

Effect of Cherry Tree Infection with Black Knot Fungus on
Growth of the Specialist Eastern Tent Caterpillar, *Malacosoma
americanum*

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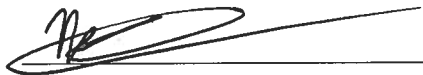
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Abstract *(no need to fill this in; I'll do it before giving the form to Adam)*

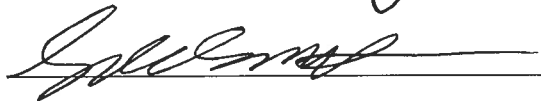
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**EFFECT OF CHERRY TREE INFECTION WITH BLACK KNOT
FUNGUS ON GROWTH OF THE SPECIALIST EASTERN TENT
CATERPILLAR, *MALACOSOMA AMERICANUM***

**Jessica Yan
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Abstract

Plants have both mechanical and chemical defenses, and their chemical defenses can be effective against both herbivores and pathogens. Plants have developed a complex defense-signaling system, which consists of different signaling pathways that interact with each other to fine-tune the plant's response to one or more attackers. These pathways include the jasmonate acid (JA) response, which protects against both herbivores and pathogens, and the salicylic acid (SA) response, which primarily protects against pathogens. These two signaling pathways can interact via cross-talk, and while each pathway typically produces an effective defense response to the corresponding attacker, it is not clear whether cross-talk between pathways enhances or hinders effective response to simultaneous attack by herbivores and pathogens. We measured the growth of the eastern tent caterpillar (*Malacosoma americanum*) fed leaves from black cherry trees (*Prunus serotina*) that were either infected or not infected with black knot fungus (*Dibotryon morbosum*). Caterpillars feeding on leaves from an infected tree grew significantly less due to lower digestive efficiency and conversion efficiency than caterpillars feeding on leaves from an uninfected tree. We conclude that the defense response of black cherry trees to black knot fungus increases defense against herbivory by reducing the ability of *M. americanum* to digest foliage and convert digested material into caterpillar biomass. Therefore, it seems likely that natural selection should favor females who lay their egg masses on uninfected trees.

Introduction

Plants are the foundation of all terrestrial communities and must cope with abiotic stressors, such as light and heat, and biotic stressors, such as herbivores and pathogens. These stressors can vary in intensity and importance to the plant over space and time. In response to these threats, plants have evolved a variety of defense mechanisms. Plant defenses negatively affect the attacker's physiology or behavior, either through mechanical or chemical methods. Both types of defenses are effective against insect herbivores. Attackers can be physically hurt or killed by mechanical defenses, or they can be deterred or killed by chemical defenses, which are also known as secondary compounds (Bennett and Wallsgrave 1994). Against most phytopathogens (parasites that cause disease), plants must rely on chemical defenses because mechanical defenses often operate on too large a scale to effectively protect the plant.

There are two main types of chemical defenses: carbon-based defenses and nitrogen-

containing defenses. A classic example of a carbon-based defense is tannins. They are among the most prevalent secondary metabolites found in plants and are particularly interesting because they can affect large mammals as well as insects in noticeably different ways (Barbehenn & Constabel 2011). Tannins are able to defend against insects through deterrence and/or toxicity, and against larger vertebrates by decreasing protein digestion (Barbehenn & Constabel 2011).

Plants have an arsenal of defense mechanisms in response to microbes. The general mechanism involves plants sensing pathogen-associated molecular patterns (PAMPs), which triggers a response (Thomma et al. 2011, Li et al. 2017). This response activates various genes, some of which typically add reinforcement to the plant's cell wall or degrade the cell walls of invading pathogens (Baron & Zambryski 1995). A closer look at plant response to herbivores and pathogens reveals that the signaling for both follow similar pathways; these responses overlap in function to defend against future attacks more effectively.

In natural conditions, plants can be attacked by multiple kinds of pathogens and insects. When plants are attacked by either organism, signaling pathways are activated to set up defensive responses against that specific attacker (Koornneef & Pieterse 2008). Since plants frequently encounter multiple stressors simultaneously, there are some defense-signaling responses that are known to protect plants. Two of the responses are the jasmonic acid (JA) response and the salicylic acid (SA) response (Thaler et al. 2004). JA protects against herbivorous insects, such as chewers, piercers/suckers, and cell-content feeders, and pathogenic infection from fungi and bacteria through a different pathway (Walling 2000, Pieterse & Loon 1999); SA, on the other hand, primarily protects against pathogens (Thaler et al. 2004). Cross-talk, or the interaction between signaling pathways, allows a plant to regulate and adapt to attackers (Bostock 2005, Koornneef & Pieterse 2008). By using cross-talk, the plant can control its defenses with more flexibility, even when being attacked by simultaneous or subsequent aggressors. However, cross-talk is not always constructive. For example, in *Arabidopsis* (*Arabidopsis thaliana*), SA was triggered when the plant was infected by the pathogen *Pseudomonas syringae*, which made the infected tissue vulnerable to a different pathogen *Alternaria brassicicola* because the SA defenses were suppressing the JA pathway (Bell 1993, Spoel et al. 2007).

Black cherry trees (*Prunus serotina*) are commonly attacked by both insect herbivores and fungal pathogens. *P. serotina* contains cyanogenic compounds, and the major insect

herbivore of black cherry is the eastern tent caterpillar (*Malacosoma americanum*) (Fitzgerald 1995). *M. americanum* emerge in the spring from egg masses that were deposited during the previous summer (Fitzgerald & Willer 1983). The larvae construct large silk tents (shown in Figure 1) after hatching and begin to feed; while the caterpillars mainly feed on black cherry tree leaves, they can feed on other nearby trees if they defoliate their original tree (Futuyma & Wasserman 1981). These communal tents may hold 50 to 300 or more caterpillars while they go through their five to seven week period of development (Fitzgerald & Willer 1983).

Dibotryon morbosum is a common fungus that causes black knot disease, as seen in Figure 1. The pathogen infects trees during the spring and overwinters in the infected branches, and it is a widespread and destructive disease in North America (Zhang et al. 2005, Koca 2006). The fungus produces indole acetic acid, which causes overgrowth of affected tissue in plants, particularly in plum and cherry trees (Crady and Wolf 1959). The overgrowth consists of a rough, greenish/blackish gall on the twig or branch which develops during the winter (Zhang et al. 2005).



Figure 1: Images of eastern tent caterpillar tent and black knot fungus on black cherry

This paper investigates the effect of a phytopathogen on a herbivore through plant defenses, specifically the effect of *D. morbosum* on *M. americanum* feeding on *P. serotina*. The goal is to understand the effects on the biological response of growth from simultaneous attacks by two different biological stressors and how the presence of one attacker may influence the success of another, though the two never directly interact.

Therefore, in this study we ask:

1. Does black knot fungus have any effect on growth of the eastern tent caterpillar?

2. If so, is the effect due to altered consumption rate, digestive efficiency, and/or conversion efficiency?
3. Do the effects of black knot fungus infection differ between young and old caterpillars?

Materials and Methods

Caterpillar sampling

We collected five *M. americanum* tents from different uninfected black cherry trees in a field surrounding Popal Road, located just off Robinson Road east of Pellston, Michigan. We sampled five tents in order to represent, to some extent, the genetic diversity of the caterpillar population. We chose tents only from trees that appeared to be uninfected with black knot fungus because we did not want any caterpillars to have previous experience feeding on leaves from infected trees. Twelve caterpillars were removed from each tent, weighed, and paired by weight. Within each pair, we assigned one caterpillar to eat leaves from uninfected trees and the other to eat leaves from trees showing symptoms of black knot disease.

Leaf sampling

In the same field, we identified four uninfected trees and four trees infected with black knot fungus, none of which contained tents or evidence of prior herbivory. Uninfected trees were defined as having no visible signs of black knot fungus, and both trees types had no other noticeable anomalies. Twice weekly, we cut branches from these eight trees using a large pair of pruning shears and brought them back to the lab; there, we removed leaves using a razor blade to minimize chemical defense induction due to mechanical damage. These leaves were weighed prior to feeding. In order to estimate leaf dry weight, we weighed approximately 10 grams of similar sized leaves, dried them in a drying oven at 60° C for at least 48 hours, then reweighed them. Their wet to dry weight ratio (about 40%) was used to estimate the initial dry weight of leaves in the feeding trials.

Feeding trials

A total of 60 caterpillars started in our feeding trials. Prior to weighing the caterpillars at the beginning of the trials, we starved them for one to two hours to clear their guts of frass, since frass is not a part of the body weight included in the biomass. We placed each caterpillar in an

individual 11 cm petri dish and added 0.3 to 1.0 g wet weight of leaves from either an infected or uninfected tree (one caterpillar from a weight-matched pair on each). We lined the bottoms of the petri dishes with filter paper, which we kept moist with deionized water.

For two weeks, we kept the petri dishes containing caterpillars and leaves in an environmental chamber which was set to mimic natural conditions (16 hours of light at about 22°C, eight hours of dark at about 18°C). Daily, we checked that each caterpillar still had adequate food and that the filter paper was sufficiently moist, adding leaves and water as needed. Twice weekly (every third or fourth day), we transferred the caterpillars into new petri dishes with fresh leaves and filter paper to minimize potential bacterial and fungal growth. During these transfers, we collected frass, molt skins, and uneaten leaf material, which we dried and weighed. We weighed the caterpillars a week after feeding began (the halfway point) and we weighed them again as they began to pupate. Upon first sign of pupation, we froze the caterpillars to euthanize them before drying. After drying for at least 48 hours, we weighed the caterpillars. We estimated caterpillar dry weight at the start and midpoint of the feeding trial by multiplying their corresponding wet weights by 20%. These weights are our primary growth metric.

In order to determine the success of the herbivores we will employ mass balance equations, specifically those used by G. P. Waldbauer (1968). These equations will be used to find any potential differences in the caterpillars' relative consumption rate, digestion efficiency, conversion efficiency, and relative growth rate. Using the estimated dry weights of caterpillars at the start and end of the feeding trial, estimated dry weights of leaves provided during the feeding trial, dry weights of leaves not consumed by the caterpillars during the feeding trial, dry weights of the caterpillars' frass, and duration of the feeding trial, we calculated relative growth rate (RGR), relative consumption rate (RCR), digestive efficiency (DE), and conversion efficiency (CE) (Waldbauer 1968), defined as:

$$RGR = RCR * DE * CE$$

$$RCR = \frac{\text{initial leaf weight} - \text{final leaf weight}}{\text{time}}$$

$$DE = \frac{(\text{initial leaf weight} - \text{final leaf weight}) - \text{frass weight}}{\text{initial leaf weight} - \text{final leaf weight}} * 100$$

$$CE = \frac{\text{weight gained}}{\text{initial leaf weight} - \text{final leaf weight}} * 100$$

Effect of black knot fungus on caterpillar growth

We looked at the differences in ingestion, egestion, and growth through their proxies of leaves consumed, frass collected, and differences in weights of leaves before and after consumption. With all of these aspects of the growth equation known to a degree, comparison can be made for all metrics between the treatments using paired t-tests. To evaluate whether black knot fungus affects growth of *M. americanum*, we conducted a paired t-test on caterpillars' RGR. We also assessed RCR, DE, and CE using paired t-tests for the uninfected and infected trials to see if differences in growth were due to any or all of these growth metrics. In these paired t-tests, a pair consisted of the two caterpillars size-matched at the start of the feeding trials. To meet our assumption of normality, we tested the normality of differences in the growth metrics between infected and uninfected feeding trials.

Susceptibility of young and old caterpillars to effects of infection

To evaluate whether infection of black cherry trees affects young and old caterpillars differently, we conducted a linear regression to evaluate the relationship between initial weight and the difference in growth of infected and uninfected trials.

Results

In our analysis, we used a total of 20 pairs of caterpillars. Over the duration of the experiment, a total of 18 caterpillars died, half of which came from the uninfected tree trials and the other half from the infected tree trials. The most shocking deaths were all from a single tent, where all 12 caterpillars perished. These caterpillars had been parasitoidized by wasps, which began to appear in the petri dishes about 7-10 days after the caterpillars' deaths. The remaining six caterpillars died of unknown causes. Of these, there were two pairs (four caterpillars) and two individuals. Since we used paired t-tests, we removed any pair where one caterpillar died, so our analyses were based on 20 pairs of caterpillars.

The paired t-tests we conducted were split into two time frames: initial to middle (first week) and initial to final (first and second week). We had no way of standardizing pupation stop time, which all occurred during the second week. This was due to our class schedule and pupation that occurred overnight. To put it in perspective, 11 of our 40 caterpillars were nearly

complete with their cocoons before being noted.

Initial → Middle

Effect of black knot fungus on caterpillar growth

The differences in all growth metrics between paired caterpillars fed uninfected vs. infected leaves during the first week of feeding trials were normally distributed (Shapiro-Wilk $p=0.12, 0.13, 0.13, 0.49,$ and 0.55 for weight gain, relative consumption rate, digestive efficiency, conversion efficiency, and relative growth rate, respectively). Therefore, paired t-tests were conducted.

M. americanum caterpillars did not consume leaves from uninfected vs. infected trees at a significantly different rate (RCR paired $t = 1.24, d.f. = 19, p = 0.23$; Table 1). However, caterpillars digested leaves from uninfected trees significantly more efficiently (DE paired $t = 6.91, d.f. = 19, p < 0.001$; Table 1). Additionally, caterpillars fed leaves from uninfected trees were significantly more efficient at converting absorbed food into biomass (CE paired $t = 2.22, d.f. = 19, p = 0.04$; Table 1). Since relative growth rate is the product of these three metrics, this resulted in caterpillars fed leaves from uninfected trees to grow at a significantly greater rate than caterpillars fed leaves from infected trees (RGR paired $t = 7.079, d.f. = 19, p < 0.001$; Table 1).

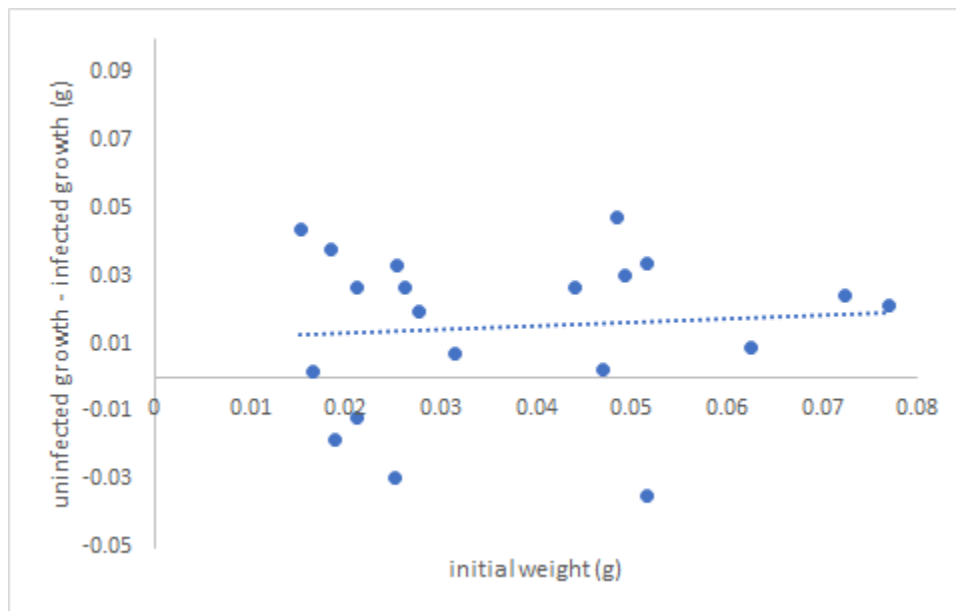
| Growth Metric | Growth Metric Mean for Uninfected | Growth Metric Mean for Infected | p-value (2-tailed) |
|-------------------------------------|-----------------------------------|---------------------------------|--------------------|
| Weight Gained (g) | 0.117 ± 0.022 | 0.104 ± 0.024 | 0.011 |
| Relative Consumption Rate (g/g/day) | 0.00037 ± 0.0001 | 0.00035 ± 0.0001 | 0.231 |
| Digestive Efficiency (%) | 79.6 ± 8.38 | 64.6 ± 8.00 | < 0.001 |
| Conversion Efficiency (%) | 8.28 ± 1.51 | 7.63 ± 1.49 | 0.039 |
| Relative Growth Rate (g/g/day) | 0.000024 ± 0.000005 | 0.000017 ± 0.000003 | < 0.001 |

Table 1: Paired sample t-tests comparing various growth metrics between caterpillars fed leaves from uninfected or infected trees. Initial → Middle refers to the time period of feeding trials to which these results pertain; this data was collected during the first week of trials

before pupation began.

Susceptibility of young and old caterpillars to effects of infection

Our linear regression analysis of whether there was a relationship between magnitude of effect from consuming leaves from uninfected vs. infected trees and the initial weight of the caterpillars revealed that there was very little to no correlation between these two factors ($R^2 = 0.007$, $p = 0.719$; Graph 1). Thus, we did not find a strong relationship between caterpillar age and effect of fungal infection on food source. For the purposes of this analysis, the initial pair weight, which we assumed to be indicative of caterpillar age, was found by averaging the weight of caterpillars within pairs.



Graph 1: Linear regression analysis on the difference in weight gained within pairs during the first week of feeding trials vs. initial average weight of pair.

Initial → Final

Effect of black knot fungus on caterpillar growth

The differences in all growth metrics between paired caterpillars fed uninfected vs. infected leaves during the first week of feeding trials were normally distributed (Shapiro-Wilk $p=0.15$, 0.23 , 0.17 , and 0.50 for weight gain, relative consumption rate, digestive efficiency, and relative growth rate, respectively), with the exception of conversion efficiency (Shapiro-Wilk $p = 0.008$). Therefore, paired t-tests were valid for all except conversion efficiency, for which we used a Related-Samples Wilcoxon Signed Rank Test.

Caterpillars did not consume leaves from uninfected vs. infected trees at a significantly different rate (RCR paired $t = 1.06$, d.f. = 19, $p = 0.31$; Table 3), nor did one group more efficiently convert food absorbed into biomass more than another (CE Wilcoxon d.f. = 19, $p = 0.37$; Table 4). Conversely, caterpillars digested leaves from infected trees significantly more efficiently (DE paired $t = -18.573$, d.f. = 19, $p < 0.001$; Table 3), resulting in them growing significantly more than those fed leaves from uninfected trees (RGR paired $t = -18.573$, d.f. = 19, $p < 0.001$; Table 3). However, the difference in total growth was not significant (Weight Gained paired $t = 0.16$, d.f. = 19, $p = 0.88$; Table 3).

| Growth Metric | Growth Metric Mean for Uninfected | Growth Metric Mean for Infected | p-value (2-tailed) |
|-------------------------------------|-----------------------------------|---------------------------------|--------------------|
| Weight Gained (g) | 0.201 \pm 0.064 | 0.198 \pm 0.064 | 0.875 |
| Relative Consumption Rate (g/g/day) | 0.00022 \pm 0.00004 | 0.00021 \pm 0.00004 | 0.305 |
| Digestive Efficiency (%) | 41.3 \pm 11.7 | 85.2 \pm 5.03 | < 0.001 |
| Relative Growth Rate (g/g/day) | 0.000024 \pm 0.000005 | 0.000017 \pm 0.000003 | < 0.001 |

Table 3: Paired samples t-tests comparing various growth metrics between caterpillars from uninfected or infected trials. Initial \rightarrow Final refers to the time period of feeding trials to which these results pertain; this data was collected during the entire feeding trial, including after pupation began.

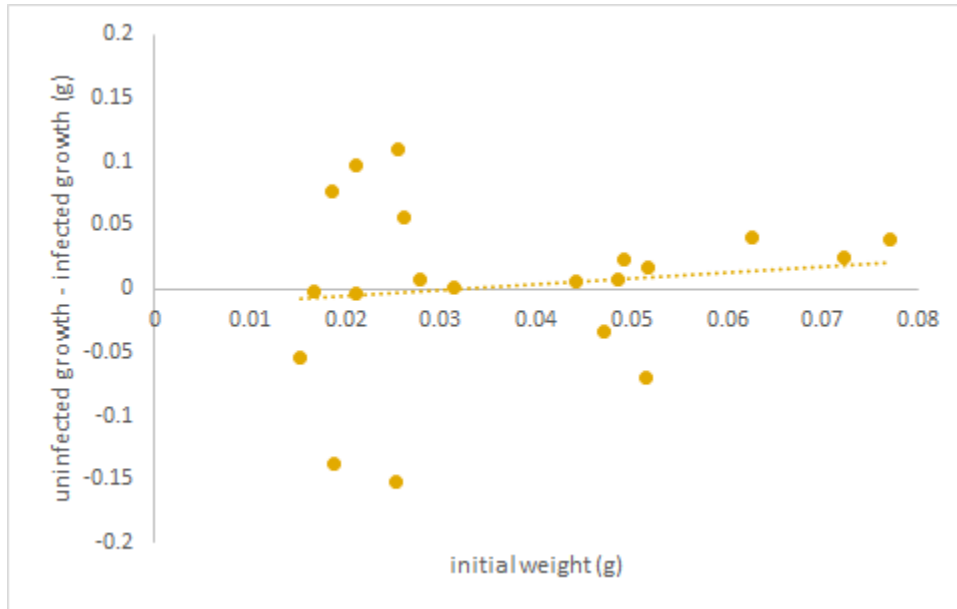
| Growth Metric | Growth Metric Mean for Uninfected | Growth Metric Mean for Infected | p-value (2-tailed) |
|---------------------------|-----------------------------------|---------------------------------|--------------------|
| Conversion Efficiency (%) | 13.7 \pm 2.05 | 14.9 \pm 5.03 | 0.370 |

Table 4: Nonparametric t-test (Related-Samples Wilcoxon Signed Rank Test) comparing conversion efficiency between caterpillars fed leaves from uninfected or infected trees.

Susceptibility of young and old caterpillars to effects of infection

Our linear regression analysis of whether there was a relationship between magnitude of effect from consuming leaves from uninfected vs. infected trees and the initial weight of the caterpillars revealed that there was very little to no correlation between these two factors ($R^2 =$

0.019, $p = 0.566$; Graph 2). Thus, we did not find a strong relationship between caterpillar age and effect of fungal infection on food source. For the purposes of this analysis, the initial pair weight, which we assumed to be indicative of caterpillar age, was found by averaging the weight of caterpillars within pairs.



Graph 2: Linear regression analysis on the difference in weight gained within pairs during the first and second week of feeding trials vs. initial weight of pair.

Number of molts between groups

The differences between number of molts within pairs were not normally distributed (Shapiro-Wilk $p < 0.001$); thus, we conducted a Related-Samples Wilcoxon Signed Rank Test. This test revealed that caterpillars fed leaves from infected trees molted more than those fed leaves from uninfected trees by a significant margin (Wilcoxon d.f. = 19, $p = 0.01$).

Discussion

In our study, we initially intended to measure caterpillar weights both one week and two weeks after beginning our experiment. However, after taking the weight for the first week, caterpillar pupation began unexpectedly. We decided to record the final weight at first note of pupation. Caterpillars were not monitored overnight, which allowed some caterpillars to have more time pupating before being noted and stopped. Furthermore, caterpillars pupated at different times across the second week, resulting in some having greater time to eat and grow, influencing the final weights considerably. This calls into question the validity of our final

weights as a useful metric to answer the questions we asked initially. Although we found significant differences in some growth metrics for the two week period, the differences during the first week are more representative of our study system since they are less susceptible to inappropriate variation. Due to this, only the first week of trials will be used for discussion.

Caterpillars consuming leaves from uninfected trees grew at faster rates than those eating leaves from infected trees. Relative consumption rate does not show a significant difference, indicating that the presence of infection in the tree does not affect the rate at which the caterpillar eats the leaves of the cherry tree. However, digestive efficiency, conversion efficiency, and relative growth rate do show a significant difference. The mean differences for digestive efficiency, conversion efficiency, and relative growth rate were all positive, meaning that caterpillars from uninfected trials were more effective at digesting food, converting that food to weight, and had a significantly higher rate of growth compared to caterpillars from infected trials. Given these results, it seems likely that the response of a plant to one specific attacker can positively influence the future defense against a separate attacker, even when the two attackers differ greatly in their methods. Due to this, herbivores and phytopathogens that appear on the same host could negatively influence each other's fitness. We see evidence to support that the presence of black knot fungus results in poorer habitat conditions for the eastern tent caterpillar population. Determining the rates and trends of the fungus in regions where the caterpillars exist could inform researchers about the quality of habitat for the caterpillars.

Since the relative consumption rate of leaves was not significantly different between infected and uninfected leaves, then the presence of black knot fungus in black cherry trees does not affect how much the eastern tent caterpillar eats. From the point of view of the black cherry tree, being infected with black knot fungus is neither beneficial nor harmful when it comes to defending against herbivory as it results in the same rate of consumption and so the same amount of foliage is eaten. However, in terms of the caterpillar, feeding from a tree that has been infected is detrimental to their overall growth. They have trouble digesting the leaves, converting those resources to energy, and using that energy for growth.

It is likely that a compound present in the leaves of infected trees is inhibiting caterpillar growth. The chemical defenses within the black cherry tree leaves could be produced in response to the presence of black knot fungus. This response could trigger a defense-signaling pathway to activate genes that add defenses to the cell wall of the leaves, which could explain why digestive

efficiencies were different. In *Arabidopsis* (*Arabidopsis thaliana*), expression of cellulose synthases could be induced through certain infections or chemical treatments (Schenk et al. 2000). *Arabidopsis* does not represent all plant defense responses, but a similar response might be present in the black cherry trees we used in our tests. In a previous study by Slansky and Wheeler (1991), they found that velvetbean caterpillars (*Anticarsia gemmatilis*) had increasing difficulty digesting food with increasing dietary cellulose. If a defense response by black cherry trees to pathogens is in fact expression of genes that reinforce cell walls, then the eastern tent caterpillar would have more difficulty digesting those leaves. In terms of consumption rate, addition of cellulose to cell walls does not necessarily explain the lack of difference between trials. However, cellulose is internal, so there is no difference in feeding stimulant or deterrent presence to affect how much the caterpillars eat.

Differences in conversion efficiency between trials is not explained by cellulose either. A possible explanation for conversion efficiency is an induced chemical defense in the black cherry trees. If the tree is producing a toxin in response to the black knot fungal infection, then the caterpillars would use more energy to combat the toxins in the leaves they ate, so that energy is not used for growth. In a similar study concerning cabbage loopers (*Trichoplusia ni*) and wild parsnip (*Pastinaca sativa*), caterpillars eating damaged leaflets, or leaflets with induced chemical defenses of various furanocoumarins, grew one fourth the size of caterpillars eating non-induced leaflets (Zangerl 1990). Black cherry trees contain cyanogenic leaf tissue that defends the plant when damaged by herbivory, but these chemicals are used in cyanogenesis by the eastern tent caterpillars to sequester and use for defense (Peterson et al. 1987). The trees also have tannins permanently present in their leaf tissue (Halarewicz et al. 2013). There is little research on the effect of tannins on eastern tent caterpillars, but one study found that the winter moth caterpillar (*Operophtera brumata*) was negatively affected by small amounts of tannins in their diet (Feeny 1968). The effects of tannins on insect growth in this experiment does not represent all natural conditions, and there could be other factors, but tannins could be a possible explanation for the inhibition of eastern tent caterpillar growth.

We also found that on average, that caterpillars feeding on leaves from uninfected leaves molted 0.29 times more than those feeding on leaves from infected leaves. This result is, for one, a biologically small amount, and the difference is not likely great enough to create much variation from our found results. Additionally, the more often a caterpillar molts, the less time

they will spend feeding since they stop for some time to prepare and perform the actual molt. However, this reduced time feeding was not enough to result in our study finding insignificant differences between the growth rates.

Areas for improvement

While all efforts to reduce noisy and confounding variables were made, there were some aspects of our design that potentially influenced our results. One noisy variable that was present during our experiment was leaf age. Younger leaves tend to have more nutrients compared to older leaves; eastern tent caterpillars have a preference for younger leaves and individuals that feed on mature leaves have lower weights, grow less efficiently, and often survive less often (Peterson 1987). Since we did not have the ability to provide only young leaves, we varied the age of leaves (determined by size) to ensure all caterpillars received as equal nutritional levels as possible. The average age of the leaves increased as we progressed through our trial, but it occurred at the same rate so it influenced both trials equally. Since we did not find any difference in the RCR, it seems unlikely that one of the leaf treatments was not preferred. A feeding trial offering both treatments simultaneously to individuals could add support to that claim.

Another possible noisy variable is the presence of prior herbivory on the trees we harvested leaves from. If there were signs of herbivory on a tree, we avoided using those leaves to prevent any herbivore-induced defenses affecting our experiments. This variable was difficult to control for, as other herbivores could feed on the tree leaves during our trials. However, our data still showed significance, so the possible presence of induced chemical defenses were not so severe on the uninfected trees as to affect our results.

Of the potential confounding variables that we found at the start of the experiment, we successfully eliminated or reduced them to noisy variables.

Areas for future research

There were a few questions that were asked during the progression of our experiment that we unfortunately do not have the time to research ourselves. One of the most interesting outcomes of the experiment was having one entire tent die due to parasitoid wasps. The wasps successfully killed the 12 caterpillars between the uninfected and infected trials. This leads to the question of whether parasitoid wasps can also be harmed by black knot fungus. Minimal work

has been done on the direct or indirect effect on the natural enemies of herbivores by the host plant. One feeding trial experiment conducted with “woolly bear” caterpillars (*Grammia incorrupta*) was interested in comparing the growth and resistance of *G. incorrupta* to an entomopathogenic nematode (Gassmann et al. 2010). There were four trials and in each trial one group was fed lettuce (*Lactuca sativa*) and the other group was fed threadleaf groundsel (*Senecio longilobus*), which contains pyrrolizidine alkaloids (Gassmann et al. 2010). The end result of this study was that in all four trials *G. incorrupta* that were fed *L. sativa* gained more weight than those who were fed *S. longilobus* (Gassmann et al. 2010). In one of the four trials, caterpillars fed *S. longilobus* actually had a higher resistance to nematodes, and nematode reproduction was lower on these carcasses (Gassmann et al. 2010). While this was only one study, it would still be interesting to see if parasitoid wasps grow less efficiently when their egg is placed within a caterpillar on an infected tree rather than one on a uninfected tree.

Recent discussions of climate change prompted us to ask how our system may be affected in the future if climate change continues at its current rate. Specifically, if and how the fungus-tree-caterpillar interaction might change. While no studies seem to have focused on black cherry trees in the face of climate change, there are quite a few articles discussing the general impacts of trees in forests and urban areas. One experiment looked at urban tree species in and around Philadelphia and their levels of susceptibility to climate change . It was found that there will be 10 tree species whose fitness will decline and two species that will thrive in the predicted climate in Philadelphia. This study was also interested in the potential change in pests and diseases in regards to these trees. In the end, they claimed their method for predicting pests and diseases was simplified, but they found that in general, pests and diseases will become more problematic (Yang 2009). Other studies have shown that global increase in temperature and CO₂ levels is associated with higher pathogen susceptibility in plants and a greater ability for pathogens to infect their hosts (Ghelardini et al. 2016). One study in particular projected the future of Norway spruce (*Picea abies*), which is frequently infected by *Endoconidiophora polonica*, a fungus specifically associated with this species of tree. Using computer modeling and experimentation, this study showed that the fungus will cause both more lesions and higher mortality in its host tree (Linnakoski et al. 2017). While it is unknown whether or not black cherry trees will be greatly affected by climate change, these studies suggest that many trees may be more negatively impacted by their specific fungi than normal. If this ends up being the case with black knot

disease and black cherry trees, the end result will likely be devastating for both the black cherry tree and the eastern tent caterpillar.

However, it is also possible that a higher prevalence of black knot disease will not necessarily have a very detrimental effect on eastern tent caterpillars. One study of forest tent caterpillars (*Malacosoma disstria*) found that poorer growth as a caterpillar does not necessarily correspond to smaller size as an adult (Etilé & Despland 2007). If growth and related metrics are the only components of caterpillar health that are negatively affected by consuming leaves from infected trees, it is possible that a fungal infection on the tree might not actually impose a selective pressure on the caterpillars. If incidences of black knot fungus do increase, eastern tent caterpillars have two potential options. The first is that natural selection might push female moths to change their oviposition behaviors and somehow allow them to notice uninfected trees more successfully. The second possible option is for caterpillars to adapt to higher rates of black knot fungal infection, and even benefit from its presence, much like they do with the cyanogenic glycosides already present in black cherry tree leaves.

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