

Oviposition Substrate Choice by Bean Beetles

Instructor's Notes

Consult "A Handbook on Bean Beetles, *Callosobruchus maculatus*" for detailed information on growing cultures and handling techniques (available for downloading at: <http://www.beanbeetles.org/handbook.html>). In addition, tips on identifying the sexes including pictures of a male and female that could be used in class are available at: <http://www.beanbeetles.org/handbook.html#IS>.

The experiment requires 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.

Experimental Design

Questions that students generally address in their experiments include:

- Do females prefer to lay eggs on a particular species of bean?
- Do females actively avoid laying eggs on a particular species of bean?
- Is oviposition substrate choice by females determined by the size of a species of bean?

In their experimental designs, students should consider the following questions:

- How would you control for female preference for the bean species from which she emerged?
- How would you control for the possibility that females will lay their eggs on the first species of bean they encounter?

Oviposition will readily occur during a 48-hour period when adult females are provided with single layer of beans in a small covered dish. Although most adult females in an active culture will have been inseminated, there are always some females that may have only recently emerged (and be infertile) and others that are near the end of their adult life (and laid most of their eggs). Students should consider the following questions in their experimental designs:

- How can you account for variation among females in the number of eggs they lay?
- If females lay eggs preferentially on a particular bean species, how will you detect that preference?

Data collection

The actual number of eggs laid on each of ten bean species during a 48-hour period could be evaluated in an oviposition preference experiment in which a female is presented with an equal number of each bean species. In this experiment, we do not use the natal bean species to control for a bias toward that species. Generally, about 10 beans of each species in a 150mm Petri dish are appropriate. If the beans are randomly arranged throughout the dish, females will be equally likely to encounter each bean species. Egg laying data do not need to be collected immediately

after 48-hours but the females should be removed from the experimental arenas, so students can evaluate the initial bean species choices. The eggs are glued to the beans and will remain intact on the beans. Therefore, students may count the eggs one (or even two) weeks after the start of the oviposition experiment. A 48-hour period for egg laying ensures that sufficient numbers of eggs are laid.

Data analysis

The data from the experiment should be the number of eggs laid on each bean species. The appropriate statistical analysis for the egg count data is a chi-squared test to determine whether the distribution of eggs on the bean species differed from random. The null hypothesis is that females will lay an equal number of eggs on each bean species. The chi-squared test can be carried out for individual replicates or for all replicates pooled. The difference in the average number of eggs on each bean species across replicates also could be compared with a one-way ANOVA (or the non-parametric Kruskal-Wallis test) with bean species as the factor and number of eggs as the dependent variable.

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 per unit) or dissection microscopes
- 15 bean beetle cultures with newly emerged adults
- 15 plastic 150mm Petri dishes for picking adults females from cultures
- 30 plastic 150mm Petri dishes for replicates of the oviposition substrate choice experiment
- 30 plastic 35mm Petri dishes for holding isolated beetles
- 16 ounces of each the following bean species, dried and organically grown, if possible: mung beans, adzuki beans, black-eyed peas, garbanzo, kidney, pinto, black beans, soy beans, urad beans, fava beans, lima beans, and green pea
- 30 small paint brushes
- 30 soft forceps, Bioquip™ featherweight forceps ([Catalog No. 4748 or 4750](#))
- 30 vernier calipers for measuring bean characteristics
- 0.1mg analytical balance for weighing beans