Female *Malacosoma americanum* Selection of Oviposition Sites

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Abstract

Selection of an appropriate oviposition site is critical in the life cycle of semelparous organisms because larvae rely entirely on their host without the benefit of parental care. To maximize fitness, females may demonstrate preference for a suitable host when ovipositing. *Malacosoma americanum* females oviposit mostly on *Prunus serotina*. In order to test whether preferences exists between trees, and identify which variables might influence this choice, data was gathered on the relative number of egg masses and tree characteristics. A Chi-squared test allowed for the rejection of a random scenario and a stepwise linear regression identified significant correlations between tree characteristics and relative number of egg masses. Females preferred to oviposit on trees with a smaller surface area to volume ratio, trees that stood further west, and were in close proximity to a conspecific with a smaller DBH. A discussion follows on the evolutionary benefits to the offspring as a result of these preferences.

Introduction

The majority of animals on Earth are semelparous organisms, with one chance to reproduce during their lifetime. This strategy has been preferred by natural selection because it allows the majority of a species' energy to be allocated for reproduction. A trade-off occurs between early reproduction and the ability to grow larger, live longer, and reproduce later in life. Because early reproduction involves sacrificing future potential, it is paramount that a semelparous organism reproduces successfully. Since the mother often dies before the first offspring have been born, there is no parental care. The vulnerable offspring rely entirely on the environment into which they begin their life (Karowe 2008a). This means that a female's fitness relies greatly on where she lays her eggs. If the environment is not suitable, there is a great risk that her genes will not be passed on. For this reason, natural selection may favor the existence of female preference for certain birthing locations.

One species for which this may be true is the eastern tent caterpillar (*Malacosoma americanum*). *M. americanum* females deposit egg masses onto the branches of black cherry trees (*Prunus serotina*) during the months of June and July (Fitzgerald 1995). Immediately after laying her eggs, the female dies. The eggs lie dormant during the winter and emerge in April and May of the year following their oviposition (Fitzgerald 1995). Each egg mass is comprised of hundreds of individuals, which cooperate to form a colonial tent upon hatching (Fitzgerald 1988). Previous research has investigated the placement of these tents on branches (Fitzgerald 1995), as well as the directional location in the tree (Aker et al. 2002), sunlight distribution (Moore 1988), and optimum temperature gradients (Knapp and Casey 1986).

Developing a Null Model

These previous studies do not address the confounding issue of whether female oviposition on a given tree is determined by choice or at random. Assuming random oviposition, there would be a higher probability of the moths finding a larger tree. In this scenario, the percent of the total surface area occupied by the tree would be equal to the percent of the total egg masses found in that tree. If female oviposition were random, there would be a linear relationship between tree surface area and the number of egg masses in the tree (Figure 1).

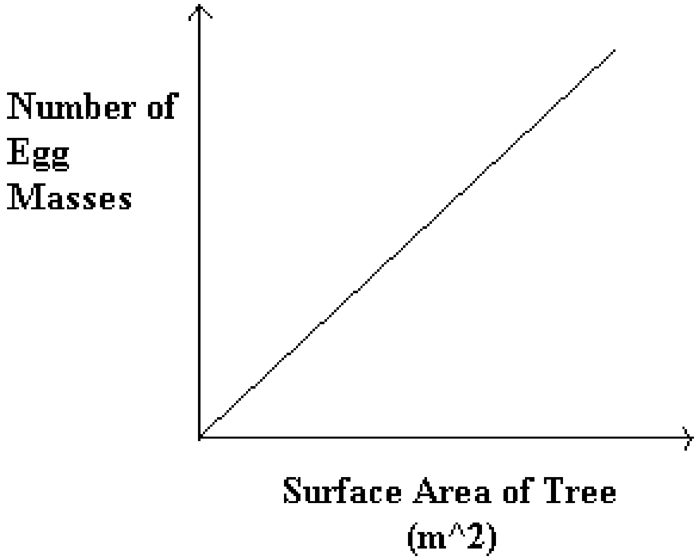

Figure 1: The linear relationship between the number of egg masses and the surface area of the tree if oviposition occurs randomly.

If the results indicate that oviposition is not random, it becomes necessary to identify which factors would suggest that female choice determines oviposition location. These factors fall into three main categories: resource availability, predator avoidance, and leaf quality. This study examines the following tree traits: height, average leaf density, surface area to volume ratio, distance to the closest tree, diameter at breast height (DBH) of the closest tree, distance from the road, relative distance east, water weight, and nitrogen content of the leaves. By quantifying these variables and then comparing them with the relative abundances of egg masses, we hope to determine which (if any) factors influence female oviposition site selection.

Resource Availability

Average leaf density may be an important factor influencing female choice. It can be advantageous for females to deposit their eggs in trees with a higher average leaf density because this provides more resources for newly hatched caterpillars. However, greater leaf density could also create more shade and a lower temperature, which is potentially detrimental to ectotherms (Knapp and Casey 1986). In addition, there is a danger of cyanide poisoning for young caterpillars, which would result from eating too many cyanide-rich leaves in a short amount of time (Fitzgerald et al. 2002). This may favor oviposition in trees with a lower leaf density. Thus, it is possible that a higher leaf density may be more detrimental than beneficial.

Assessing surface area to volume ratio may also provide insight into oviposition preference. A smaller surface area to volume ratio corresponds to a spherical shape, a longer average branch length, and more available leaves. Female moths oviposit a mean distance of 10 cm from the tips of the branches (Fitzgerald 1995). A spherical shape would prevent a large number of females from laying their eggs, reducing competition and increasing resource availability. For this reason, it is expected that there would be a preference towards trees with a smaller surface area to volume ratio.

The distance to the closest cherry tree may affect female preference for a certain tree. Tent caterpillar moths may prefer to lay their eggs in a tree that is closer to another tree because often caterpillars completely defoliate their original host trees (Fitzgerald and Peterson 1988). If this occurs, they must move to neighboring trees for more resources. Hence, females may choose trees in close proximity to another cherry tree.

Taking this into consideration, we expect that the DBH of the closest cherry tree may also be a factor influencing female choice for many of the same reasons. A larger DBH is associated with an older tree and a greater total tree biomass (Packer and Clay 2003). Thus, it is expected that female moths will prefer trees near a conspecific with a large DBH.

Predator Avoidance

Height could be a factor in determining oviposition because the higher the caterpillars are located in a tree, the further they are from ants that have been shown to prey on the larvae (Fitzgerald 1995). Therefore, we hypothesized that more egg masses would be found on taller trees.

The distance the cherry tree is from the road could also affect where females oviposit. Roads create open spaces, which simulate clearings in nature and favor caterpillar survivorship by decreasing habitats used by enemy parasitoids and parasites (Roland 1993). For this reason, we would expect to see that trees closer to the road have more egg masses.

Because wind blows from west to east in northern Michigan, it may be energetically favorable for moths to fly in the direction of this flow. If this behavior is preferred, it could be expected that a higher volume of egg masses would be found on the westerly trees in our plot.

Leaf Quality

The water and nitrogen contents of the leaves of a tree may also be important factors that influence female choice. Water and nitrogen-deficient leaves are less nutritious (Fitzgerald 1995). Therefore, caterpillars must ingest greater quantities in order to receive the nutrients they need to survive. For this reason, it would be expected that trees that have leaves with greater water and nitrogen content will have more egg masses because their leaves are more nutritious.

Materials and Methods

Data was collected May 25, 28, and June 5, 2008 on Riggsville Rd in Pellston, MI, approximately 3 miles southwest of the University of Michigan Biological Station. On May 25, 2008 twenty black cherry trees between 1 and 6 meters tall were randomly selected from a single clearing. Identical measurements were taken for each of the twenty trees including height, trunk height, ten measurements of canopy radius, number of egg masses per tree, and leaf density. Tree heights were calculated using a clinometer. Leaf density was determined by averaging the number of leaves within the 10 cm space beginning at a distance of 10 cm from the tip of 10 randomly-selected branches on each tree. The surface area and volume of each of the trees were determined by approximating the tree shape as a cone (Figure 2).

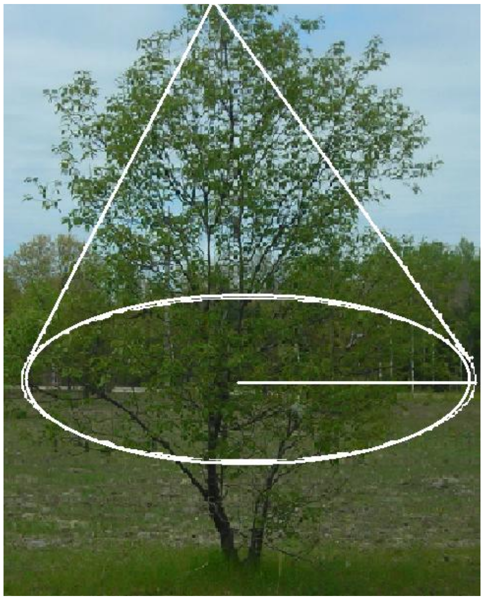

Figure 2: Geometric model to calculate surface area

On May 28 further measurements were made. These included distance from the road, distance to the nearest cherry tree, the DBH of the nearest cherry tree, and the distance from the most eastern tree. A transect line was created from north to south using the eastern-most tree as a reference. Using a compass, several marking flags were placed to indicate the north and south directions. The perpendicular distance from the transect line was then measured for each tree. These measurements could not be made four trees of the trees because the transect line could not be extended due to traffic interference.

Finally, on June 5, 10 leaves were collected from each tree from 1.5 meters high on the western-most side of the tree. Two trees no longer had any leaves, so they were excluded from these calculations. Collected leaves were weighed twice. The fresh weight was recorded immediately after collection and the dry weight after a 24-hour drying period where the leaves were kept in a 75° C drying oven. Nitrogen content was measured (using a mass spectrometer) from a leaf powder made by grinding the dried leaves in a coffee grinder.

Statistics Calculations

To test whether female oviposition occurs randomly, trees were grouped into five size classes, each with a total surface area of approximately one-fifth of the total surface area of the twenty trees. If female oviposition were random, we would expect that there would be an equal number of egg masses in each unit of surface area. A Chi-squared test was run to determine the deviation from the expected number of egg masses.

In order to test the individual variables and their effect on female preference, the five separate size classes were maintained. Because each size class had a different number of trees, each variable was averaged to create the best representation of the trees within a given class. For each variable, a linear regression was run to see if there was a relationship between female preference and the variable. Female preference was quantified using the deviation from the expected number of egg masses assumed for random oviposition relative to the surface area. A stepwise linear regression was conducted comparing the egg mass deviation to the average value of each of the following: height, surface area to volume ratio, leaf density, distance to the closest tree, DBH of the closest tree, distance to the road, relative distance east, water content, and nitrogen content.

Results

Null Model

The results of the Chi-squared test indicate that the pattern of female oviposition is not random. The p-value for the Chi-squared test was between 0.01 and 0.005, so the null hypothesis was rejected. From the individual Chi-squared results for each tree group, we see that tree group two has many more egg masses than expected while group 5 has fewer (Table 1).

Group	TSA (m^2)	% of TSA	(Observed-Expected)	X^2
1	78.37	0.18	-0.74	0.08
2	71.76	0.16	6.83	7.55
3	72.91	0.17	-1.27	0.26
4	86.77	0.20	2.54	0.86
5	132.40	0.30	-7.39	4.79
Total	442.21			13.55

Table 1
*TSA=Total Surface Area

A stepwise regression indicated that the DBH of the closest tree (p=0.001), the distance from the most eastern tree (p=0.001), and the surface area to volume ratio (p=0.004) are all significant factors influencing female choice when considered together. The correlation value for the DBH of the closest cherry tree and the deviation from the expected number of egg masses is -2.836. Therefore, as the DBH of the closest tree increases, female preference for the tree decreases. The correlation value for the distance from the most eastern tree to the deviation from the expected number of egg masses is +0.29. Hence, the more west the tree is found, the higher the female preference for it.

The correlation between the surface area to volume ratio and the deviation from the expected number of egg masses was -0.473, meaning that as the surface area to volume ratio increases, the preference for the tree decreases. Below are the graphs of the deviation from the expected number of egg masses versus each of the traits being analyzed.

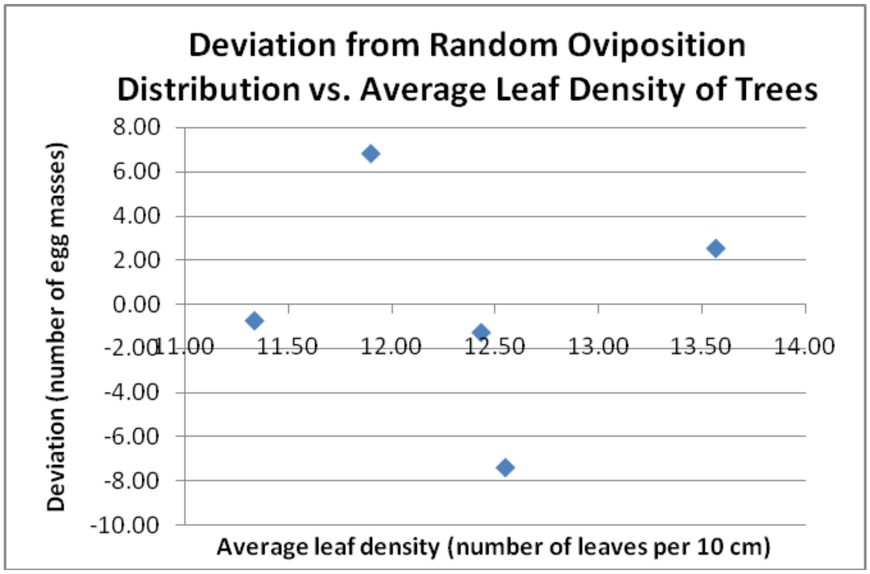

Figure 3- The effect of the average leaf density of the trees on female oviposition preference.

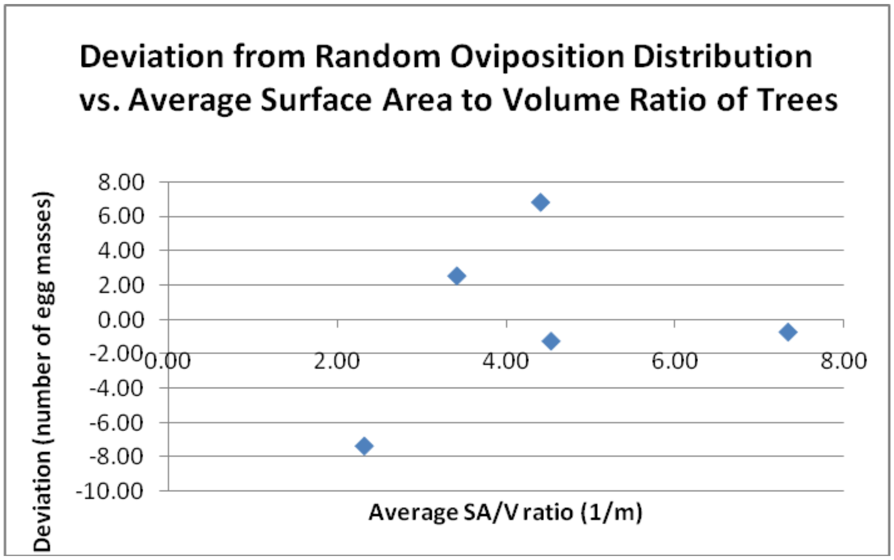

Figure 4- The effect of the average surface area to volume ratio on female oviposition preference.

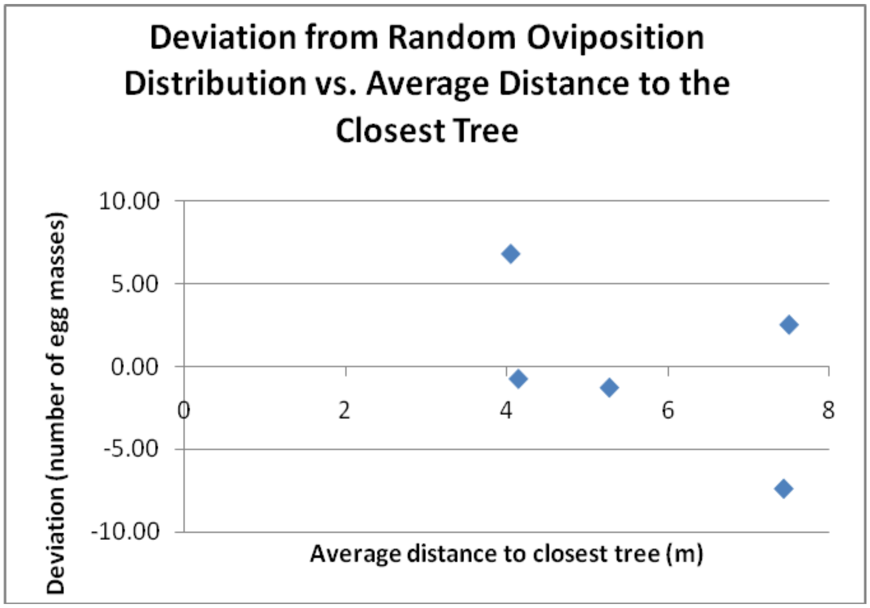

Figure 5- The effect of the distance to the closest tree on female oviposition preference

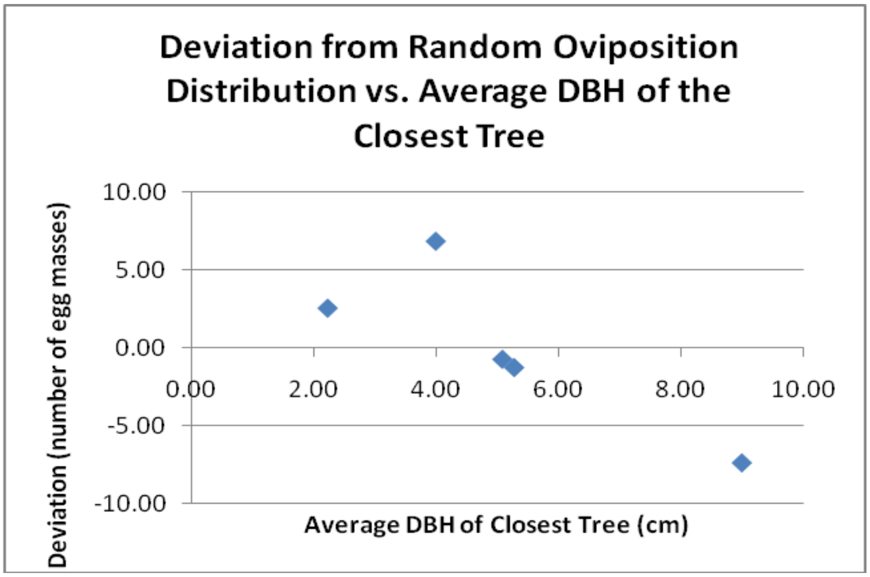

Figure 6- The effect of the average DBH of the closest cherry tree on female oviposition. The p-value for this linear regression was 0.071. As can be seen from the graph, the trend shows that as the DBH of the closest cherry tree decreases, the number of egg masses, and thus the female preference for the tree, increases.

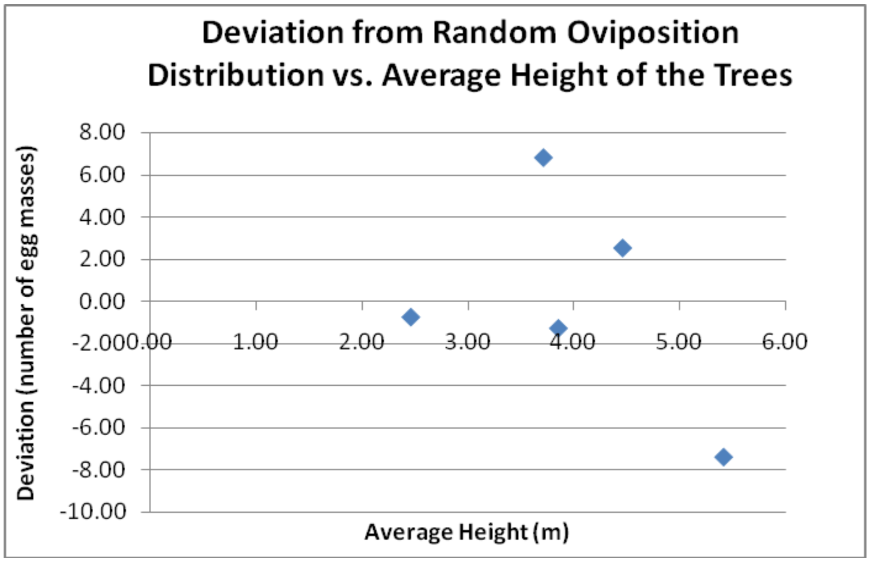

Figure 7- The effect of height on female oviposition preference

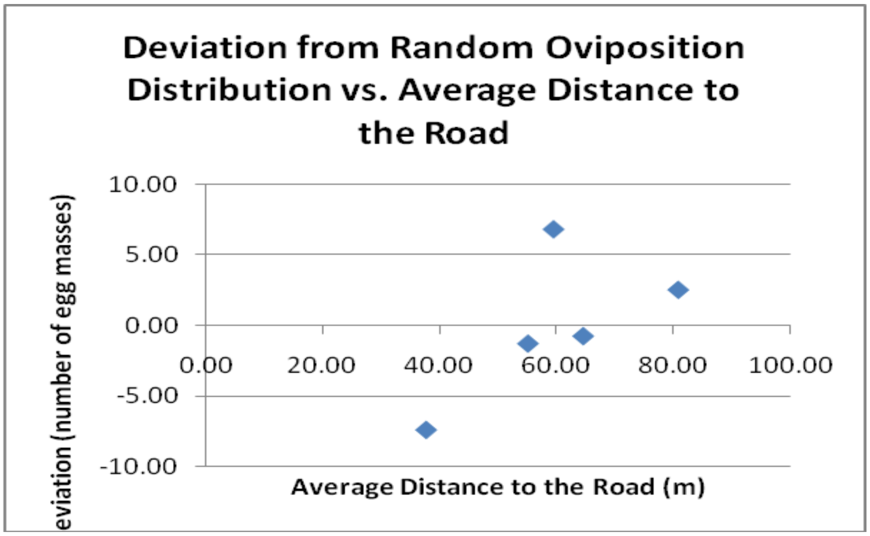

Figure 8- The effect of the distance to the road on female oviposition preference.

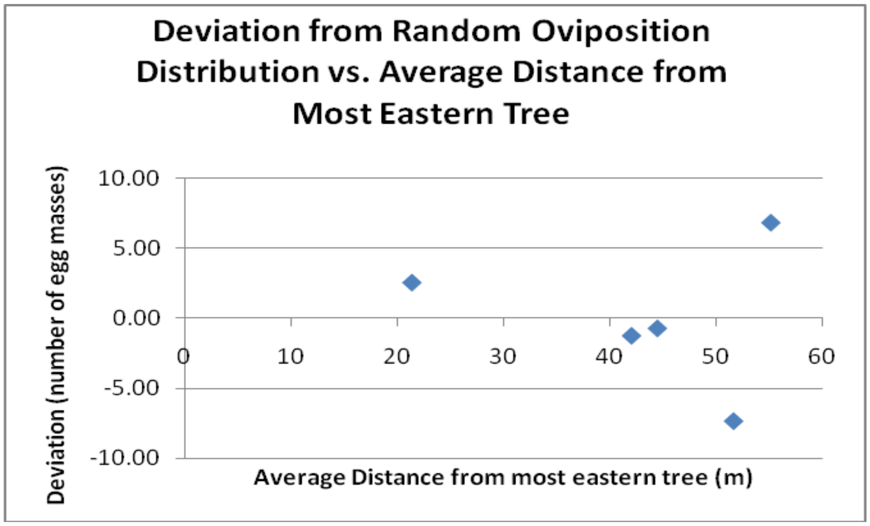

Figure 9- The effect of the average distance from the most eastern tree on female oviposition preference.

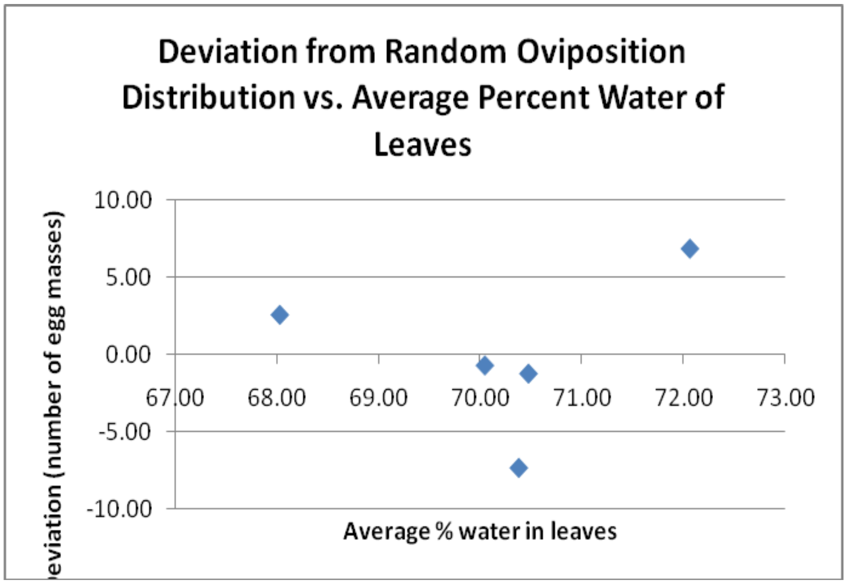

Figure 10- The effect of the percent water in the leaves on female oviposition preference.

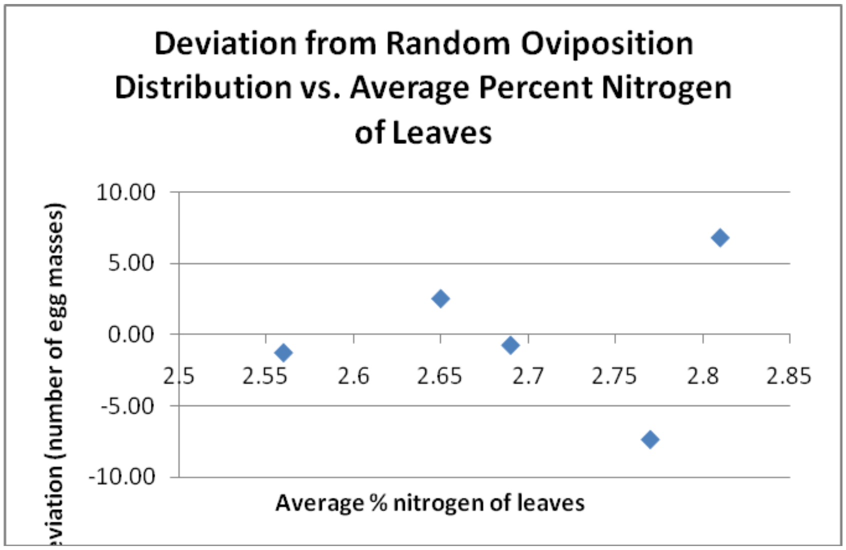

Figure 11- The effect of the percent nitrogen in the leaves on female oviposition preference.

Discussion

One consideration for possible oviposition preference was the leaf density of the tree. It was expected that females would choose to oviposit on trees that have a greater number of leaves per centimeter of branch over trees that on average have a relatively sparse amount of leaves. This female choice was hypothesized to have evolved so that the mother could place her eggs in a tree that would provide the greatest volume of food for her offspring. This possible preference was not apparent in our findings; it may be that the mother chooses a tree based on a more moderate leaf density ideal. It is possible that too many leaves per unit branch could result in rapid overeating of leaves and in turn cyanide poisoning of the larvae. While the caterpillar is capable of rapidly depleting the cyanide content of the leaves between the foregut and midgut of the digestive process, some cyanide remains, which indicates that some danger exists for the caterpillar (Fitzgerald et al. 2002). The assertion that the mother prefers to oviposit on trees that have a medium leaf density is met with the possibility that the female does not take into consideration leaf density at all when choosing where to oviposit. Studies would require a larger sample size and a more in-depth consideration of the variation in leaf density of cherry trees to provide support either way.

The results indicate that the distance to the closest black cherry tree did not influence female choice. If the particular tree in which the egg masses were laid has a sufficient amount of resources, the caterpillars would not need to go looking for another tree. Another possible explanation is that there has not been a need for this evolutionary response.

Research has shown that height may play an influential role in determining oviposition preference for semelparous insects (Horner and Abrahamson 1999). In the case of this study, the taller the tree, the greater the preference for a female to oviposit on it. Because caterpillars are often prey for predatory ants that live on the ground, a higher locus in a tree would distance larva from predators (Fitzgerald 1995). Our analysis indicates that females do not show preference for taller or shorter trees. This observation could be related to a low predator threat in the sample environment. In a low predator environment, it would still be beneficial to be deposited on taller trees to avoid the risk of predation; however this benefit would be less than in a high predator threat environment. With medial threat, there is a trade-off between being high and far from predators or lower in the tree, closer to the ground, and more able to access resources from neighboring trees.

The distance a cherry tree stood from the road was not shown to correlate with female preference. Females may choose to oviposit on trees that were nearest to the road because the open space it creates simulated a natural clearing in the woods, which is a desirable location for the tent caterpillars. Spaces on the edges of forests or in clearings are safer environments because there are fewer parasitoids and predators to eat or otherwise harm the caterpillars (Roland 1993). However, our findings did not support this hypothesis, which may be an indication that there was not a great enough variation in the distances the twenty trees stood from the road. It is possible that all trees stood in a sufficiently open space away from other trees, implying that the female may look for trees that meet a certain standard distance to a clearing but are not necessarily more desirable the farther they are above this standard. This would suggest that there is a predator tolerance threshold and, as long as trees provide a level of protection below this threshold, there will be no negative impact in offspring survival. It may be that the females do not take into consideration distance from a road if there are other more pressing preferences, such as food availability, when determining where to oviposit.

Nitrogen is a limiting resource for the growth of living organisms and water can be limiting for many insect species. Spring leaves of black cherry trees are known to have the greatest nutritional value in the early spring when the leaves have just budded (Karowe 2008b). In synchrony with leaf budding, caterpillar hatching occurs in June and July (Fitzgerald 1995). Although nitrogen and water content has contributed to the phenological adaptation of hatch time, research showed that the nitrogen and water content of leaves does not determine the tree onto which a female moth deposits her egg masses (Karowe 2008b). This observation is likely because *M. americanum* populations are not near carrying capacity. With an abundance of leaves to eat, the quality of individual leaves is not as important a factor as it would be if leaves were limiting. Instead of eating fewer better leaves, caterpillars eat leaves in mass quantities and still receive the water and nitrogen required for growth. For future experimentation, it would be beneficial to test whether nitrogen and water content of leaves play a role in oviposition in tent caterpillar outbreak years where populations do reach carrying capacity.

In the stepwise regression it is observed that when the three variables are considered together, females prefer trees with the closest cherry tree having a smaller DBH, that are located more to the west, and that have a smaller surface area to volume ratio. The DBH of a tree is positively correlated both with the age and the total biomass of the tree (Packer and Clay 2003). Herbivorous caterpillars induce the production of phenolics in the leaves of cherry trees, which also induces the production of phenolics in neighboring trees (Karowe 2008b). These phenolics signal the location of caterpillars to predators. If a surrounding tree has a smaller DBH, it has fewer leaves to induce a phenolic signal (Karowe 2008b). Smaller neighboring trees also have less room to house predators as well as other caterpillars that may compete for resources. Finally, caterpillars are thermo regulators, relying on the sun to maintain their body temperature. Large neighboring trees may shade the trees, preventing caterpillars from receiving energy from the sun. Any of these reasons may account for this preference for neighboring trees with a smaller DBH.

There are several factors that may explain the preference for trees located more to the west. In northern Michigan, the wind travels from west to the east, meaning that trees located to the west are upwind of other trees. Looking to phenolics, it is possible that there is a preference for trees located upwind of conspecifics. This would be advantageous because upwind host trees would not be induced by the phenolics of other trees. Additionally, it may be energetically favorable for moths to fly in the direction of the wind. Finally, it is known that the caterpillars come out in the afternoon when the sun is located in the western part of the sky (Fitzgerald 1995). It would be advantageous for caterpillars to be located in the more western trees if they were receiving significantly more sunlight because this would help them maintain a constant body temperature.

The preference for a smaller surface area to volume ratio, which is associated with a spherical shape, can be explained in various ways. Because the female moths oviposit about 10 cm from the outer edge of branches, a spherical shape would minimize competition because there would be a smaller surface area on which caterpillars could lay their eggs. At the same time, however, the tree would provide a greater volume of resources for the young caterpillars. Finally, the larger volume may provide protection from predators.

Since eastern tent caterpillars are semelparous organisms, the behaviors surrounding the ovipositional process are crucial to the survival of the species. Because the mother dies shortly after oviposition, the relative adequacy of the chosen tree offers the feeble larvae the sole remnant of maternal concern for her offspring. Thus certain tree-selecting behaviors could be evolutionarily favored, especially those that pertain to resource availability and avoidance of predators. Simultaneously, the host trees may be selected for on the basis of their ability to avoid the dangers of herbivory; therefore, the interactions between host and caterpillar are much more complex than can be viewed through the scope of the ovipositional process. Nevertheless, semelparity is a risky reproductive strategy that owes some of its overwhelming success to behavioral traits, such as the environmental preferences that have evolved to increase offspring survivorship.

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