Larval Consumption and Growth in Host-Shifted Herbivorous Insects: a Test with the Eastern Tent Caterpillar, *Malacosoma americanum*

Christina Carson, RahulGondalia, Andrew Schwartz, Elizabeth Thom

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Abstract

Laboratory experiments were conducted to analyze the ability of *Malacosoma americanum*, the eastern tent caterpillar, to consume, digest, convert, and grow on the leaves of non-host tree species found in close proximity to *M. americanum* populations in northern Michigan. Test tree species included *Acer saccharum* (sugar maple), *Acer pensylvanicum* (striped or moose maple), *Fagus grandifolia* (American beech), *Quercus rubra* (red oak),*Elaeagnus angustifolia* (Russian olive), and *Prunus serotina* (black cherry). Findings showed significant differences in relative growth rate, relative consumption rate, approximate digestibility, and efficiency of conversion of digestive matter in all test species aside from *Q. rubra* when compared to the specialized host *P. serotina.* No significant difference was found in comparing the same factors in test caterpillars fed *Q. rubra*with those fed *P. serotina*. These results suggest possible existence of alternate available host species for *M. americanum*. This finding is consistent with previous observations of *M. americanum* feeding on oak species in nature during later instars.

Introduction

Current estimates figure the number of herbivorous insects rangesfrom 4 to 30 million species. While the vast majority of mammalian herbivores demonstrate a generalist eating pattern, specialization to specific host plants appears to occur in a much higher percentage of insect herbivores (Vojtech et al., 2002, Menken, 1995). This disparity between the two animal taxa raises the question of what selective forces in the evolutionary past have led to millions of insect herbivore specialists.

There are several different theories that attempt to explain the prevalence of host plant specializations among insect herbivores. The 'arms race' theory suggests insect-plant co-evolution resulted from successive evolutionary advances in plant defenses and counter-adaptation by insects, leading to alternating periods of plant and insect adaptive radiations. The plant defenses select for feeding specialization among the insects feeding on them**.** Another theory postulates that feeding specialization arises from a long-term association of an herbivore with a particular host. Over time, the preference of an organism for a specific host species may lead to the loss of genetic variation required to use alternate hosts. This could result from genetic drift or the absence of selective pressures acting to retain the alleles required for feeding on multiple host plants. Such alleles could code for specific digestive enzymes to process host plant chemical defenses and/or behavioral recognition of a leaf as a suitable source of nutrition (Mayr, 1997 in Yotoko et al., 2005).

Lepidoptera (butterflies and moths) have a greater proportion of specialists than any other order of insects (pers. comm. D. Karowe). Because of the limited mobility of the larvae, the female's plant oviposition choice will determine the primary food source for her offspring (Rausher, 1979). Since Lepidoptera provide no parental care to their offspring, natural selection would favor the utilization of a host plant that enhances the fitness of their offspring. If the female chose a host plant for her offspring that provided poor nutrition to the larvae and they were unable to grow adequately, they would have lower fitness.

One example within Lepidoptera is *Malacosoma americanum*, the eastern tent caterpillar, which is native to the eastern half of the United States and parts of southeastern Canada. Even among specialists, it is a relatively extreme insect herbivore.

The female moth oviposits her single egg mass exclusively on plants of the Rosaceaefamily, typically those of the genus *Prunus*. Larvae hatch in synchrony with the bud break of their host tree due to increased nutritional content of young leaves. An additional benefit comes from the ease of digesting these young leaves because they are more tender than the tough leaves found later in the season (Fitzgerald 1995). Although the species is highly specialized, late instar larvae will disperse from their natal tree after defoliation and may feed on more than 50 species outside ofRosaceae(Tietz 1972 in Fitzgerald 1995).

There are a few possible explanations for this observed oviposition preference. Since larval Lepidoptera use olfaction via maxillary palps to locate their food, there may be a missing stimulant in non-host species (Roessingh et al., 2007). Conversely, there could be a deterrent in the leaf, perhaps in the cuticle, that would cause the caterpillar to avoid such a plant. A physiological barrier might also exist if, after consumption, the larva is either harmed by toxic plant defenses or simply unable to absorb nutrients without a prerequisite digestive enzyme. Research completed at the University of Michigan Biological Station by Gannon et al. in 1993 has shown that, if larvae moved from their host trees to *Populustremuloides*(trembling aspen), *M. americanum* will eat and grow more effectively than on *Prunus serotina*(black cherry). Aside from this study, we are unaware of any research quantitatively evaluating *M. americanum's*abilityto consume, digest, and convert food to tissue, andtherefore grow on species other than those in the family Rosaceae.

This study will examine the relative consumption and growth of a specialist insect herbivore on its host plant compared to its performance on non-host species found in the surrounding area. In doing so, we will explore how important nutrition and growth are to

an insect herbivore specialist for determining its possible host plants.Therefore we ask: **1** On which tree species in the local environment, other than those in the Rosaceae family, can *M. americanum* feed? **2** How does the ability of *M. americanum* to perform on non-host species compare to its specialized host species, *P. serotina*?

Materials and Methods

Study Sites and Organisms

Caterpillars were collected from two sites along Riggsville Rd. in the area surrounding the University of Michigan Biological Station (UMBS) in Pellston, MI. Site 1 was located 1.4 miles northeast of the UMBS campus entrance while site 2 was located 3.8 miles southwest. Both locations contained *P. serotina* trees with*M. americanum*tents. These roadside clearings were flanked by deciduous forest habitats that were seemingly vacant of *M. americanum.* In using test caterpillars from more than one location we hoped to have test caterpillars from more than one population of *M. americanum*.

To determine the relative ability of *M. americanum* to consume, digest, convert food to tissue, and therefore grow on non-host species, larvae were fed *Acer saccharum*(sugar maple), *Acer pensylvanicum*(moose maple), *Fagusgrandifolia*(American beech), *Quercusrubra* (red oak),*Elaeagnusangustifolia*(Russian olive), and*Prunusserotina* (black cherry). We chose *A. saccharum, A. pensylvanicum, F. grandifolia,* and *Q. rubra*for their abundance within a short distance of populations of *M. americanum* throughout northern Michigan while *P. serotina* functioned as our control species. We included *E. angustifolia,* an invasive species,due to curiosity about the potential differences between native northern Michigan

tree species and thoseintroducedanthropogenically (TMWC). If *M. americanum* was to thrive on *E. angustifolia*, there could be the possibility of its use as a biological control agent against the invasive.

Collection and Standardization of Leaf Size

Leaves from *A. saccharum, A. pensylvanicum, F. grandifolia,* and *Q. rubra* were collected from trees found on UMBS property. *P. serotina*leaves were obtained from Site 2 and *E. angustifolia* were collected from the Little Traverse Conservancy Colonial Point Nature Preserve near Burt Lake. Forty leaves of each species were collected in moist plastic bags to prevent water loss, as it was important to maintain the most natural state possible. Ten leaves of similar size from each species were chosen for use in the feeding trial. The remaining leaves were kept on branches in the lab with the ends of the branches submerged in water to maintain hydration.

Collection and Standardization of Larvae

Five *P. serotina* trees containing tents were chosen from each site and 20 caterpillars of comparable size were removed from each tree. We used multiple tents in an attempt to ensure genetic variation within the sample as caterpillars from one tree most likely came from a small number of egg masses and therefore would be closely related. The caterpillars were then transported back to UMBS where they were separated by relative head capsule size. In an attempt to prevent the test caterpillars from molting during the feeding trial, we used individuals with a large head capsule relative to bodysize as they were more likely to be close to the beginning of an instar (Dyar, 1890 in Fitzgerald 1995). The caterpillars were then starved for three hours to clear their digestive

tracts. Finally, from each of the 10 test trees, the six larvae having the largest head capsules relative to body size and having most similar masses were selected for inclusion in feeding trials. The rest of the caterpillars were weighed, frozen, dried, and re-weighed to determine the wet to dry conversion factor for estimating initial dry weight of the test caterpillars. Ten caterpillars from each of the 10 tents obtained during our initial collection were first frozen for 24 hours at -45˚C to ensure the least painful death. They were then weighed, dried at 70˚C for 72 hours, and re-weighed. From this data we determined the average percent water of the caterpillars in each tent, from which we were then able to estimate initial dry weight of the test caterpillars.

Determining Tree Species Eaten by M. americanum

The 10 leaves per species set aside for the feeding trial were each placed in a separate Petri dish. Those from *P.serotina*and*E.angustifolia*were placed in 9cm Petri dishes, and the rest were placed in 20cm dishes to maintain proportionality between leaf size and dish size. Leaves were kept hydrated by filling a 1.5 mLmicrocentrifuge tube with water and placing the petiole into the water through a hole drilled in the cap. One caterpillar from each of the 10 test trees was placed in a Petri dish for each leaf species. The Petri dishes were then put into an environmental chamber set at a constant temperature of 25˚C and with 16 hours of light and 8 hours of dark. The feeding trial was run for 72 hours and the larvae were checked a minimum of four times a day (morning, afternoon, evening, and night) to ensure that the leaves were fully hydrated and the caterpillars had enough food. As necessary, additional leaves from the initial leaf collection were used to replace consumed leaves during the 72 hour period. Additional leaves were weighed prior to use in the feeding trial.

Determining Growth, Consumption, and Digestive and Conversion Efficiency

After the 72 hour feeding trial, the caterpillars were removed from the Petri dishes and placed in plastic cups. They were then starved for three hours in order to clear their digestive tracts. This was done to eliminate the possibility of undigested food being included in caterpillar growth. To determine actual growth we measured the dry weight of the caterpillars by first freezing them at -40˚C for 24 hours. Upon removal each one was weighed, placed in a 70˚C drying oven for 72 hours, and re-weighed to obtain the final dry weight.

From each feeding trial, all uneaten leaf material was weighed, dried in a 70˚C drying oven for 72 hours, and re-weighed to obtain their dry weights. Initial dry mass of leaves used in the feeding trials was estimated by first collecting ten leaves per species of equivalent size and mass as those leaves used in the feeding trial. These leaves were then dried in a drying oven as above and weighed to determine the mean water percentage for each species. This was used to estimate the dry weight of the trial leaves before the trial began. A different method was used for *Acer pensylvanicum* due to the very small amount of leaf eaten by each test caterpillar. Equivalent leaves were obtained and the portions of the leaves eatenduring the feeding trial were “traced” and cut out of the intact leaves. These small pieces of leaf blade were then dried as above and weighed to estimate the dry mass of *A. pensylvanicum* eaten.

In order to determine digestive efficiency of the caterpillars, it is necessary to knowthe amount of food excreted. For this reason, we collected the frass of all test caterpillars at the end of the feeding trial. Frass from each larva was dried at 70° C for 72 hours and weighed to obtain its dry weight.

To determine growth, consumption, digestion, and conversion we compared nutritional indices of the test caterpillars on each of the non-host species to those of the test caterpillars fed *P. serotina.*Nutritional indices calculated included relative growth rate (RGR: grams of tissue gained per gram of caterpillar per day), relative consumption rate (RCR: grams of leaf consumed per gram of caterpillar per day), approximate digestibility (AD: percent of the mass of leaves consumed that is digested), and efficiency of conversion of digested matter (ECD: ability of test caterpillar to convert digested food into tissue) (Waldbauer 1968 in Karowe 1989).

$$RGR = \frac{larval\ weight\ gain}{(average\ larval\ weight\ during\ trial) * (number\ of\ days)}$$

$$RCR = \frac{weight\ of\ food\ ingested}{(average\ larval\ weight\ during\ trial) * (number\ of\ days)}$$

$$AD = \frac{weight\ of\ food\ ingested - weight\ of\ frass}{weight\ of\ food\ ingested}$$

$$ECD = \frac{larval\ weight\ gained}{weight\ of\ food\ ingested - weight\ of\ frass}$$

Second Feeding Trial

A second feeding trial was conducted after a substantial number of the test caterpillars in our first trial molted and as a result, spent much of the trial not eating. For the second trial, we focused on the tree species consumed in at least moderate quantity by the test caterpillars during the first trial:*A. saccharum, F. grandifolia, Q. rubra* and *P. serotina* (the control). In order to double the sample size, caterpillars were collected from ten trees at each of the two sites. For all species except*P. serotina*, one caterpillar from each tree was fed each species. Two caterpillars from each tree were fed *P. serotina* in

attempt to ensure that we had a control from each tree with which to compare the performance of larvae fed non-hosts. To decrease the chance of the test caterpillars molting, instead of a 72 hour trial, we allowed the caterpillars to feed for 48 hours. We also reduced the drying time from 72 hours to 48 hours due to time constraints.

Statistical Analysis

Due to the high level of molting in our first feeding trial, that data was only used to qualitatively determine the lack of consumption of *M. americanum* when fed *A. pensylvanicum* and *E. angustifolia*. Data statistically analyzed was therefore only from the second feeding trial. Before beginning the statistical analysis, we reviewed the data for elements that could be inaccurate. The first variable considered was three test caterpillars for which negative consumption was calculated. All negative numbers were between -0.05 and 0, a small enough differenceto allow us to assume there was no consumption. This negative value is believed to come from a slightly inaccurate wet to dry conversion factor that only affected very small figures. Due to removal of consumption in our data set, RCR, AD, and ECD were also dismissed. Next, our data showed some growth rates that resulted in high percentages of total body weight being lost over the two-day trial. As this was thought to be an impossibly large percentage of body weight, we decided to remove all data from any test caterpillar with a percent body weight loss greater than 25%. For some other data points, a substantial difference was seen when wet and dry growth were compared. For situations where wet and dry growth did not share the same sign, alterations were made. We determined an alternate wet to dry conversion factor using the wet and dry weights measured at the end of the feeding trial for that specific caterpillar. This was used to replace our previously estimated conversion

factor. Finally, most likely due to the small values in data from some species and therefore the increased impact a small error in wet to dry conversions would cause, we received some impossible results for AD. As AD is a percent, any AD data not between 0 and 100 were eliminated from the data set. The largest removal was seen in *A. saccharumand F. grandifolia*as those were the species with the smallest consumption and therefore greater chance of error.

After those changes were made, the first step in our statistical analysis was to check for normalcy in our data. From this, we determined that our data was not normal and non-parametric tests were used for the entire analysis. We first ran a Kruskal-Wallis test to compare all species within each nutritional index. After finding that our data was significantly different in all cases, Mann-Whitney U tests were run to compare each species to the others in terms of all four nutritional indices separately.

Results

Experiment 1 Results

M. americanum did not consume *E. angustifolia* and had little to no consumption when given *A. pensylvanicum*. Larvaeconsumed*P. Serotina, F. grandifolia,* and *A. saccharum*. The molting of 50% of the larvae resultedin a decreased sample size. The data from Experiment 1 was unreliable due to the reduced sample size, so Experiment 2 was conducted with a larger sample size to reduce error when calculating nutritional indices.

Experiment 2 Results

*P. serotina*vs. *F. grandifolia*

M. americanum larvae had a higher relative growth rate (RGR) (MWU = 27.5, p<.0001), relative consumption rate (RCR) (MWU = 18.0, p<.0001), and efficiency of conversion ofdigested matter (ECD) (MWU=21.0, p=.007) when fed *P. serotina* compared to the RGR, RCR, and ECD on *F. grandifolia*. The growth rate for *F. grandifolia* was negative, though there was a positive mean consumption rate (*Figure 5).* To see all species related to one another regarding RGR and RCR, see *Figure 3* and *4.*Between the two species, there was no significant differencein approximate digestibility (AD) (MWU=21.0, p=.220). TheAD and the ECD gave unreliable results for *F. grandifolia* as explained in materials and methods.

P. serotina vs. A. saccharum

RGR (MWU=15.0, p<.0001) and RCR (MWU=35.0, p<.0001) both demonstrated significantly higher rates when *M. americanum* larvae were fed *P. serotina* as compared to those fed *A. saccharum*. Although nearly significant to suggest the opposite, there was no significant difference for AD (MWU=.000, p=.077) and ECD (MWU=.000, p=.077) in *P. serotina* as compared to *A. saccharum.* However, due to data removed as explained in the materials and methods, sample size was 1 for both AD and ECD in *A. saccharum* making the results unreliable.

*P. serotina*vs. *Q. rubra*

When fed *P. serotina* and *Q. rubra, M. americanum* did not yieldasignificantly different RGR(MWU=147.5, p=.254), RCR (MWU=170.0, p=.601), and ECD (MWU =

131.0, p = .823). Though the ECD for Q. rubra was approximately 3.3 times the ECD value of P. serotina with our sample size, there was no significant difference (*Figure 1)*. Also, there was no significant difference in AD between the species (MWU = 29.0, p < .0001), which can be seen in *Figure 2*.

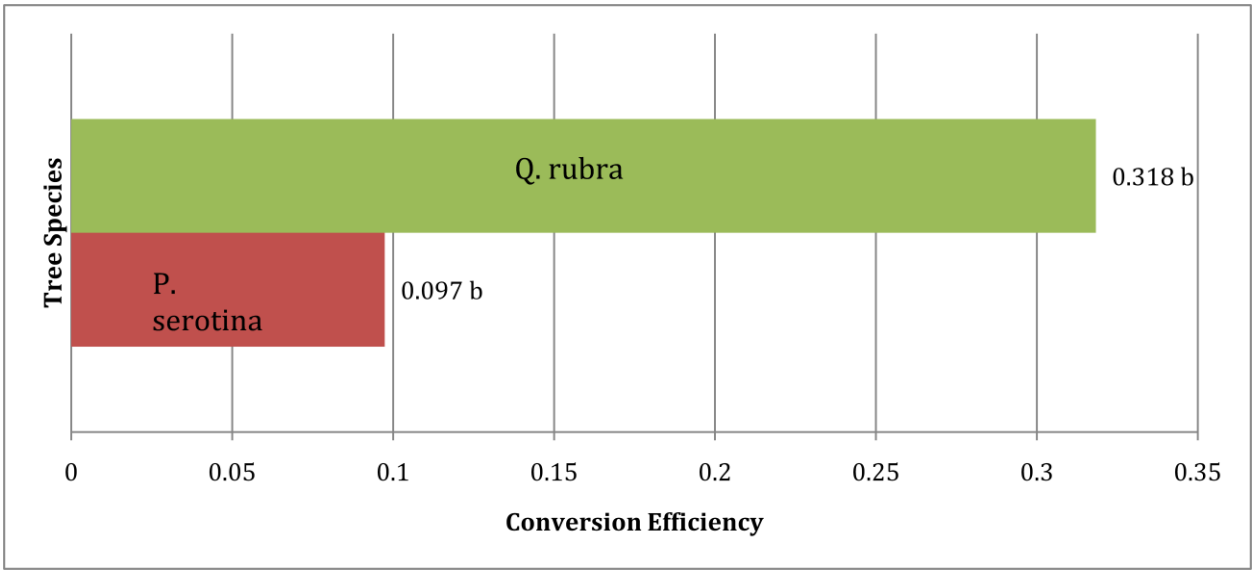

Figure 1. The efficiency of conversion of digested matter between *Q. rubra* and *P. serotina* suggested no significant difference.Within our sample, ECD was greater in *Q. rubra*. Means followed by the same letter are not significantly different.

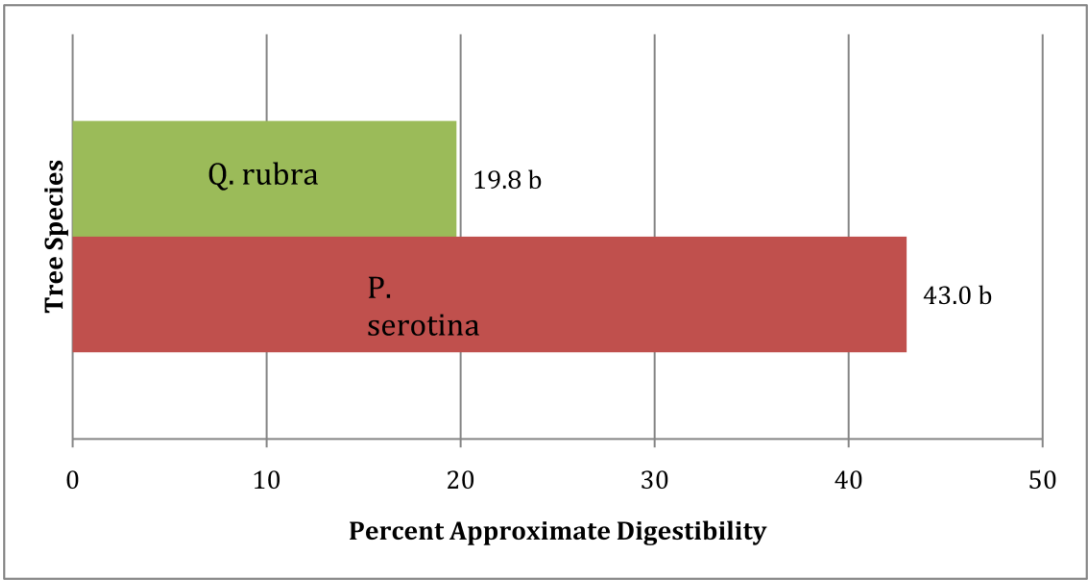

Figure 2. Approximate digestibility (AD) was greater in *P. serotina*compared to *Q. rubra* in our sample, although the data was not significantly different .Means followed by the same letter are not significantly different.

*Q. rubra*vs. *F. grandifolia*

Data showed that *F. grandifolia* had a significantly lower RGR (MWU=14.0, p<.0001), RCR (MWU=0.0, p<.0001), and ECD (MWU=1.0, p<.016) compared to *Q. rubra*. However, AD was not significantly different between the two (MWU = 12.0, p=.484).

*Q. rubra*vs. *A. saccharum*

Q. rubra had a significantly greater RGR (MWU= 6.0, p<.0001) and RCR (MWU=2.0, p<.0001) than *A. saccharum* when fed to *M. americanum*. The RGR for *A. saccharum* was negative even though it had a positive RCR. There was significant data when looking at AD (MWU=0, p=.111) and ECD (MWU=0, p=.111).

*F. grandifolia*vs. *A. saccharum*

For all nutritional indices, the feeding of *F. grandifolia* and *A. saccharum* to *M. americanum*did not yield a significantly different RGR (MWU = 58.0, p=.181), RCR (MWU=72.0, p=.537), AD (MWU=1.0, p=.655), and ECD (MWU=1.0, p=.655).Both species had similar, yet disadvantageous nutritional indices when comparing them to *P. serotina* and *Q. rubra*.

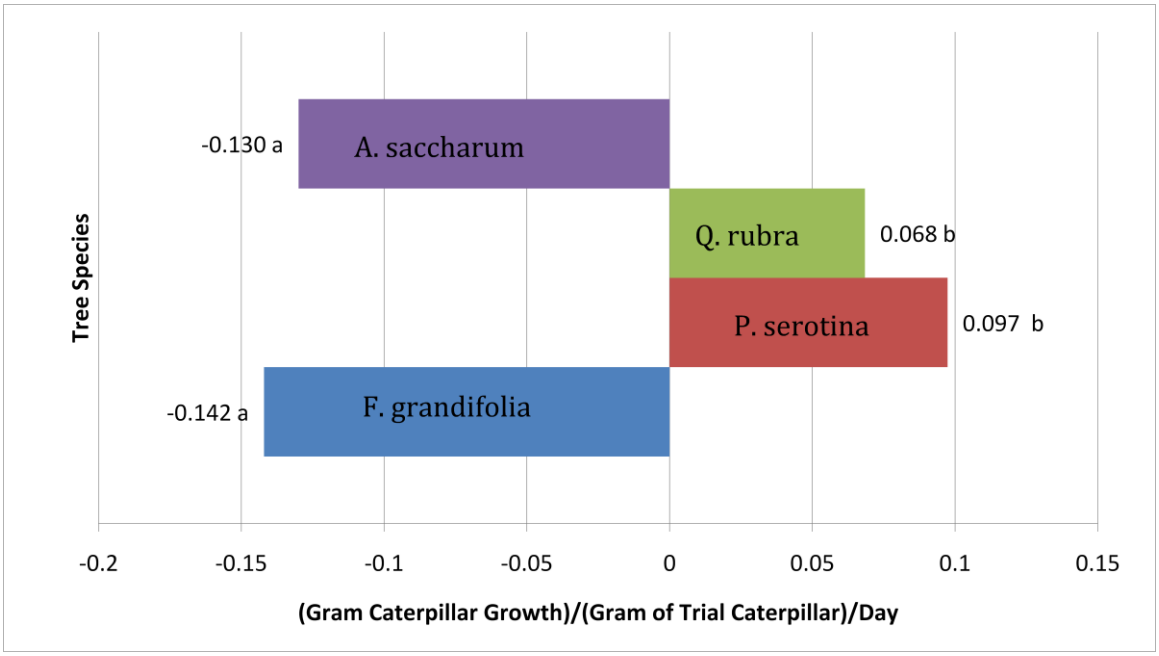

Figure 3. The highest growth rate means for *M. americanum* were *Q. rubra* and *P. serotina*, while *A. saccharum* and *F. grandifolia* had negative growth rate means *A. saccharum*and *F. grandifolia* had no significant difference in RGR, as well as *Q. rubra* and *P. serotina.*Means followed by the same letter are not significantly different.

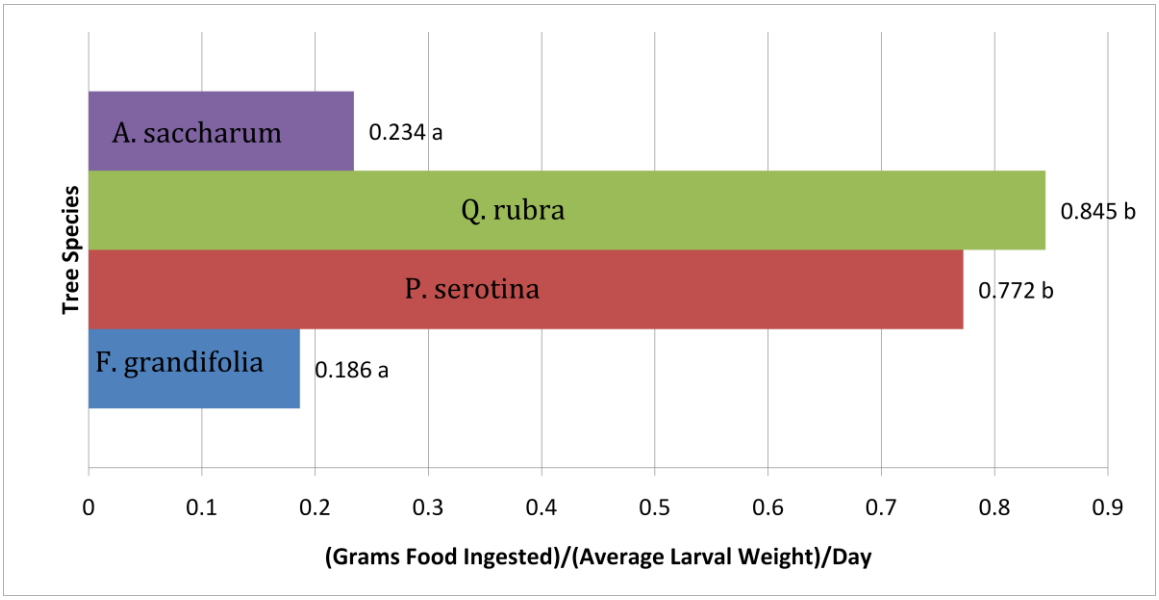

Figure 4.M. americanum had the highest consumption rate with *P. serotina* and *Q. rubra*, while *A. saccharum* and *F. grandifolia* were consumed less. *A. saccharum* and *F. grandifolia* had no significant difference in RGR, as well as *Q. rubra* and *P. serotina.*Means followed by the same letter are not significantly different.

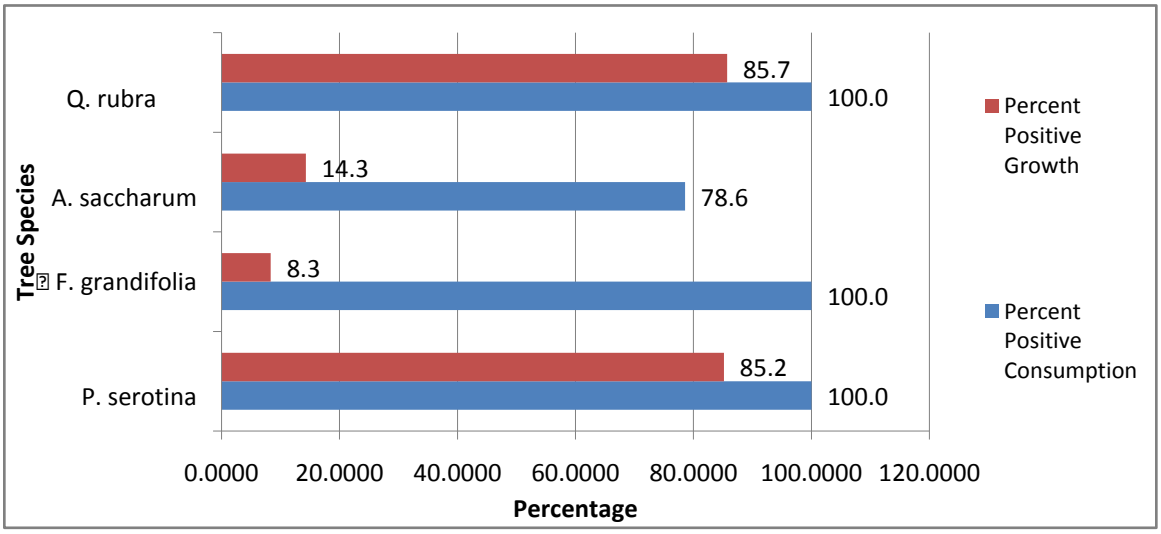

Figure 5. Though there is a high percentage for all species that had *M. americanum* larvae positively consuming, the growth of larvae, especially for *A. saccharum* and *F. grandifolia*, did not have a similar percentage of growth.

Discussion

Each of the five test tree species assayed within our experiments fell into one of three general categories. Caterpillars fed *E. angustifolia* and *A. pensylvanicum* exhibited little to no consumption or growth. While larvae offered *A. saccharum* and *F. grandifolia* did consume leaves of each species, their consumption and growth as measured by the nutritional indices were significantly lower than those of the larvae offered *P. serotina* leaves. Only *Q. rubra* demonstrated no significant difference to *P. serotina* in all but one nutritional index (AD). These data suggest that the *Q. rubra* could potentially serve as a valuable resource for *M. americanum*.

Seeing that *E. angustifolia* experienced no herbivory whatsoever from the larvae, we thought it probable the leaf lacked a stimulant. Within the order Lepidoptera numerous specialist herbivore species have been known to require a feeding or ovipositional stimulant of some nature (Spencer, 1996). Often times the stimulant takes the form of a chemical embedded within the waxy cuticle of the leaf. Caterpillars will sense this with their maxillary palps and feeding will ensue (Roessingh et al., 2007). It is likely, then, that the eastern tent caterpillar would have a similar strategy for determining adequacy of its food source. Another possible reason for no observed feeding on *E. angustifolia* concerns the fact that it is an invasive species. Since the tree is non-native to northern Michigan the larvae may not be accustomed to a novel compound or deterrent in the leaves. *M. americanum*would not have the necessary adaptations to bypass such a foreign compound because of the relatively short amount of time that the two have existed in the same environment (Leather, 1986). Unlike *E. angustifolia*, test caterpillars provided *A. pensylvanicum* did eat some of the leaf blade, though none ate more than

0.012 g dry weight. One rationale for this low-level performance is that *A. pensylvanicum* contains a chemical defense the larvae are unable to detoxify. Being a specialist on a different genus and family than the test species makes it even more likely that *M. americanum* would be unaccustomed to toxins found within the leaves of the *A. pensylvanicum*; thus, these compounds would have a much greater affect on the performance of this species (Ehrlich and Raven, 1964).

As mentioned above, the assays performed on *A. saccharum* and *F. grandifolia* showed larval performance that was significantly lower than for larvae fed *P. serotina*with respect to all four nutritional indices. Most surprisingly, while caterpillars given each of the two species consumed a moderate amount of the leaf mass offered to them, they actually lost weight. A hypothetical explanation for this result is that *A. saccharum* and *F. grandifolia* contain a time delayed toxin. Potentially, the caterpillars could have begun feeding on the leaves only to become fully affected by the compound minutes or hours later. It is also possible the weight decrease observed in these larvae could negatively impact later growth and development. Yet another factor that could have contributed to this trend is that nutrients within the leaf blade, such as nitrogen, might not have been accessible to *M. americanum*. This could be due to the way in which said nutrients are stored within the leaf. Finally, with *F. grandifolia*, hydration became a major issue during the feeding trials. Beech leaves tended to have shorter petioles compared to the other tree species studied and, as a result, were more prone to desiccation. Therefore, *M. americanum* may perform better on this species if water content of the leaves were kept more constant (Karban and Rackrefs, 1983).

Q. rubra provided the most promising data regarding potential for use as a food source of *M. americanum* larvae. This leads us to conclude that the presence of a detrimental toxin within *Q. rubra* leaves is highly unlikely. Additionally, we were not surprised by the larvae feeding on *Q. rubra* because research turned up numerous sources reporting M. americanum feeding and even nesting on unidentified oak species (Fitzgerald, 2005 and Shetlar, OSUE). Despite the fact the larvae ate *Q. rubra* leaves and faired just as well as those offered *P. serotina*, the low digestive efficiency observed in our experiments was unexpected considering the values obtained for the other nutritional indices. Perhaps this finding is attributable to the experimental design in which caterpillars were taken from *P. serotina* during mid to late instars and then exposed to the test species. Instead, the larvae could have been reared on the test species from the time they hatched. In such a scenario *M. americanum* could have gained a better ability to extract nutrients from its experimentally determined "host" species.

The determination that larval growth, consumption, and conversion in *Q. rubra* were similar to *P. serotina* led us to posit an important question: why are*M. americanum* females neglecting this seemingly valuable resource within their habitat? If larvae can perform as well on *Q. rubra* as on the host species one might initially assume that individual fitness would be increased by expanding the niche to include another host with comparable nutritional qualities. However, this is not necessarily the case. Consideration of the existence of local and global optima is one manner from which to approach this topic. Within the adaptive landscape for *M. americanum*, use of *P. serotina* could represent a local optimum while concurrent use of *Q. rubra* (possibly *P. tremuloides*) couldrepresent a global optimum. In this case, female *M. americanum* would not be

ovipositing on *Q. rubra* because the population would have to cross an adaptive valley (Wright, [1931] 1986 and Wright, [1932] 1986 in Skipper, 2004). Crossing an adaptive valley would likely require *M. americanum* to first adopt a more generalized ovipositional preference. However, due to the fact that the larvae perform well only on a few species, a more generalized ovipositional pattern would result in reduced fitness for those females ovipositing on trees unsuitable for larval growth and development. Since natural selection favors genotypes with highest fitness, stabilizing selection would act on the population removing alleles associated with indiscriminate ovipositional behavior. Despite the extreme limitations to the likelihood of the expansion of *M. americanum* onto *Q. rubra*, if it were to occur (and *Q. rubra* did indeed represent a global optimum) the species would benefit from a larger realized niche.

Although our results appear to demonstrate *Q. rubra* would be an important food source for eastern tent caterpillars, the possibility remains that some other factor influences ovipositional preference of female *M. americanum*; thus, explaining the exclusive use of *P. serotina* and other rosaceous plants. Another factor likely to shape ovipositional preference is predator avoidance. Perhaps *P. serotina* provides a larval habitat and food source burdened by fewer natural enemies of *M. americanum* larvae than *Q. rubra* would. Existence of the defense compounds within the leaves of *P. serotina* could also act as a basis for specialization within *M. americanum*. Cyanogenic glycosides are found within many species of the genus *Prunus*. Such chemical defenses are dangerous to animals because hydrolysis of the compounds produces cyanide, which can lead to poisoning in high enough amounts (Vetter, 1999). For that reason, it is probable that by feeding on *P. serotina*, larvae are able to indirectly utilize the plant defenses to

protect themselves against predation. While *M. americanum* is not known to sequester cyanogenic glycosides within its tissues, the compounds would still be present in its body while digesting the leaf blades. This also appears to be a likely theory considering the larvae regurgitate some of their internal fluid when threatened. Said fluid has been shown to contain HCN (Zagrobelny et al., 2008). One additional reason for the lack of *M. americanum* oviposition on *Q. rubra* is that earlier instars may not perform as well on the leaves of this test species and as a result produce smaller adults. In general, smaller adults produce fewer eggs, thus, potentially decreasing their fitness. For that reason, it would be much more beneficial for female *M. americanum* to oviposit solely on *P. serotina*.

Although our research provided us with both significant and interesting results, there is room for a great deal of further study. As has been mentioned multiple times, due to time and seasonal constraints, our experiment focused on the act of host-shifting. If further research were to be conducted, it would be of importance to rear the larvae from birth on non-host species. If it was not possible for a researcher to carry out future experimentation in this way, it could also be important to perform an experiment similar to ours that looks at each of the larval instars separately. These alterations in experimental design would ensure determination of the ability of M. *americanum*larvae in nature to carry out their entire life cycle on that non-host tree species. On a separate note, as much of our discussion focused on the presence/absence of stimulants and deterrents on the leaves of our various test species, further research in that area would be of importance. Due to significantly similar consumption in *Q. rubra* and *P. serotina* it appears likely there is a chemical similarity in stimulants present in leaves of the two tree species. Therefore, determining the chemical basis of any stimulants or deterrents present

in these test species or others would give expanded validity to the explanations of these compounds having such an effect on the specialization of *M. americanum.*

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