The Effect of Black-Eye Pea Bean Environment on Female Oviposition Preference in Bean Beetles (*Callosobruchus maculatus*)

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Introduction

Oviposition refers to an organism's ability to lay larvae eggs in an environment that is best suitable for its survival (Yee et al., 2021). Careful selection of an oviposition site is required because organisms are exerting energy and shortening their lifespans with the hope of producing viable offspring. The two stages of oviposition consist of pre-oviposition and post-oviposition. Pre-oviposition refers to identifying the best oviposition site, while post oviposition is when the female deposits the egg, hence the larvae try to maximize its surroundings to develop on its own (Lancaster and Downes, 2013). Since larvae are the juvenile form of an insect, they do not have the means to provide their own food; they depend on their selected deposit sites. The physical environment will determine if the larvae have enough food to survive, thus reaching the adult stage of development.

Further, oviposition site choice has been studied in beetles, specifically in bean beetles (Callosobruchus maculatus). Scientists examined the egg-laying mechanisms of bean beetles based on bean type and larval density (Paukku and Katiaho, 2008). This study allowed the researchers to determine if bean color or size influenced the number of eggs deposited. They found that the females were more likely to lay eggs on larger bean species due to increased surface area and preference. The larger the bean, the more likely the larvae will be able to develop based on the abundance of resources. Moreover, female bean beetles can acquire nutrients and water from male ejaculation (Ursprung et al., 2009). Accordingly, for our experiment, we aimed to investigate whether a bean beetle would be more likely to deposit its eggs in an environment with additional resources, such as providing water as a resource.

Based on this information, we hypothesized that bean beetles are more likely to deposit their eggs in a moist habitat when presented with both wet and dry bean environment due to increased hydration benefits. By depositing their eggs in the moist environment, the larvae will not have to compete for water, thus increasing their likelihood of survival. The female bean beetle aims for the larvae to have maximized resources, which increases their chance to develop to adulthood. The dry environment will not contain water, only beans. Hence, in the dry environment, the larvae would be less likely to survive due to their ability to only feed on the bean. We predict that there will be a higher number of eggs deposited in a wet environment than dry environment. To test our hypothesis and prediction, we provided an option for female bean beetles to lay their eggs on black-eyed pea beans placed in either wet or dry environments.

Methods

General Setup

We used a live culture of bean beetles, ***Callosobruchus maculatus***, for our experiment. We sexed the bean beetles and placed the female beetles in a separate petri dish, we collected 12 female beetles. In a petri dish, we added two cotton balls: dry and wet cotton ball. For the wet cotton ball variable, we submerged a cotton ball in water and squeezed any excess water. We labelled the side with the wet cotton ball as "WET" underneath the petri dish with tape (Figure 1). On each cotton ball, we added three black-eye pea beans per section. We prepared 11 more petri dishes to have a total of 12 samples. In each petri-dish, we added a female beetle. We left the petri dishes for 48 hours on a table to avoid excess sunlight from the window.

Collecting Data

After 48 hours, the female bean beetles laid their eggs on the beans. We removed the females from the petri dishes and waited another 24 hours for the eggs to darken. Then, we counted the eggs under a microscope and recorded how many eggs were laid. For our statistical analysis, we performed a t-test to compare the means of the number of eggs laid in the two different environments.

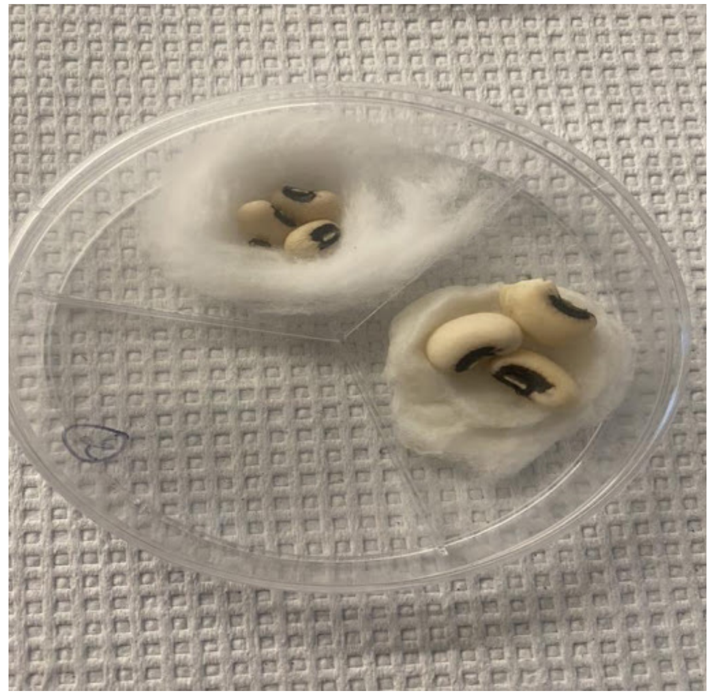

Figure 1. Black-eyed peas experimental set-up. Three beans in a dry environment (dry cotton ball) and three beans in a wet environment (dampened cotton ball).

Results

When given the option, we expect females to oviposit more in moist environments than dry environments. However, there is a higher mean number of eggs in dry environments (M = 3.83, SD =4.91) than wet environments (M = 1.92, SD =5.16). There was a large variation within the bean environment samples. There is no statistical significance between both bean environments (t = 2.20, p = 0.41).

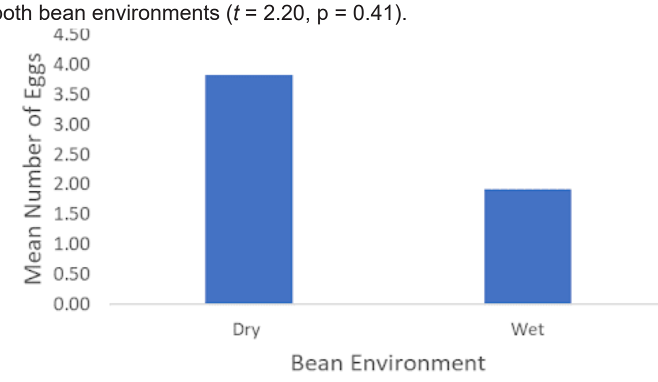

Figure. 2. The bar graph above shows the mean number of eggs in both the dry and wet environments. There were more eggs laid on the dry beans than the wet beans. The dry and wet beans had means of 3.83 and 1.92 eggs, respectively. The standard deviation of the dry and wet beans was 4.91 and 5.16, respectively. There was no statistical significance between the two different environments (t = 2.20, p = 0.41).

Discussion

In this study, we investigated how bean environmental differences affected oviposition site choice in bean beetles. Our hypothesis stated that bean beetles would exhibit increased egg deposition on beans in wet environments than dry environments due to greater resource availability of water. The data didn't support our hypothesis. Specifically, the results suggest that bean beetles prefer dry bean environments over wet environments, but this difference in preference is nonsignificant. Therefore, our study shows that there is no environmental effect on bean oviposition site choice among bean beetles.

The observed tendency for bean beetles to prefer dry oviposition sites aligns with previous research in which the same beetle species showed a significant increase of egg deposition on dry beans than moist beans when bean size was controlled (Hudaib et al., 2010). However, it's important to note that the referenced study controlled the beans moisture, while we created a moist environment which did not manipulate the bean's properties. Although our study showed a similar trend for dry bean preferences, the data was ultimately insignificant, and this may be because bean size was not controlled. Previous studies have shown that bean beetles tend to deposit their eggs on beans larger in size with more surface area (Paukku and Kotiaho, 2008). Therefore, differences of bean size used in the experiments serve as a limitation of our study. This is because some beans in our study may have had more surface area than others and therefore directly influenced bean selection. Future experimental replications should consider controlling bean size and surface area to mediate this confounding variable. Another study used a different bean beetle species to assess both the effects of temperature and humidity on oviposition in azuki beans. The researchers found that there is a preferred temperature and humidity range for beetle oviposition (Mainali et al., 2015). High levels of humidity in the referenced study may induce "wet" environments on the bean sites tested, similar to our experimental wet condition. Based on this, perhaps there is an optimal or favorable degree of wetness and temperature that bean beetles may prefer to deposit their eggs. Future studies should investigate whether there is a favorable balance of wetness and dryness in the bean environment.

In conclusion, our results did not statistically support our hypothesis regardless of the observed trend that reveals female bean beetles prefer to deposit their eggs on beans in dry environments. This is in opposition to our hypothesis and may stem from previous research that has shown *C. maculatus* beetles evolved to oviposit in dried, stored beans which makes these species a common agricultural pest (Tuda et al., 2006). However, our data was insignificant, which may also be due to the limitations previously acknowledged. Therefore, the application of our results is limited. Still, the current study adds to literature on bean beetle oviposition preferences, which is an essential aspect of bean beetle reproduction.

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