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Ecological Impact of American Chestnut Hybridization on Insect Communities

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Abstract

The American chestnut tree (*Castanea dentata* (Marsh.) Borkh.), a keystone species of the Appalachian mountains faced near extinction with the introduction of blight in the early 1900s. Recent hybridization with the Chinese chestnut (*Castanea mollissima* Bl.) has allowed for the development of a blight-resistant variety. The impact of this hybridization on insect communities needs to be evaluated as these hybrids are planted through restoration efforts. In the summer of 2023, we conducted a study across three stands featuring American chestnut trees with varying degrees of hybridization (75%, 94%, or 100% American chestnut). We evaluated insect biodiversity aboveground, measured herbivory, and evaluated Asian chestnut gall wasp infestation in each of the stands. Our findings indicated that different hybrid varieties did not have a significant impact on surrounding insect diversity or herbivory on the leaves. However, the Asian chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu.) infestation was significantly different among the stands, with the 75% hybrid stand having the largest infestation and the 100% American chestnut tree stand having the least infestation. These results suggest that hybridized American chestnut trees may not have a significant effect on the overall insect diversity or insect herbivory, but can alter Asian chestnut gall wasp infestation levels. Understanding the effect of hybridized American chestnut trees on insect communities is crucial for anticipating potential pest problems and assessing possible implications for the local ecosystem.

Introduction

The American chestnut tree (*Castanea dentata* (Marsh.) Borkh.) was once one of the most important trees in the Appalachian ecosystem. Western North Carolina was known to have some of the largest American chestnuts, with one reaching 17 feet in diameter (Detwiler 1915). They could live up to 400 years (Lunsford 1999) and were once a keystone species providing food and habitat for wildlife. Chestnuts that are covered with a thorny husk were dropped in the fall and consumed by many organisms like deer, bears, squirrels, and many insects. American chestnut trees also reached 120 feet in height providing much-needed habitat for wildlife (Detwiler 1915). They have also been vital for people, being used to build railroads, houses, ship masts, and furniture because of their durable, rot-resistant wood (Davis 2005). American chestnuts contributed greatly to the economy through these uses and the blight caused a devastating impact on mountain communities (Wilhelm 1982**)**. In the early 1900s, a fungus was introduced (*Cryphonectria parasitica)* causing chestnut blight (Murrill 1906). It wiped out 4 billion trees causing significant damage to forest ecosystems and the people utilizing the trees for food and timber (Russell 1987). They are considered functionally extinct because they grow from old-growth rootstock but are unable to grow past a shrub-like stage before the blight kills them (Newhouse 1990).

There have been developments in backcross hybridization with a blight-resistant variety, the Chinese chestnut (*Castanea mollissima* Bl.) (Diller and Clapper 1969). The third backcross hybrid had 94% of its genome made up of *C.dentata* with 6% of *C.mollissima* and when Diskin and others (2006) compared diagnostic morphological traits they found no significant difference from American chestnuts. With similar morphological attributes showing hope for a successful reintroduction, many aspects of ecological effects were not investigated (Worthen et al. 2010)

Hybridization of the American chestnut and Chinese chestnut could allow the once vital keystone species to thrive once again in the Appalachian mountains. With different levels of backcross hybridization, it is crucial to use a hybridized variant that is not only resistant to blight but maintains ecological integrity. For the American chestnut to make a healthy recovery through hybridization it needs to be able to support the same ecological functions as pure American chestnuts.

While morphologically similar, American chestnut hybrids have slightly different maximum leaf size potential and curl compared to pure American chestnuts (Kane et al. 2019). Understanding how these leaf differences could affect insect herbivory is crucial to knowing if hybridization would change the associated insect community. Leaf size and shape have been seen to affect insect herbivores through their abundance and richness, which was impacted by different plant genotypes (Brown et al. 1991). Studies of foliar chemistry have found that American chestnuts have higher concentrations of carbohydrates and lower tannin levels compared to hybrids, which means pure American chestnuts could be a higher quality host for generalist herbivores but have a limited defense to insect herbivory (Rieske et al. 2003). Genetic differences between American chestnut trees and the hybrids could impact the dependent insect herbivore community (Knops et al. 1999) and their predators (Price 1980).

Following the theory of bottom-up interactions (McQueen et al. 1986), impacts on insect herbivores would affect insects that prey on them. Genetically controlled plant traits have previously been studied and shown that hybridization can impact the distribution and abundance of associated insect species as well as insect diversity (Fritz and Simms 2012, Whitham et al. 1994). The insect diversity can be affected due to the accumulation of insects that would be more unique to each parental species when there is plant hybridization (Whitham et al. 1994). There are already many introduced insects from the native region of the Chinese chestnut, further supporting these invasive species could pose a problem for reintroduction. For a successful introduction of American chestnut hybrids, we should understand the impact on insect diversity and the effects insects could have on them.

Besides the possible interactions between hybrid American chestnuts and native insect diversity and abundance, one of the most dangerous and well-known pests of chestnut trees is the Asian chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu, Hymenoptera, Cynipidae). Previous studies examining different plant hybridizations and the effects it has on parasite infestation have had variable outcomes (Fritz et al. 1997). The Asian chestnut gall wasp is considered one of the most successful invasive species (Avtzis et al. 2019). They are a species of parasitic wasp that create galls resulting in greatly reduced green biomass (Gehring et al. 2018). These parasitic wasps, in high infestations, damage the canopies impacting chestnut production (Riga et al. 2013) as it leads to decreased flower and leaf production (Gehring et al. 2018). In heavy infestation the Asian chestnut gall wasp damages the tree's survival and reproduction abilities, ultimately killing chestnut trees (Riga et al. 2013). Understanding if American chestnut hybrids are impacted differently by this pest is paramount for reintroducing this species.

In this study, we examined insect communities at different levels of American chestnut hybridization with the Chinese chestnut. We examined how local insect diversity and abundance are affected by hybridization and the potential difference in herbivory between hybrids and pure American chestnut trees. We also compared how hybridization affects the Asian chestnut gall wasp infestation. This study provides a better understanding of how insect communities, insect herbivory, and Asian chestnut gall wasp infestation are affected by American chestnut hybridization compared to pure American chestnuts.

Methods

1.1 Site Description

At Dupont State Recreational Forest (35.1900,-82.6035) there are three stands of American and Chinese chestnut tree hybrids. Each stand consists of either 100% American chestnut trees (35.21939,-82.59705), 94% American chestnut/6% Chinese chestnut tree hybrids (35.21192,-82.59662), or 75% American chestnut/25% Chinese chestnut tree hybrids (35.21606,-81.59480)(Table 1). The stands are integrated in hardwood and coniferous mixed forests with natural Southeastern vegetation, which made up our three separate treatments. Prior to planting, stands were cleared of any larger trees or vegetation that would obstruct the chestnut trees from growing. To keep ecological integrity, plants that were outside the stand or did not interfere with the potential growth of the chestnut trees were left undisturbed.

The stand containing 100% American chestnut trees (100% AC treatment) was established at Dupont State Recreational Forest in 2013 and came from collections of chestnuts from the Blue Ridge Parkway. This stand was slightly shaded and separated into three adjacent sections, with one having a large presence of ant hills. The heights of the trees at the time of this study ranged from 12 cm to 96 cm with basal diameter ranging from 1 mm to 21 mm. The stand consisting of 94 % American chestnut tree hybrids (94/6% AC treatment) was established in 2014 and had minimal shade. These trees ranged in height from 0.8 meters to 7.7 meters with dbh ranging from 0.03 cm to 7 cm. The 75% percent American chestnut tree hybrid (75/25% AC treatment) stand was planted in 2009 and was slightly shaded with more dense vegetation. The chestnut trees in this stand had heights ranging from 0.6 meters to 11.62 meters with a dbh between 4 mm and 16.5 cm.

Table 1. Stand information on American and American-Chinese Chestnut treatments

Treatment ID	Stand Genetics	Year Planted	Mean Height	Number of Trees

100 % AC	100% American Chestnut	2013	0.3 m	46
94/6% AC	94% American Chestnut/ 6% Chinese Chestnut	2014	3.9 m	62
75/25% AC	75% American Chestnut/ 25% Chinese Chestnut	2009	4.2 m	30

1.2 Insect Diversity

We deployed four pyramid traps for each of the three treatments (Tedders Pyramid Trap, GL-5000-06, Great Lakes IPM, Vestaburg, MI, USA). Each trap was constructed with four 4-foot tall triangular pieces of upright black cardboard leading to a plastic trap cup at the top. These traps were evenly distributed along a central transect throughout each treatment. The traps were placed on May 15, 2023, and collections continued until August 29, 2023.

Each trap was staked to the ground within a meter of an American chestnut tree or a hybrid. The traps were left in the field for roughly ten days at a time and were cleaned in between collections. All traps were collected on the same day and insects were identified to order once frozen. Insects that were too mutilated in the traps to identify were discarded. Traps that had fallen between collections due to natural elements were excluded from the data.

Statistical analysis was conducted to compare the differences in insect diversity and abundance among the different treatments. For the Shannon Index, Simpson Index, and Order Evenness comparing biodiversity a one-way ANOVA was used, and a Kruskal-Wallis test was used to compare Order Richness among the treatments. Jaccard dissimilarity index was calculated and compared using permutational multivariate analysis of variance. The significance level was set at $\alpha = 0.05$. All calculations and statistical analyses were performed using R statistical software (R Core Team, 2021).

1.3 Herbivory

To evaluate herbivory between each treatment, we collected ten leaves from different trees in each treatment every month from June 2023 till August 2023. Each collection was based on a stratified random sample of trees and leaves were selected through blind collection. To do the blind collection we would stand by the selected tree and reach out at varying degrees with our eyes closed, the first leaf touched was the leaf collected from that tree. All the leaves were collected from below nine feet on the tree.

After the collection, leaves were laid flat in the freezer at 0°F for 24 hours to preserve and flatten. To capture images without shadows, we constructed an examination box, a cardboard box with a light source inside, and a paper-covered top. Another box without a bottom surface was placed on top creating an enclosed space. Photos were taken using an iPhone 13 with dual 12MP cameras through a hole cut in the top box. Each leaf was placed in between the boxes on the illuminated surface.

We processed these photos using the Bioleaf Application with the setting of no reflection to find the percent of herbivory for each leaf (Machado 2016). If a leaf's edge was defoliated the missing portion was estimated based on the opposite side of the leaf. Only herbivory attributed to insect herbivory was calculated, while herbivory likely caused by mammals or other natural elements was excluded. To determine if it was herbivory caused by insects, we examined known insect herbivory patterns that cause more rippled sections of damage, compared to mammal herbivory or tears that are larger, straighter cuts.

We used a Kruskal-Wallis test to determine if there were significant differences in the percentage of herbivory on the leaves with the presence of herbivory between the different treatments. To compare the amount of leaves with herbivory and without herbivory between the treatments a Fisher test was performed. These statistical analyses were computed in R statistical software (R Core Team, 2021) with a set significance level of $\alpha = 0.05$.

1.4 Asian Chestnut Gall Wasp

In each treatment, we used stratified random sampling to select ten trees. Those ten trees were then monitored for galls formed by the Asian chestnut gall wasp. Gall counts were recorded every ten days from May 15, 2023, until August 29, 2023. Only current-year gall growth was considered.

Gall counts were separated into four categories of infestation based on average tree height for each treatment and categories were adjusted based on former gall impact studies (Foss 2004). With an average height of 4.2 meters in the 75/25% AC treatment, the infestation levels were as follows: None(0), Low(1-6), Medium(7-42), or High(>42).In the 94/6% AC treatment, the average height was 3.9 meters so the infestation levels were adjusted as follows: None (0), Low (1-5), Medium (6-39) or High (>39) For the 100% AC treatment, the average height was 0.5 meters so the infestation levels were adjusted as follows: None(0), Low(1), Medium(2-3), or High(>3). On August 29, 2023, we conducted an examination of each tree in every treatment to determine the presence or absence of galls. Both new and old gall growth were considered as present in this second assessment.

To compare the differences in infestation levels and the proportions of the presence of galls among the treatments, a Fisher test was conducted. All statistical analyses were performed using R statistical software with a significance level set at $\alpha = 0.05$ (R Core Team, 2021).

Results

2.1 Insect Diversity

There was a total of nine orders of invertebrates collected which were Arachnida, Blattodea, Coleoptera, Collembola, Diptera, Hemiptera, Hymenoptera, Lepidoptera and Orthoptera (Figure 1). Of these nine orders, Hymenoptera was the most abundant, while Coleoptera was the second most abundant and Blattodea the least.

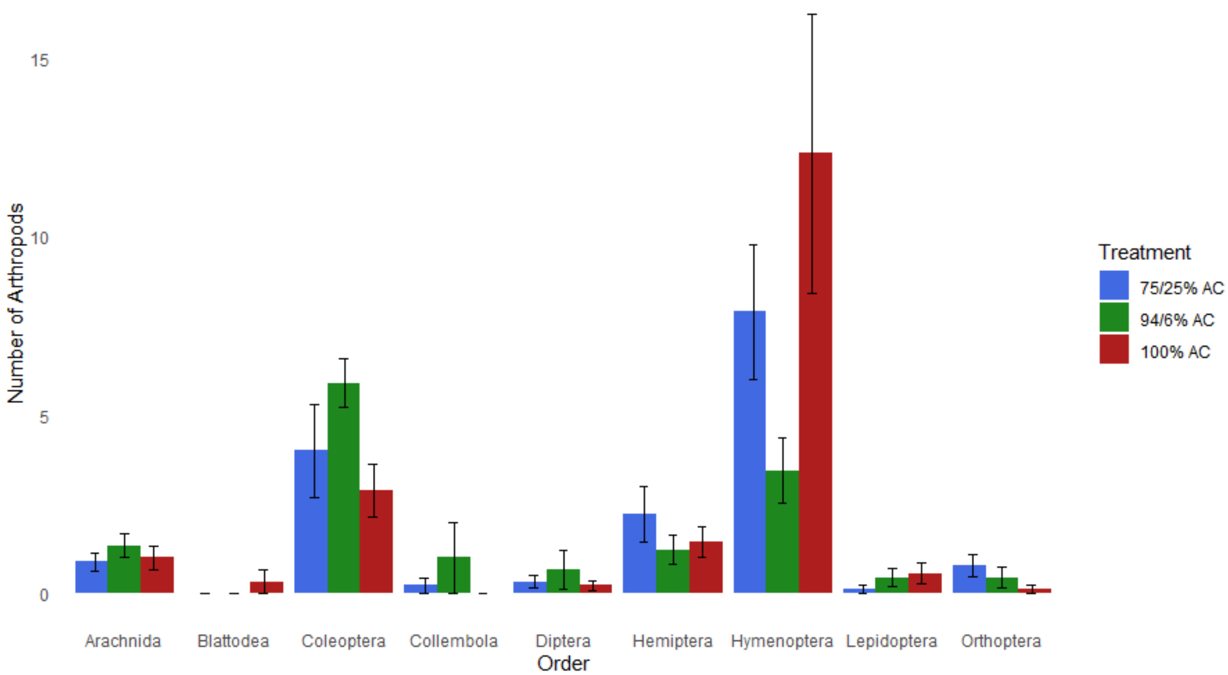

Figure 1. The mean number of each order of arthropod collected in pyramid traps for each Chestnut treatment. Error bars represent standard errors.

We calculated the mean Simpson Diversity Index for each treatment, finding it was the highest in the 100% AC treatment and lowest in the 94/6% AC (Table 2) (Figure 2A). As indicated by a one-way ANOVA test there was no significant difference in the Simpson diversity index values between the treatments, $F(2, 24) = 1.335$, $p = 0.28$. The average Shannon Diversity Index was highest in the 94/6% AC and lowest in the 100% AC (Table 2) (Figure 2B). Which also did not have a significant difference between treatments indicated by the one-way ANOVA, $F(2, 24) = 0.7217$, $p = 0.49$. Order evenness was calculated for each treatment, the average order evenness was highest in the 94/6% AC treatment, and lowest in the the 100% AC treatment (Table 2) (Figure 2C). The one-way ANOVA comparing order evenness did not show significant differences between the treatments ($F(2, 24) = 2.641$, $p = 0.09$). Order richness was similar throughout all treatments ranging from 4.33 to 4.11 (Table 2). There was not a significant difference between the treatments when a Kruskal-Wallis test performed

(Figure 2D) (χ^2 = 0.57272, df = 2, p = 0.75). The PERMANOVA conducted on the Jaccard Dissimilarity Index showed significant differences in the the community composition of the treatments ($F(2, 24)$ = 1.7195, p = 0.03) allocating 12.5% of the difference to treatment and 87.5% to residual variation.

Table 2. Average Simpson Diversity Index, Shannon Diversity Index, Order Richness, and Order Evenness for each treatment

	Simpson Diversity	Shannon Diversity	Order Evenness	Order Richness
75/25% AC	0.422	1.18	0.753	4.33
94/6% AC	0.351	1.21	0.864	4.22
100% AC	0.456	1.04	0.731	4.11

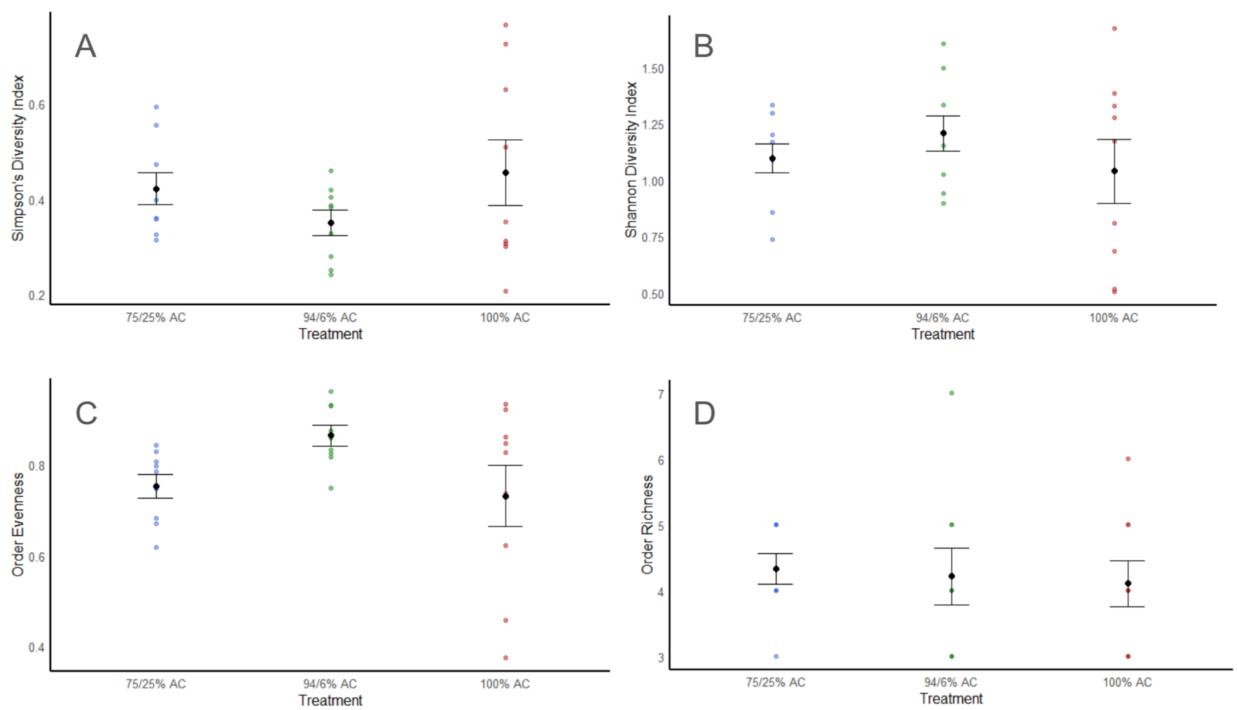

Figure 2. (A) Simpson Diversity index, (B) Shannon Diversity index, (C) Order Evenness, and (D) Order Richness. Colored dots represent samples with darker colors indicating multiple collection value overlap. The black center dot shows the mean value with standard error bars. No significant differences were found between the treatments for any of these diversity or abundance measurements(P>0.05).

2.2 Herbivory

The percentage of herbivory by insects was calculated to compare the amount of herbivory if it was present. Each treatment was compared using the Kruskal-Wallis test and did not indicate any significant difference between the treatments ($\chi^2 = 2.1791$, df = 2, $p = 0.33$). The 100% AC treatment had the highest average herbivory percentage at 2.89%. The 75/25% AC treatment had the lowest average herbivory (1.64), and the 94/6% AC treatment had two collections above 15% indicating the presence of higher abnormalities for herbivory and an average percent herbivory of 2.81 (Fig 3A). The presence and absence of herbivory on the leaves collected were compared with a Fisher exact test. The 75/25% AC and 100% AC had the same quantity of leaves with herbivory and it was not significantly different compared to the 94% AC treatment (p=0.14). The 94/6% AC treatment had more leaves with the presence of herbivory with 25/30 compared to 19/30 in the other treatments (Fig 3B).

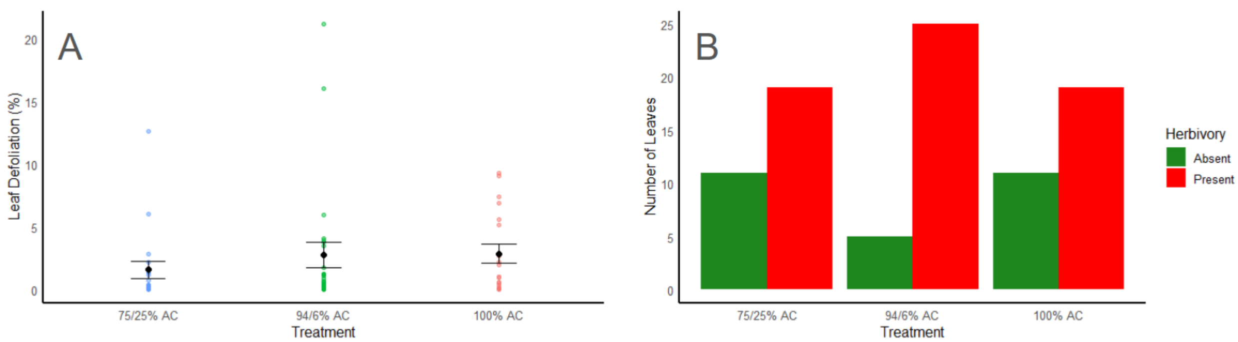

Figure 3. (A) Percent insect herbivory for each leaf with herbivory (Mean +/- 1 standard error. (B) Bar chart comparing the number of leaves with and without herbivory between the treatments. No significant difference was detected in either assessment (p>0.05).

2.3 Asian Chestnut Gall Wasp

We found that of the ten trees surveyed by the end, the 100% AC treatment did not have any galls. The 94/6% AC treatment had only one of the ten infected, with an infestation level of medium. For the 75/25% AC treatment, there were three trees infected with galls and of that one ended with a high infestation, one with medium, and the other with a low infestation (Figure 4). The proportions of different infestation levels throughout the season from each treatment were compared using a Fisher test and found significant differences between each combination of treatments. There were significant differences in the proportion of infestation levels between the 94% AC trees and 100% AC trees (p<0.01), and between the 75% AC trees and both the 94% and 100% American chestnuts (p<0.001).

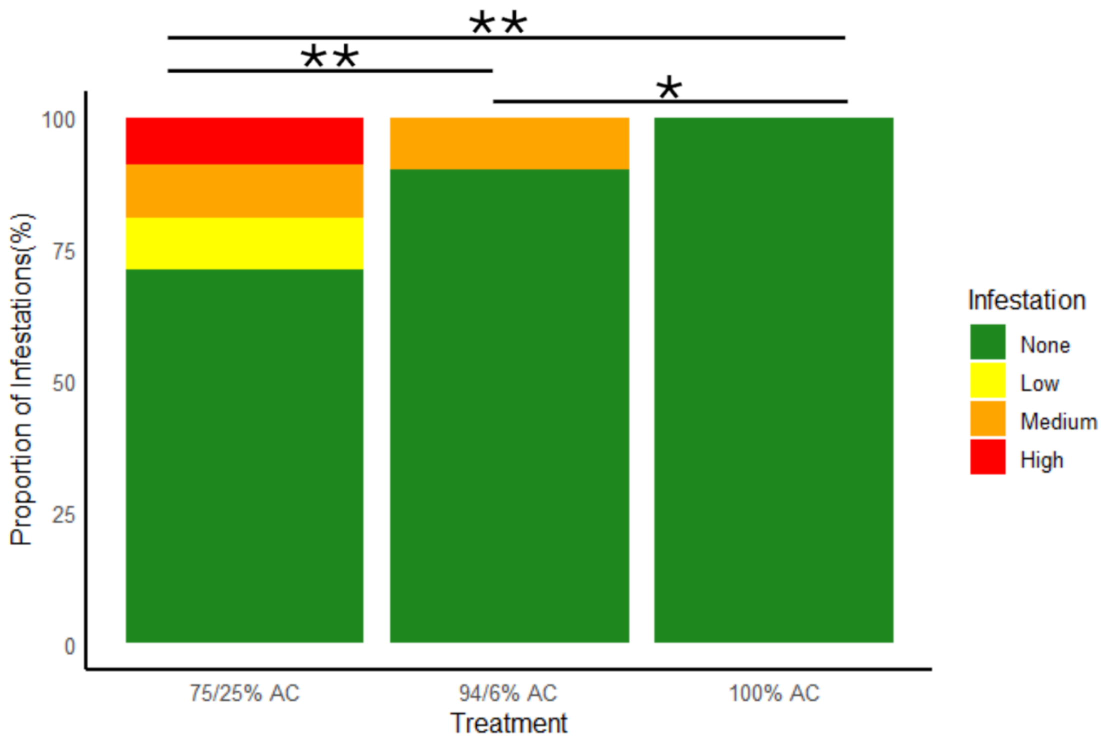

Figure 4. Proportion of infestation levels from the 10 trees monitored throughout the study. None, low, medium, and high infestation levels were based on gall abundance and average tree height per treatment. Proportions of infestation levels among each combination of treatments are significantly different from each other (*=P<0.01,**=P<0.001).

Examining the proportion of American chestnut trees and their hybrids that had been infected out of the total number of trees in each treatment we found that the 75/25% AC treatment had a significantly higher proportion infested. It had six out of the 30 (20%) trees with the presence of galls. The 94/6% AC treatment had two infested out of 62 trees (3%). The 100% AC treatment had the least with no trees being infested out of 46 (0%)(Figure 5). This end-of-season infestation presence was compared using a Fisher test and found a significant difference in proportions infested in the 75/25% AC treatment compared to 94/6% AC and 100% AC treatments (P<0.05; P<0.01 respectively).

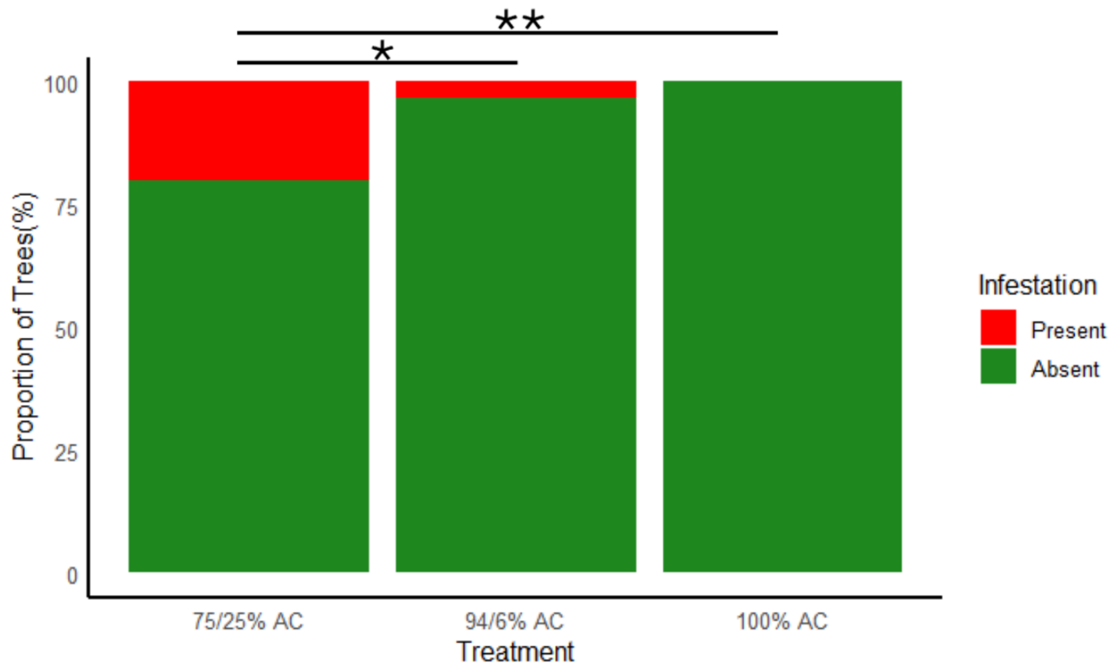

Figure 5. Proportion of all trees in each treatment with and without the presence of galls by the end of the study. 75/25% AC treatments had a significantly higher percentage of infestations compared to 94/6% AC and 100% AC treatments. (* =P<0.05, **=P<0.01).

Discussion

3.1 Insect Diversity

In this study, we aimed to assess the effects of American chestnut tree hybridization on insect communities as hybridization can affect insect diversity and abundance (Fritz and Simms 2012, Whitham et al. 1994). We compared the local insect communities associated with each American chestnut treatment. From our insect collections, we discovered no significant effect attributable to the different levels of hybridization of the American chestnut tree in that all treatments had similar levels of insect diversity and abundance.

When looking at the Shannon Diversity Index and Simpson Diversity Index we found concurring results among diversity and variation. The 100% AC treatment held the highest average Simpson Diversity Index (0.456) and lowest average Shannon Diversity Index (1.04) with the largest range of values for both measurements. Both of these diversity indices indicate that the 100% AC treatment had on average lower insect diversity but more variation. In both diversity indices, there was also an indication of higher insect diversity on average in the 94/6% AC treatment.

The Simpson and Shannon diversity indices showed a non-significant trend among the three treatments, the 94/6% AC treatment had the highest diversity and the

100% AC treatment had the lowest. It is important to note more diversity is not necessarily better as hybridization can cause more diversity (Whitham et al. 1994). To keep ecological integrity hybridization should not affect insect diversity differently than pure American chestnuts. We did not find any indication that insect diversity is affected by hybridized American/Chinese chestnut trees differently than pure American chestnut trees. If hybridization affected local insect communities, we would have expected to see either a greater difference or that the change correlated with the distance of genetic variation from the pure American chestnut trees. With 94/6% AC treatment being more genetically similar to the 100% AC treatment than the 75/25% AC treatment, our results of insect diversity do not follow a trend with the amount of variation from the 100% AC treatment. Without a trend, there is a stronger indication that Chinese chestnut genetics do not have an impact on insect diversity.

The larger range of diversity in 100% AC can be attributed to non-genetic factors. This treatment consisted of three separate plots compared to both other treatments which were only made up of one. A greater distance of insect collections then the other treatments allowed for the chance of different environmental factors affecting insect communities within the same treatment. Different tree species, shade, wind penetration, and soil temperature of the 100% AC treatment could have varied among its three plots contributing to supporting a wider range of insect communities (Zhao et al. 2023). These same environmental factors could be altered among the different treatments as well contributing to differences in insect collections. The lower average insect diversity could be due to non-genetic factors in the 100% AC treatment.

The average order richness was similar among the treatments (4.3, 4.2, and 4.1) as a large effect for diversity can be attributed to order evenness. A portion of the 100% AC treatment consisted of large ant hills which would explain the greater presence of Hymenoptera collected. This would be attributed to more Hymenoptera collected. Ants have the potential to predate and prevent other insect orders from occupying the area (Siddiqui 2021). While the 75/25% AC and 94/6% AC treatments had a similar range of order evenness, the low recordings of order evenness in the 100% AC treatment could be attributed to this factor. Non-significant differences in diversity indices in our study are more likely attributable to stand characteristics than the impact of genetic differences. The PERMANOVA conducted on the Jaccard dissimilarity index between the treatments supports this, although there was significance ($P<0.05$), it indicates only 12.5% of the difference can be attributed to the treatment and 87.5% can be linked to other variables.

3.2 Herbivory

In this study, we assessed herbivory by insects across the different American chestnut treatments. With genetic differences, other research has shown it could affect the dependent insect herbivores (Knops et al. 1999). This research aimed to understand the genetic effect of hybridization on the herbivore community by looking at the proportions of the leaf with herbivory and the occurrence of herbivory. By only including herbivory thought to be caused by insects we did not find any statistical significance when comparing the percentage of herbivory if present. There was a larger range of herbivory in the 94/6% AC treatment compared to the other treatments containing two collections above any collections from the other treatments but the 100% AC treatment had the highest average herbivory.

Similarly, when looking at the amount of leaves with and without herbivory we did not find a significant difference. The 94/6% AC treatment had more leaves collected with herbivory present while the other treatments had the same amount of leaves with herbivory. Through both comparisons, there wasn't a trend through genetic variation or any significance.

Our results show that even though chestnut hybridization can affect leaf size and foliar chemistry it does not alter the amount of herbivory per leaf or the occurrence of insect herbivory (Rieske et al. 2003, Kane et al. 2019). Without a statistical difference in herbivory patterns between the treatments, it aligns with the measure of insect diversity and abundance. The non-significant results of insect herbivory further support the lack of differences in insect community diversity found by the pyramid traps as insect herbivory and insect abundance are associated through the theory of bottom-up interactions (McQueen et al. 1986). Insect herbivores cause damage to plants in excess quantities reducing size and their ability to survive (Myers and Sarfraz 2017) but it is crucial that hybridized American chestnuts can still support the same herbivores as they are vital organisms in the food chain (Hare 2012). Our study concludes that the hybridized American and Chinese chestnuts experience similar insect herbivory as pure American chestnuts.

3.3 Asian Chestnut Gall Wasp

We monitored ten trees in each treatment (75/25% AC, 94/6% AC, 100% AC) and recorded infestation levels based on the number of galls and average tree height for the treatment. The 75/25% AC treatment had significantly more infestation than the other two treatments. Examining the proportion of recorded infestation, allowed us to take into account the levels of infestation and how quickly they developed. We found that 9% of the infestation levels recorded for 75/25% AC treatment were low, 10% were medium, and 10% were high, with the remainder of the treatment having no infestation (71%). Significantly more than 10% medium infestation in the 94/6% AC and no infestation in the 100% AC treatment ($P<0.001$). The 94/6% AC treatment also had significantly more infestation than the 100% AC treatment ($P<0.01$).

When diagnosing every tree in each treatment at the end of the season for the presence or absence of galls from the Asian chestnut gall wasp, similar results were found. By the end of the season, galls had begun to fully kill the leaves they were impacting. This made current season gall growth and previous season growth indistinguishable. This study took into account any gall formation from current or previous seasons. Similarly, the 75/25% AC treatment had a significantly higher proportion of trees infested than both 94/6% AC and 100% AC ($P<0.05$ and $P<0.01$ respectively) There were six out of the 30 trees in that treatment with the presence of galls compared to two of 62 in 94/6% AC and zero of 46 in 100% AC treatments.

Both measurements of Asian chestnut gall wasp infestation showed that a higher percentage of Chinese chestnut genetics in American chestnut tree hybrids could increase susceptibility to Asian chestnut gall wasp infestation. This research suggests that hybridized American chestnuts could be at a more serious risk of damage associated with this pest. Previous studies have found similar results in that chestnut genotypes can impact susceptibility to Asian chestnut gall wasp infestation (Lombardero 2022). However, it has been thought that Chinese chestnuts and their hybrids contain less gall density than other variations due to their coevolution (Labbate and McCullough 2022). A possible implicational factor to our study was that average tree height varied. The 100% AC treatment had a large size difference, with the average height being about half a meter compared to roughly four meters for the other treatments.

Conclusion

Understanding the entomological effects of restoration of the American chestnut tree through hybridization with the Chinese chestnut is crucial for maintaining ecological integrity. Our study investigated the impact of the hybridization of American and Chinese chestnuts on local insect diversity, Asian chestnut gall wasp infestation, and herbivory. All of our measurements comparing insect diversity and herbivory showed similar results throughout the different treatment treatments. Indicating that the genetic difference from chestnut hybridization does not affect the insect communities or insect herbivory. However, there were significantly higher Asian chestnut gall wasp infestation levels and the portion of trees infested with American chestnut hybrids compared to the pure American chestnut treatment. This could mean American chestnut hybrids are more susceptible to infestation and it warrants the need for future research.

It would be valuable to conduct species-level identification of the insect community and insect herbivores on the leaves in future research. This would allow a better understanding of the effects chestnut hybridizations may have on insect communities and potential pest problems. Additionally, examining rates of Asian chestnut gall wasp infestations at various stages of tree development would enhance our understanding of how American and Chinese chestnut hybridization influences Asian chestnut gall wasp infestation.

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