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Global Climate Change and Phenological Shifts

Abstract

The effect of global climate change on spring phenology was studied by examining the close interactions between eastern tent caterpillars (*Malacosoma americanum*) and black cherry trees (*Prunus serotina*). Eastern tent caterpillars are one example of insect herbivore specialists (herbivores that feed on 3 or fewer plant families) and will most likely be greatly affected by global climate change. We investigated how caterpillar growth and consumption could be affected if global climate change speeds up leaf budburst so that the caterpillars are forced to eat older, less nutritious leaves. We simulated asynchrony between caterpillar egg hatch and leaf budburst by stunting the caterpillars' growth and placing them in an environmental chamber at 15°C for varying lengths of time (six days, four days and two days). We compared the consumption and growth of all three trials to our control group, which was still in sync with the leaves. We discovered that although two days of asynchrony might not be enough to greatly affect the caterpillars' growth, four days of asynchrony did impact caterpillar growth. Six days of developmental delay did not significantly affect caterpillar growth. This could be attributed to varying abilities to acclimate to cooler temperatures. Additionally, six and four days of asynchrony did not create a significant difference in caterpillar consumption, however there was a difference with only two days of developmental delay. This may be a result of different levels of efficiency in digestion.

Introduction

Increased emissions of carbon dioxide and other greenhouse gasses from anthropogenic sources have caused Earth's climate to change (Malhi et al. 2002). Since the Industrial Revoltion, there has been a 31% increase in atmospheric carbon dioxide (Malhi et al. 2002). This increase has the potential to significantly modify ecosystem composition, especially in temperate and boreal regions (Malhi et al. 2002). Excess carbon leads to earlier spring phenology; it increases plant development and leads to earlier blooming in trees and other C_3 plants (Malhi et al. 2002, Visser 2006). Many interactions between trees and herbivores have therefore been affected by global climate change (Visser 2001).

Over the last decade, changes in spring phenology have been detected in various species from major taxa (Root et al. 2003). Root et al. (2003) indicated that 80% of species studied showed some form of a spring phenological shift, and on average, ontogenetic stages among all taxa occurred five days earlier than they did ten years ago (Root et al. 2003). The extent of phenological shifts varies among different species (Root et al. 2003).

About 15,000,000 species of insect herbivores have become specialized to develop on a specific plant family (Karowe, pers. comm.). Several interactions have developed to favor specialization and allow insect herbivore specialists to better feed on the leaves of their host. These interactions can include insect resistance to plant defenses, symbiosis in which plants protect insect herbivores, and synchrony between plant budburst and larval hatching. Generalist herbivores are not dependent on a single species of plant, and in the case that any interaction is disrupted, a generalist could simply shift to a different plant species. Specialists, therefore, will be most affected by global climate change because their reproduction and growth depends on the success of interactions with their host plants (Visser 2001). A Visser (2001) study reported that winter moth caterpillars in the Netherlands hatched three weeks before the budburst of their host oak trees due to the warmer spring temperatures. The newly hatched caterpillars only survived two to three days without food, suggesting that even a small phenological shift and disruption of synchrony could be fatal for caterpillars of all species (Visser 2001).

Similar to the winter moths and oak trees, eastern tent caterpillars (*Malacosoma americanum*) hatch in sync with the budding of their host plant, black cherry trees (*Prunus serotina*) (Fitzgerald 1995). This synchrony has evolved so that young caterpillars feed on young, nutrient-rich leaves. Mature black cherry tree leaves contain reduced levels of nitrogen, water, cyanide, and sugar compared to young leaves, and at the same time have more lignin, cellulose, and fiber (Schroeder 1986). The old leaves, therefore, have less nutritional value and are harder to digest than the young leaves. For that reason it is best for the eastern tent caterpillars to hatch and begin development in sync with the budburst of their host tree. Natural history has shown that this synchrony is indeed the pattern that many specialist insect herbivores follow. The optimal time period when leaves are young and nutritious has come to be called the “window of opportunity” (Root et al. 2001).

If climate change causes the black cherry trees to bloom earlier in the spring, the eastern tent caterpillars may be at a distinct disadvantage regarding growth and development.

Based on this information, we ask:

1) Does leaf age affect the growth of caterpillars?

2) Does leaf age affect the amount of consumption of caterpillars?

Materials and Methods

To determine whether the age of black cherry tree leaves affects the growth and consumption of eastern tent caterpillars, we simulated an earlier budburst and thereby disrupted the synchrony between the budburst of leaves and the primary feeding period of eastern tent caterpillars. This allowed us to investigate how less nutritious and tougher leaves might affect the growth and the consumption of caterpillars.

Our group simulated the earlier budburst of tree leaves by delaying the development of eastern tent caterpillars so that the tree leaves would continue to develop while the caterpillars’ development was temporarily delayed. This was accomplished by placing four groups of caterpillars in an environmental chamber (Precision Scientific 818) at a temperature of 15°C for six days, four days, two days, and two hours. The two hour group was the control because they were in sync with their leaves. Placing the caterpillars in the chamber eliminated time spent in the chamber as a confounding variable, but did not stunting growth. Forty-eight caterpillars from four different trees and two different test sites were collected for each of the three delayed groups. The control group had twenty-four caterpillars. By collecting caterpillars from various locations, we ensured that our sample was random. The two collection sites were located on the property of the University of Michigan Biological Station in Pellston, MI.

After each group was in the environmental chamber for their respective amount of time, each caterpillar was weighed and then a feeding trial of three days began. We used leaves of the same size and therefore instead of weighing each individual leaf, we calculated the initial weights of the leaves based on an average weight of a twenty leaf representative sample. During the feeding trial, half of the caterpillars from each of the three groups were fed old leaves, and half were fed young leaves. This was done to determine if the length of time spent in 15°C had any confounding effect on the caterpillars' growth and/or consumption. The caterpillars from each of the three groups that were fed the young leaves served as a control for their age group; feeding the young caterpillars young leaves reversed the asynchrony that we created. After the feeding trial, all 168 caterpillars and all of the leaves were weighed again to calculate the amount of growth and consumption of each caterpillar.

Day 1	Day 3	Day 5	Day 7	Day 10
Collect 48 caterpillars from 4 trees (12 each), and place in environ-mental chamber at a 15ºC.	Collect 48 caterpillars from 4 trees (12 each), and place in environ-mental chamber at a 15ºC.	Collect 48 caterpillars from 4 trees (12 each), and place in environ-mental chamber at 15ºC.	Collect 24 caterpillars from 4 trees (6 each), and 3 for each caterpillar. Place in environmental chamber at 15ºC for two hours. Remove all caterpillars from environmental chamber. Weigh each individual and place in environmental chamber at 25ºC (night) /30ºC (day); begin feeding with collected leaves.	Remove all caterpillars from environmental chamber. Weigh each caterpillar and leaf material remaining.

Figure 1

Growth of each caterpillar was measured by subtracting its initial fresh weight from its weight after the feeding trial and then calculating weight gain as a percentage of initial weight. Similarly, consumption of each caterpillar was found by taking the difference between the fresh weights of the leaves before and after the trial. We then found a relative consumption for each caterpillar by dividing the difference in leaf weights by the initial weight of the caterpillar.

Statistical Tests

After measuring the final weights of all of the caterpillars and leaves from each of the four test groups, we compiled all of the data and checked for normality. The data were normal, and we used ANOVA tests and independent T-tests to compare means among the groups. Our most important comparisons were between the three asynchronous groups and the control group for weight gained and amount of leaves eaten. This was analyzed with an ANOVA test. We also compared each group individually with the control group for both weight gained and amount of consumption. This was done by using an independent sample T-Test. Additionally, we compared each subgroup (asynchronous and synchronous) within each group to determine if we had a confounding variable. This was also done by using an independent sample T-test.

Results

After verifying the normality of our data, we analyzed our data by using both independent sample T-tests and ANOVA tests.

<u>Testing the growth of the caterpillars:</u>

We compared all three groups to the control in order to determine if there was a difference between the caterpillars' growth. We determined that there was a significant difference between the growth of the four-day delayed group and the control group. There was also a nearly significant difference between all three groups (six-day delay, four-day delay, and two-day delay) and the control group. To see all results from each comparison, refer to table 1. To see mean and variance values for each group, refer to figure 2.

Group A	Group B	Group C	Group D	F	P-value
6-day delay	4-day delay	2-day delay	Control	2.438	0.077
6-day delay	Control	-	-	3.345	0.487
4-day delay	Control	-	-	5.323	0.029*
2-day delay	Control	-	-	0.246	0.630

Table 1

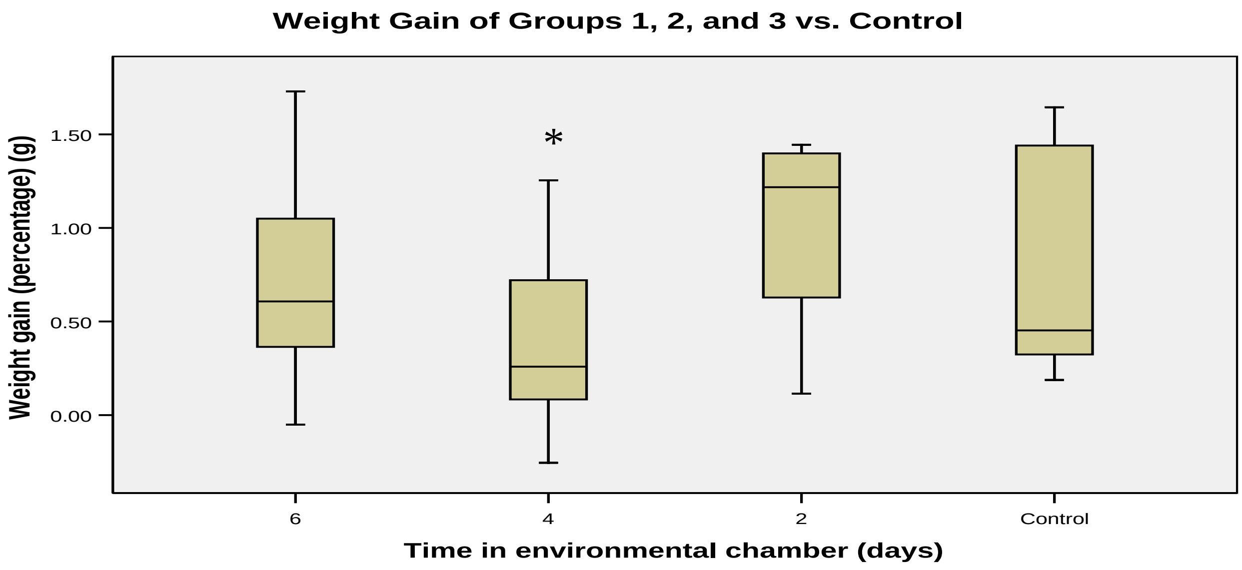

Figure 2

Testing the consumption of the caterpillars:

We compared all three groups to the control in order to determine if there were differences in the caterpillars' consumptions. We determined that there was a significant difference between the three groups (six-day delay, four-day delay, and two-day delay) and the control group. To see which trials contributed most to this difference, we compared each trial individually to the control and found that there was also a significant difference between the group delayed two days and the control group. To see all results from each comparison, refer to table 2. To see mean and variance values for each group, refer to figure 3.

Group A	Group B	Group C	Group D	F	P-value
6-day delay	4-day delay	2-day delay	Control	7.317	0.0001*
6-day delay	Control	-	-	1.211	0.366
4-day delay	Control	-	-	3.743	0.931
2-day delay	Control	-	-	22.620	0.010*

Table 2

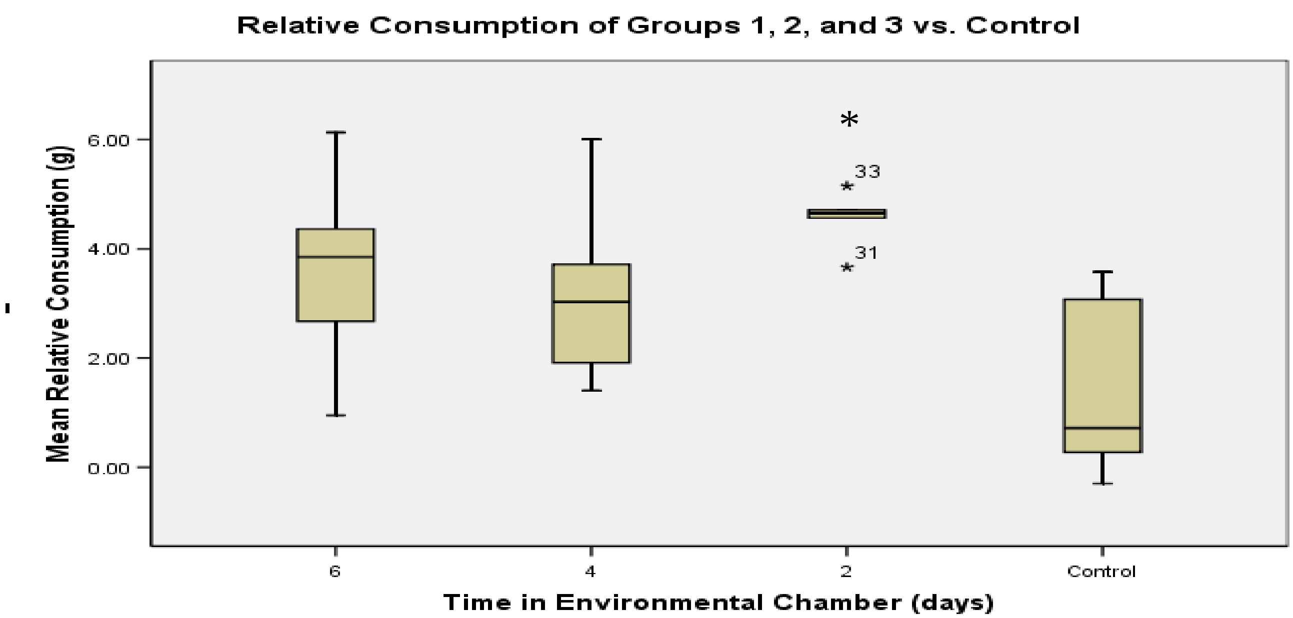

Figure 3

Testing the Confounding Variable:

The possible confounding variable was amount of time spent in the environmental chamber at a temperature of 15°C. We compared each subgroup (asynchronous and synchronous) within each trial in order to determine if there was a confounding variable. We found that there was no significant difference between any of the subgroups of the three groups for neither growth nor consumption. This means that amount of time spent in the environmental chamber at 15°C may be a confounding variable. If it were not, we would expect the asynchronous groups to grow less and differ in consumption from the synchronous groups. To see all results from each comparison, refer to table 3. To see mean values for each group, refer to figures 4 and 5.

Growth/Consumption	Asynchrony	Synchrony	F	P-value
Growth	6-day delay	6-day delay	0.487	0.417
Growth	4-day delay	4-day delay	6.396	0.138
Growth	2-day delay	2-day delay	0.094	0.800
Consumption	6-day delay	6-day delay	0.325	0.386
Consumption	4-day delay	4-day delay	0.818	0.816
Consumption	2-day delay	2-day delay	3.354	0.105

Table 3

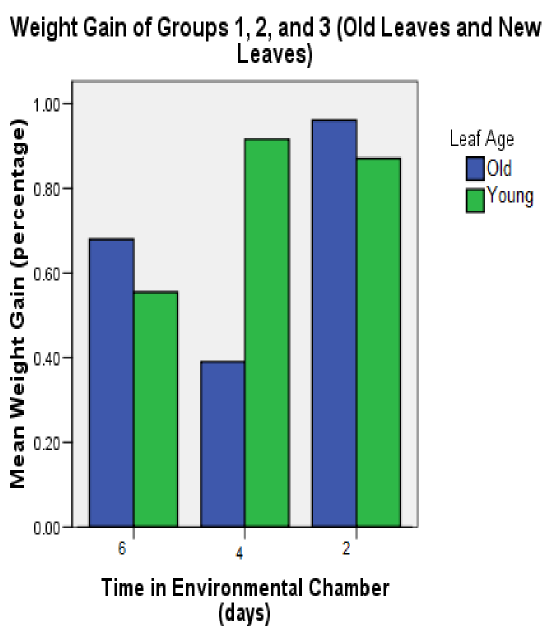

Figure 4

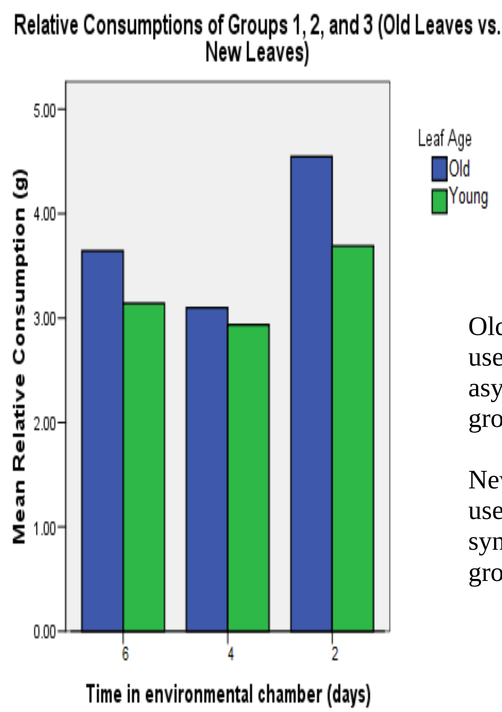

Figure 5

Old leaves were used for the asynchronous groups.

New leaves were used for the synchronous groups.

Discussion

Growth

Our first comparison was between the growth of all three asynchronous groups and the control group. Our results show that there was a nearly significant difference between these four groups. After performing an ANOVA test, we made a graph of the four groups' growth means and variances. Our results suggest a growth pattern contrary to that which we had hypothesized; instead of being linear with the caterpillars most out of sync (six-day delay) growing the least, our data showed an oscillating pattern of growth means.

Only the group that was in the environmental chamber for four days was significantly different from the control group in terms of growth. This can most likely be attributed to differences in the caterpillars' abilities to acclimate to cooler temperatures in the short-term. Group 1, which was the youngest group to be put in the environmental chamber, most likely had a greater ability to respond to environmental stresses such as cooler temperatures. Group 2, which was developmentally two days older than Group 1, may not have had the ability to acclimate as well to the imposed temperature stress. This trait has most likely been selected for; caterpillars that are unable to tolerate temperature stress would not be able to survive as well as those that could. There may be no significant difference between Group 3 and the control group because two days might not create enough asynchrony to cause a change in the caterpillars' growth.

Consumption

To determine differences in the leaf consumption of our three groups, we compared all three test groups to the control group, and there was an overall difference in the amount of leaf consumption relative to initial body weight of the three asynchronous groups versus the control. Similar to our predictions of caterpillar growth, we had hypothesized that the caterpillars that were most asynchronous with the leaves would eat the least. However, there was an oscillating pattern of consumption. The group that was six days out of sync actually ate more than the group that was four days out of sync. Once again, this may be due to younger caterpillars' ability to tolerate temperature stress. Additionally, the group that was two days out of sync ate the most and was the only group that was significantly different than the control.

All groups that were out of sync ate more than the control. This pattern may be a result of the young caterpillars need to eat more of the old leaves in order to obtain the required amount of nutrients; as leaves age, they decrease substantially in nutritional value. Also, as caterpillars age, physiological changes allow them to become more efficient at processing food for new tissue. Therefore, the in sync caterpillars may not need to consume as many nutrients in order to grow.

Confounding Variable

After comparing asynchronous caterpillars to synchronous caterpillars in each of the three trials, we concluded that amount of time spent in the environmental chamber may be a confounding variable for both growth and consumption. Because there was no significant difference between the growth or consumption of the asynchronous and synchronous groups, we cannot conclude that the results were due to the amount of asynchrony between the caterpillars and the leaves. However, we also cannot not conclude that our results are due to this confounding variable.

Noisy Variables

While analyzing our data, we encountered many noisy variables that may have affected our results. For instance, many of the caterpillars molted during the feeding trial and were not included in any of our statistical tests. Although each trial started with 48 caterpillars from four different trees at two different sites, eliminating the caterpillars that molted created variation in the ratios of caterpillars from the various sites and trees. In one of our treatments, for example, one entire tree was eliminated because all six caterpillars from that tree molted. Some groups contained up to 24 caterpillars, but others were consisted of as few as five. Such a small sample size could have greatly influenced our results.

Another noisy variable caused by molting is that some caterpillars might have been preparing to molt toward the end of the feeding trial. This means that they could have stopped eating for up to 12 hours prior to the end of the feeding trial. If a disproportionately large amount of caterpillars from one group were preparing to molt, it may have affected the overall growth or consumption of that group.

Initial caterpillar age may have also been a noisy variable. Our intentions were to collect caterpillars that were two days apart in age. However, we had no way of determining the exact age of every caterpillar when we did our collection. This may have thrown off our results because without knowing the exact age of each caterpillar, we cannot ensure that the trials were equally asynchronous. Also, if the caterpillars were of different ages, then they would have eaten and grown different amounts because, in general, caterpillars in later instars grow and eat more (Fitzgerald 1995). Similarly, we could not determine the exact age of the leaves when we collected them, and as a result, we could only assume that the "old leaves" were older than the caterpillars that were eating them, and the "young leaves" were younger than the caterpillars that were eating them.

We were also unable to determine which stage of an instar each caterpillar was in upon collection. During each instar, caterpillars vary in rates of both growth and consumption. Growth and consumption are highest toward the beginning of the instar and level off as the caterpillar prepares to molt (Fitzgerald 1995). Therefore, if there was a large range of relative instar stages, both overall growth and consumption could have been affected.

Finally, although we starved the caterpillars for three hours prior to measuring both initial and final weights, the contents of their guts may not have been completely emptied. The remaining material in each caterpillar's gut could have had an impact on both initial and final weights. This could have affected the data because it is possible that the caterpillars were more full one of the two times that they were weighed. It is also likely that each caterpillar was not equally full, thus making the data uneven.

Conclusion

Although our results were not what we predicted, if our experiment were modified and repeated, there could be more conclusive results. For instance, if caterpillar and leaf age were more definitively known from the beginning, the asynchrony between trials would be more exact. Also, we believe that a two day feeding trial would be better than three days for this experiment because the amount of caterpillars molted would be significantly decreased and the sample size would therefore be larger.

We were able to conclude that if the global climate were to change enough to speed up the budburst of black cherry tree leaves by four days, there could be an adverse effect on the growth and consumption of the caterpillars. This is ecologically significant because without nutritious leaves, eastern tent caterpillars could eventually be wiped out of the ecosystem which could greatly affect the ecosystem as a whole. Our results could be indicative of the effects that climate change may have on species interactions globally.

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