

How would this change your hypothesis (if at all) regarding differences between male and female bean beetle behaviors towards the Parasitoid body wash?

If you wanted to test for the efficacy of the combination of parasitoid body-wash and an essential oil solution (5%), which essential oil would you choose? Why? How would you design such an experiment?

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Data Collection

Be sure to submit your groups data using the Google Form Links Below

Data Collection Sheet (Small Choice-chambers)

Chamber	Sex	Number of Beetles	
		Control (Acetone:Hexane)	Par. BW
1	M		
2	M		
3	M		
1	F		
2	F		
3	F		
	Mean		
	Median		

[Google Form Link](#)

This concludes the first authentic research experience in this course. Using the materials available on Moodle, your group can now use the information learned from the first half of this semester to produce a scientific poster and present your findings to your lab section at the end of the semester. You will be given access to this year's results as well as those from previous semesters. You have access to the course weevil library, have built your own reference library using Mendeley software, and have learned to utilize resources in the library and Google Scholar. I look forward to your future presentations!