

Substrate Size Selection by Bean Beetles

Instructor's Notes

Consult “A Handbook on Bean Beetles, *Callosobruchus maculatus*” (Beck and Blumer, 2010) for detailed information on growing cultures, handling techniques, and methods of safe disposal (available for downloading at:

<http://www.beanbeetles.org/handbook> and in the supplemental materials for this major workshop). In addition, tips on identifying the sexes including pictures of a male and female are available at: <http://www.beanbeetles.org/handbook/#IS>.

The student handout is written as a guided inquiry that allows students to design their own experiments, rather than instructors giving students explicit directions on how to conduct their experiments. No matter the exact experiment that students design, the experiments will require having dense cultures of bean beetles from which females can be isolated. If new cultures are initiated approximately 2 months before the lab period, there will be sufficient time for two generations of beetles, which will result in dense cultures. When possible, we supply one culture to each group of students. However, cultures should have sufficient beetles for multiple groups. Newly emerged cultures work better for this experiment than older cultures.

Instructors should caution students to prevent the accidental release of bean beetles from the laboratory environment. *Callosobruchus maculatus* is a potential agricultural pest insect that is not distributed throughout the United States and Canada. It is essential that you keep your cultures secured in a laboratory environment to ensure that they are not released to the natural environment. Disposal of cultures (and beans (seeds) exposed to live beetles of any life cycle stage) requires freezing (0°C) for a minimum of 72 hours prior to disposal as food waste. If you have any questions about the handling or disposal of bean beetles, please contact Larry Blumer at lblumer@morehouse.edu or 404 658-1142 (voice or FAX). Information also is available at: www.beanbeetles.org in the Handbook section.

Experimental Design

Our students have successfully conducted this experiment using both very short-exposure and long-exposure protocols. Students typically design experiments in which female beetles choose between two categories of beans and are permitted to lay eggs on those beans. The species of bean is the same but the quantity of the bean resource is different. For example, we have had students conduct experiments in which each replicate contained 15 whole mung beans and 15 split mung beans in a 60mm dish (or 10 of each category in a 35mm dish). A female and a male bean beetle were introduced to each dish and the female was permitted to lay eggs for a minimum of 24 hrs or as long as 7 days. Alternatively, students could sort whole mung beans (visually judging size) to create sets of large beans and small beans. A similar experiment with 15 large and 15 small beans per replicate could be conducted, and run for 24 hrs to 7 days. If similar numbers of beans of each size category were presented to a female, the experiment could be permitted to run until each female laid all her eggs and died (7-10 days from adult emergence). The difference in the quantity of the bean resource may be documented at the start of an experiment by weighing the beans in each size category or measuring

beans with vernier calipers. In all experiments, the data collected are the number of eggs laid on beans in each category of size.

This experiment may be readily modified for more advanced students by using it as an alternative means of studying intra-specific competition. Beck and Blumer (Beck and Blumer, 2009 and Blumer *et al.*, 2010) previously presented a competition protocol in which female beetles chose between beans with or without an egg from another female, so females were choosing based on the number of competitors already present. In the current study, female beetles would choose between beans based on differences in the resources actually available, not the number of potential competitors. The experiment could be elaborated by using other bean species (such as black-eye peas or adzuki beans) and by evaluating differences in successful development and adult body size for eggs laid on different size beans.

Data analysis

The total number of eggs laid on each of the two size categories may be analyzed using a binomial test. This is more appropriate than a chi-squared test since there are only two categories in a given experiment. Alternatively, the mean number of eggs laid on each bean size category per replicate may be evaluated using a paired-t test or a non-parametric pairwise test (Wilcoxon sign-rank test). These statistical tests are easily performed using the on-line VassarStats website <http://faculty.vassar.edu/lowry/VassarStats.html>.

Equipment and supplies

For a class of 30 students working in pairs:

- 30 magnifiers 2.5x, 4" diameter self-standing with folding base ([Fisher #14-648-19](#) or [VWR #62379-535](#), approximately \$50.00 US per unit) or dissection microscopes
- 15 bean beetle cultures with newly emerged adults (Carolina Biological [#144180](#))
- 60 plastic 60mm Petri dishes for new cultures
- Plastic 35mm Petri dishes for isolating adults (minimum 60, but reused)
- 32 ounces each of dried mung beans and split mung beans
- 30 small paint brushes
- 30 soft forceps, Bioquip™ featherweight forceps ([Catalog No. 4748 or 4750](#))
- 0.1mg analytical balance for weighing beans (more than one balance would permit more efficient data collection by students)
- 30 vernier calipers for measuring bean size
- permanent markers for labeling Petri plates