Oviposition preference of dry over wet kidney beans in *Callosobruchus maculatus*

Maeve Barbera, Sarah Burchart, Nathaniel Kregar, and Nada Reffat
Lake Forest College
Lake Forest, Illinois 60045

Introduction

The bean beetle (*Callosobruchus maculatus*) is native to regions of Africa and Asia (Barbosa, 2022). Inseminated female bean beetles oviposit (lay) their eggs on beans, and their larvae must feed, grow, and mature in the selected bean. Her larvae's success is based on the bean's quality. The bean's quality is measured by size and nutritious value. In previous research, these beetles have been known to be selective in the type and size of bean they choose to oviposit on (Cope and Fox, 2003). Bean beetles are an excellent organism of study in determining oviposition behavior. All feeding is done in the larval stage of development, and adults do not require food or water (Mitchell, 1975). Due to the known selective behavior of bean beetles in previous research, we decided to run an experiment to determine if the moisture of beans affected the oviposition behavior of the bean beetle.

This experiment used sedentary (flightless) female bean beetles to determine if moisture influenced oviposition behavior. The beans chosen for this study were kidney beans due to their darker color and uniform size; the dark color of the bean allowed for ease in counting white eggs on the bean surface. Additionally, the uniform size of kidney beans decreased variability in our experiment. Beans with increased moisture provided more water for the larvae, whereas the dry beans did not. We therefore predicted that beetles would show a preference for moist beans due to the increased water availability. We hypothesized that if the beans were moister, we would find a more significant number of eggs on the wet bean's surface than the dry bean.

This study shows interest in the bean beetles' oviposition preference and how it can determine increased success in the larvae. Because the beans used in the study were all the same, besides the addition of moisture, our findings determine how necessary increased amounts of water are for the survival and development of the larvae.

Methods

Prior to formulating an experiment, our group began by listing characteristics of beans that would be of importance for the female bean beetle when choosing which bean to oviposit her eggs. Some of these consisted of the nutrient level of the bean, the burrowing feasibility of the bean, size of the bean, color of the bean, etc. After we had discussed why these characteristics might be important to the female bean beetle and how we can incorporate these into an experiment and test them, the group came up with two potential experiments to carry out, (1) do females prefer moister or drier beans? and (2) do females prefer larger or smaller beans? With these two experiments in mind, we decided on the first of the two, since it would be easier to make the beans moist without variability, compared to making sure all the beans were the same size in experiment two.

The bean used in this experiment was a kidney bean, as it is a darker bean, making it easier to see the white eggs when collecting results. Each Petri dish was set up the same to eliminate any variability. Eight Petri dishes were used, each containing two dry beans, two "wet" beans, and two female bean beetles. The beetles had to be sexed before placing them into each Petri dish since we were only utilizing females for our experiment. As we sexed the individuals, we soaked our kidney beans in tap water for 20 minutes each. Each bean was soaked for the same amount of time to ensure that each had the same treatment and none of the beans were moister than the others, or drier than the others.

As the kidney beans were being soaked and the bean beetles were being sexed, the Petri dishes were prepped for the experiment. For the experiment we used divided Petri dishes to keep the dry and "wet" beans separated from each other. The divisions still allowed for the females to climb between all three areas of the dish. On the bottom of the Petri dish, each section was labeled to determine which beans were which. After the beans were soaked for 20 minutes and all the female bean beetles were collected, the experiment was set up. We placed two "wet" beans in each Petri dish where it had been labeled wet bean, two dry beans in each Petri dish where it had been labeled dry bean, and two female bean beetles in each Petri dish.

It typically only takes bean beetles 12 to 24 hours to lay their eggs, but waiting longer gave each female more time to oviposit; this allowed the eggs to turn white , making it easier to count the eggs. We checked the beans every two days until we noticed eggs on the beans. Once eggs were noticeable on the beans, we stopped the experiment. To do so, the bean beetles were removed from the Petri dishes that contained eggs and the beetles were then euthanized. After all the data was collected, it was analyzed with an ANOVA statistical test.

Results

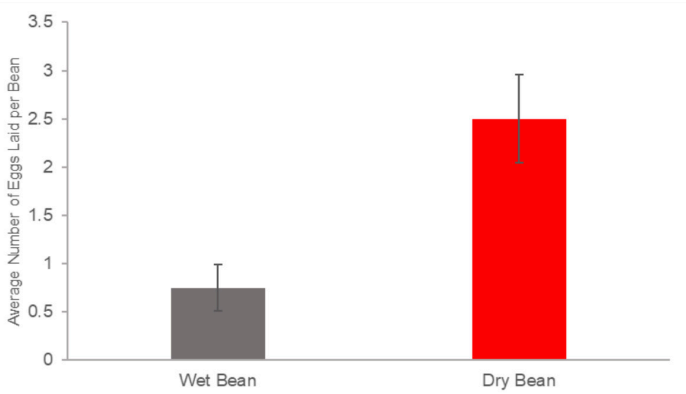

Figure 1. Average number of eggs laid on wet and dry beans (± SEM). Significantly more eggs were laid on the dry bean than the wet bean ($p<0.001$).

Mating and oviposition were not observed or guaranteed, but oviposition occurred in nearly all samples. Only one set of the eight sets of females did not oviposit. Descriptively, wet beans appeared different than dry beans, with wet beans having wrinkled, cracked, or contained broken skin while dry beans were smooth and unbroken. Female bean beetles laid different amounts of eggs on dry beans than wet beans during the testing period (Figure 1). A paired- samples t-test revealed a significant difference between the two treatments, $t(15) = -4.457$, $p<0.001$. Specifically, females laid a higher number of eggs on dry beans ($M= 2.5$, $SEM= 0.459$) than wet beans ($M= 0.75$, $SEM= 0.242$).

Discussion

In this experiment, the results showed that female bean beetles prefer to oviposit their eggs on dry beans over wet beans when both are present. We hypothesized that females would choose to lay their eggs on wet beans since all feeding is done in the larvae stage. Therefore, laying eggs on wet beans would give the larvae more water before adulthood, but our findings in this experiment did not support our hypothesis and predictions. Instead, we found that the bean beetles laid a significantly greater number of eggs on dry beans than wet beans when both were present in the experimental Petri dishes. We determined that these findings could be due to several different reasons such as the bean beetle did not like the texture of the wet beans, or the wet beans were unfamiliar to the beetles and that is why they mostly laid their eggs on dry beans. Since bean beetles are tropical and subtropical pest insects, it is safe to assume they are not exposed to wet beans as often as they were in this experiment (Beck et al., 2013). Additionally, their life cycle can be completed successfully without any food or water on dried beans suggesting the beetles may have not found it necessary to lay their eggs on beans they were not used to (Beck et al., 2013). Another reason for these findings could be that soaking the beans may have caused the beans to develop bacteria or mold that made them no longer a suitable bean to lay eggs on.

Since the number of eggs laid on wet beans in this experiment was significantly less than dry beans, we can infer that the moisture of beans can affect the oviposition behavior of the bean beetle. In nature, the climate they are living in is typically dry; in this experiment, the environment becomes wetter, and this could affect the life cycle of bean beetles because they are not used to laying eggs on wet beans. Other insects that invade beans such as mites may also prefer wet or dry beans and could be examined in future research; however, they do not lay eggs on

beans. These findings can be used to further investigate the reproductive decisions that female bean beetles make and if these decisions can affect the fitness of their offspring. Two broad decisions are typically made by female insects when it comes to egg laying which may impact the fitness of her offspring: to lay single or in clusters and to select the laying substrate (Paukku and Kotiaho, 2008). Our research can be further investigated by performing a longer study that examines if the fitness of offspring of female bean beetles is impacted by laying eggs on wet beans providing the larvae with a greater water supply instead of dry beans.

References

Barbosa, Flavia. Animal Behavior Assignment Guidelines and Laboratory Handouts. 2022. Pgs 32-35.

Beck, C.W., Blumer, L.S. & Habib, J. (2013). Effects of evolutionary history on adaptation in bean beetles, a model system for inquiry-based laboratories. *Evo Edu Outreach* 6, 5.

Cope, J. M., & Fox, C. W. (2003). Oviposition decisions in the seed beetle, Callosobruchus maculatus (Coleoptera: Bruchidae): effects of seed size on superparasitism. *Journal of Stored Products Research*, 39(4), 355-365.

Mitchell, R. (1975). The evolution of oviposition tactics in the bean weevil, Callosobruchus maculatus (F.). *Ecology,* 56(3), 696-702.

Paukku, S., Kotiaho, J.S. (2008). Female oviposition decisions and their impact on progeny life-history traits. *J Insect Behav* 21, 505–520.