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Innate food preference in the larval tobacco hornworm, Manduca sexta

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Abstract

Food preference can drive an organism to seek a specific food source even if another food, which can provide needed nutrition, is easier to obtain. Food preference can develop in different ways, including innate preference, i.e., organisms display food preference at birth, or learned preference, i.e., organisms develop food preference after previous experience with that food. The tobacco hornworm, *Manduca sexta*, shows learned preference; however, we lack information about innate food preference in *Manduca*. *Manduca sexta* larvae eat many different foods until they feed on a solanaceous plant, when they become more specific in their diet. This study focused on naive *Manduca sexta* larvae to determine if they show innate food preferences. Because early juveniles seek out solanaceous plants if they hatch on a different family of plants, I predicted that *Manduca sexta* prefer solanaceous plants over artificial food designed for captive animals. Based on trends seen in other studies, I also predicted that *Manduca* prefer plants that have not been fed upon previously. To test my predictions, *Manduca sexta* were allowed to choose from live plants with damaged leaves (to simulate previous feeding by other insects), live plants with undamaged leaves, or lab food. I recorded which food source *Manduca* approached first and latency to select food. I found that there is no significant difference between latency time, or between the plant and lab food trials. There was a significant difference between the damaged and

Introduction

Food preference can develop in different ways. One of these ways being learned food preference, which is developing an affinity for a food after having experience with a food (Nyström, E. (2013)). Another way is innate preference, which is an inheritable genetic preference for one food over another based on genetically imprinted nutritional needs (Singer, etal, (1992)). *Manduca sexta* is an important model organism that has demonstrated learned food preference (de Boer, G., (1992), Jermy, T., Hanson, F. E., & Dethier, V. G. (1968)). Which have shown that larval *Manduca sexta* prefer to feed on host plant they have already fed on (de Boer, G., (1992)Jermy, T., Hanson, F. E., & Dethier, V. G. (1968)). They also prefer non damaged plants over ones that have been fed upon by another insect. (Jermy, T., Hanson, F. E., & Dethier, V. G. (1968), Nyström, E. (2013)).However, there is a lack of available information on innate preference in *Manduca sexta*. A precedence for the theory that they show innate preference comes from the fact that if an egg is laid on a nonhost plant, the resulting larvae actually seek out a suitable host

Objectives

This study analyzed the food preferences of naive *Manduca sexta* larvae, which are larvae that have not had the opportunity to feed on a host plant.

Predictions

1. *Manduca* prefer live plants over the lab food source. Based on the fact that newly hatched Manduca larva will actively seek out a more suitable host plant after hatching on a less suitable host plant(Nyström (2013)).
2. *Manduca* prefer the undamaged plants over the damaged ones. Based on the odors given off by damaged *Solanaceae* plants, that can signal predators of the feeding herbivore, and the *Manducas* reliance on olfactory senses in food selection(Kessler and baldwin (2001), Nyström

Methods

This study utilized 11 naive *Manduca sexta* larvae,. The larvae used were acquired from Carolina Biological, and staff verified that the animals were never allowed to feed on a host plant, and were reared on their own blend of a lab prepared food source.
This study was conducted in 603A Science at the University of Southern Maine. This room is used to rear *Manduca sexta* for laboratory use and provides optimal growing conditions.
I tested three different treatments: A live undamaged tomato plant vs. a grain based lab made food, used to raise *Manduca*. A live plant with leaves damaged with a lancet to mimic predation vs. a lab made food. Finally a live undamaged plant vs. a live damaged one.
For each test, a larva was placed in the middle of the testing apparatus (Fig. 1) and was allowed to choose between the options at either end, but it was not allowed to feed on that choice. The choice was made when the larva crossed the black line seen in the fig. ,the length of time to make the choice was measured with a stopwatch, were recorded. Latency times were analyzed using JMP 12.2 statistical package(SAS institute, inc., 2012). Preferences were analyzed using the chi-squared test.

Fig. 1 The setup of the testing aparatus the larva was placed on the center line in the middle of the food choices. A chice was made when the larva crossed the black line of the food side.

Fig. 2 the damaged plant leaf, damaged with a lancet.

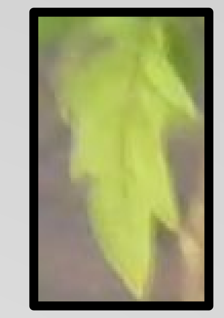

Fig. 3 The undamaged leaf

Results

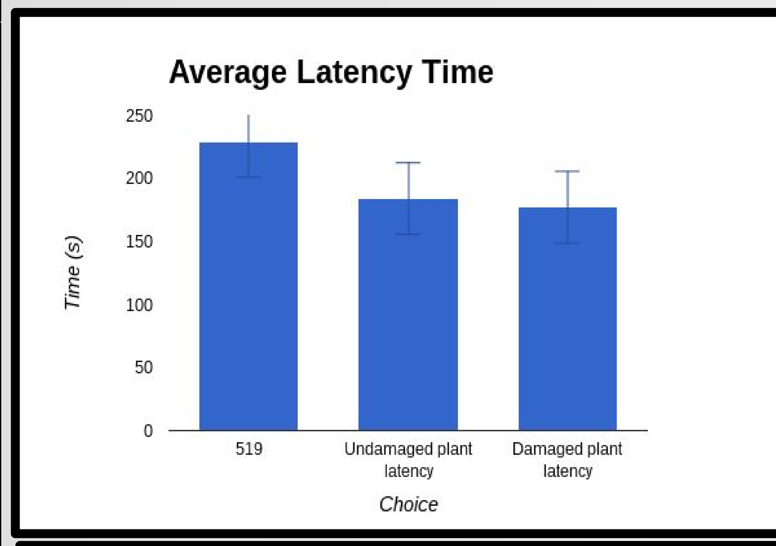

Fig. 4 The average latencies for food choice matched pairs with wilcoxon signed rank tests. Undamaged and Lab food, N=10, p=0.6250, test statistic(s) -5.5. Damaged and lab food, N=9,p=0.3008, s=-9.500. Damaged and Undamaged, N=10, P=0.4316, s=-8.5 There is no statistical difference between the latency times.

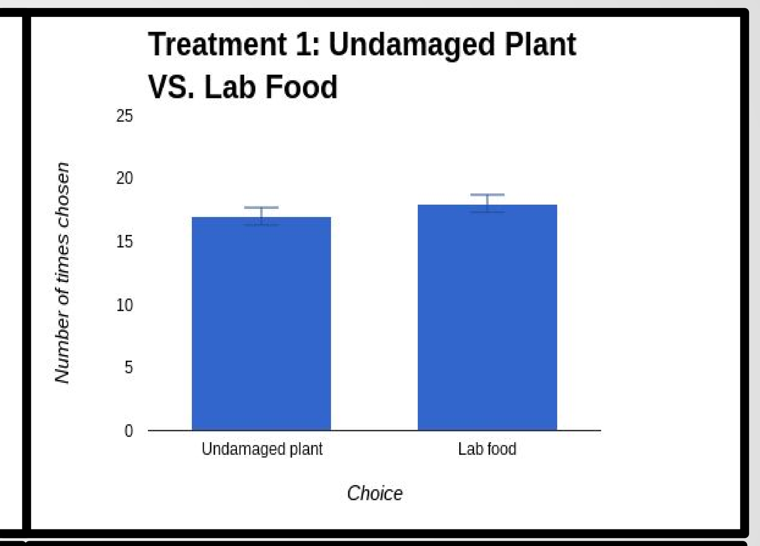

Fig. 5 The choices made in Treatment 1. Chi-squared test used to analyze. N=11, χ2=0.02857, degrees of freedom (DF)=1, chi squared critical value=3.841 chi-squared< critical value p>0.05 No statistical difference in results

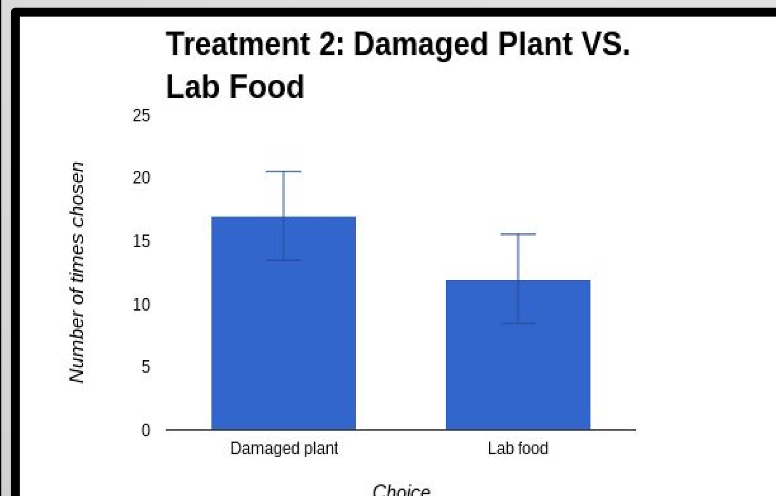

Fig. 6 The choices made in Treatment 2. Chi-squared test used to analyze. N=11, χ2=0.862, degrees of freedom (DF)=1, chi squared critical value=3.841 chi-squared< critical value p>0.05 No statistical difference in results

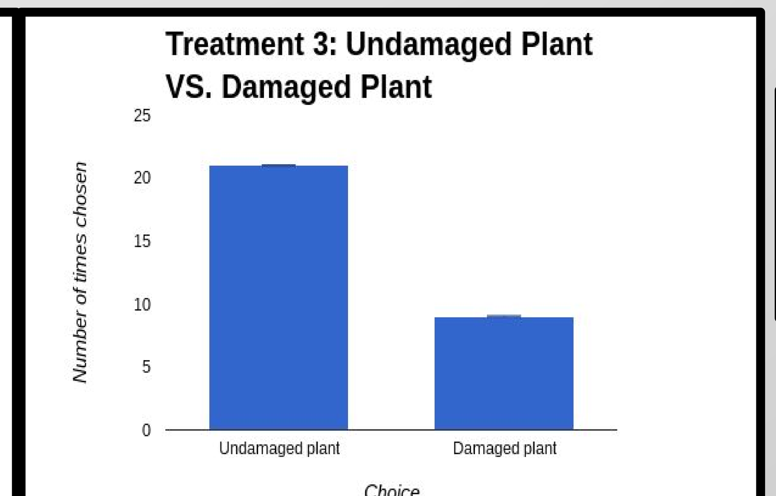

Fig. 7 The choices made in Treatment 3. Chi-squared test used to analyze. N=11, χ2=4.8, degrees of freedom (DF)=1, chi squared critical value=3.841 chi-squared> critical value p=0.05 There is a statistical difference in results

Discussion

From the data we can see that there is no significant difference in preference between the undamaged plant, and the lab food, with the probability being more than 0.05. This is also true for the comparison between the damaged plant and the lab food, where again there is no statistical difference, p>0.05. There is also no significant differences in the latency times. From this we can conclude that there is no preference for any of these different food choices. Because of this we can conclude that the first initial prediction was incorrect and that there is no inherent preference for a the tomato plants that the *Manduca* has not been exposed to. These results could be do to several different factors the most important of which is the life stage to the Manduca used in the experiment. The 9 Manduca were in the 4th instar, and 2 were in the 3rd instar, which are both late in the larval cycle, It has been observed that food preference is induced in the first instar, and that the larvae reared on an artificial medium remain polyphagous(yamamoto (1974)). This could explain why they showed no preference, because they were past the life stage where they would have been the most attracted to the novel food source, and showed no preference because each of the foods, which are roughly equivalent in nutritional value, would have been equally attractive. It is also very likely that these results are due to experimental error, and a small sample size. The testing area may not be well suited for the experiment and a different apparatus, which features air pumps, such as the one featured in the Nyström paper, this would force the olfactory stimuli to the larva, and may allow it to better choose between the two options. Also the small sample size and the fact that they all came from the same source may lead to skewed results.
The Undamaged plant VS. Damaged plant trial did give a significant result, where the undamaged plant was chosen a significant number of times more than the damaged plant, p=0.05. This is most likely due to the fact that *Manduca* rely on chemoreceptors to make food choices (De Boer (1993)), and that plants in the same family as tomatoes have been shown to release pheromones, and other volatiles when being fed upon (Kessler and baldwin (2001)). These signals being given off by the plant may drive away the larva, and the pheromones used to attract predators of the larva may also make it less likely to chose the damaged plant. The avoidance of these plants may give the larva a survival advantage, where being able to interpret the pheromone signals will allow the larva to avoid possible predators.

Conclusion

1. The prediction is incorrect, there is no preference shown between the lab food and a tomato plant. There is no statistical difference between the different choices. There is also no statistical difference between the latency times which suggests that there is no preference based on the amount of time it takes to chose, reinforcing the conclusion that there is no preference for either lab food or a live tomato plant.
2. This prediction was in fact reinforced by the results of the study. There was a clear, and statistical difference between the two choices. There is a preference for the undamaged plant, over the damaged one, while there is not a difference in latency time, there is a significant difference in the number of times the undamaged plant was chosen. It however cannot be concluded why the difference is seen.

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