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Frequency and Abundance of the Asiatic Oak Weevil on the American, Chinese, and Hybrid Chestnut in the Southern Appalachian Mountains.

Ashley E. Case, Albert E. Mayfield III, Stacy L. Clark, and Scott E. Schlarbaum
Environmental Studies
The University of North Carolina at Asheville
One University Heights
Asheville, North Carolina 28804 USA

Faculty Advisor: Dr. Barbra Reynolds

Abstract

The American chestnut, once one of the most useful and abundant trees in the Appalachian Mountains, was almost completely destroyed with the accidental introduction of the chestnut blight fungus (*Cryphonectria parasitica)*. Currently American, Chinese, and hybrid chestnut seedlings are being studied in the southern Appalachian Mountains for their field performance and resistance to the blight. Some of these plantings are sustaining notable insect defoliation. The Asiatic oak weevil *(Cyrtepistomus castaneus)* is suspected to be one of the main defoliators. This project assessed the amount of defoliation throughout the growing season on American, Chinese, and hybrid chestnut seedlings at two sites in the southern Appalachians. The study monitored the frequency of the Asiatic oak weevil occurrence on trees three times throughout the growing season. This research also monitored the abundance and emergence timing of *C. castaneus*.

"Chestnut Trees grow very tall and thick, mostly, however, in mountainous regions and high land. Its wood is very lasting, and its fruit exceptionally sweet"
-William Byrd (1737)[9]

"A grove of chestnuts is a better provider than a man-easier to have around, too" [9]
-Unknown Mountain Woman

1. Introduction

The American chestnut (*Castanea dentata)* was once one of the most prominent trees throughout hardwood forests in the eastern United States. Some estimate that chestnuts dominated 200 million acres of land from Maine to Mississippi[4]. Pollen records have shown the chestnut to have been largely present during eastern forest post-glacial periods[8]. *Castanea dentata's* distribution was well documented in the late 19th and early 20th centuries due to its lumber, which is rot-resistant, durable, and easy to work[8]. The nuts of the trees were a commodity, used to barter and sell, and an important food source for families and their livestock[6].

Near the turn of the century an exotic blight was introduced to the United States through importation of Japanese chestnuts (*C. crenata)*. In 1904 a forester at the New York Zoological Park first reported the disease after observing a significant amount of chestnuts on the park's land that were dead and dying[4]. The fungal disease, first named *Endothia parasitica* and later renamed *Cryphonectria parsitica*, spread quickly through eastern forest, reaching the Appalachian Mountains in the first part of the 20th century. By 1915 noticeable damage was recorded in eastern Tennessee. By the 1930s it had spread in to Georgia and by the 1940s there was scarcely a chestnut in the Appalachian Mountains that was not dead or dying[3].

In response to the blight, efforts to breed blight-resistant trees were initiated by various state and federal agencies [5]. The American Chestnut Foundation (TACF) was formed with the mission of breeding a blight-resistant tree by

initially crossing *C. dentata* with blight-resistant Chinese chestnut (*Castanea mollissima*) [1]. The traits of the American chestnut are then recovered with a series of back crossings, to the American chestnut parents, while screening to retain the blight resistance from the Chinese parent. Back crossing will generally reduce the Chinese alleles by an average of one-half per generation[5]. The most recent generation of hybrid chestnuts is a third intercross and a third backcross generation, commonly referred to as BC_3F_3. On average these trees are 15/16 *C. dentata* and are anticipated to show stable blight resistance[2]. A series of experiments was established from 2009 to 2011 to test the blight resistance, field performance, and morphological characteristics of back crossed (hereafter referred to as hybrid trees) at within the National Forests in the southeastern region. More than 4,500 trees were planted across 11 sites, consisting of the two parental species, American and Chinese chestnuts, and their backcrossed and intercrossed progeny hybrid chestnuts, including the BC_3F_3, have been planted and are currently being studied[2].

In the late growing season of 2011, we noticed excessive herbivory on the chestnut saplings at one of the planting sites. The Asiatic oak weevil (*Cyrtepistomus castaneus*) was in abundance at the time of noted herbivore damage and was hypothesized to be largely responsible. Although trees in the family Fagaceae are commonly utilized by many groups of insects[7] the sites showed extreme amounts of herbivory, that could potentially damage performance of the saplings.

Little is known about *C. castaneus* in American forests. The weevil is indigenous to Japan[10]. The first specimen recorded in North America was found July 1935 in Montclair, New Jersey. *Cyrtepistomus castaneus* has since been recorded in many eastern states[10]. The insect's life cycle produces one generation per year. A study in Delaware noted adults to begin emerging from leaf litter as early as May 10th; significant numbers of adults appear by June 18th. *Cyrtepistomus castaneus* will first feed on seedlings mostly in the Fagaceae family. As their numbers increase, they have been found on larger trees, shrubs, weeds, and cultivated crops[10].

The current project was developed with three major objectives in mind. The first objective was to assess defoliation on chestnut saplings from the American, and Chinese species, and the different generations of hybrids. Secondly, the study assessed the frequency of *C. castaneus* observed on the American, Chinese, and hybrid chestnut saplings. Lastly, the research monitored the abundance and temporal patterns in emergence of Asiatic oak weevils. The study took place at two of the previously established sites, at three intervals during the growing season (early, mid, late).

2.Methods

2.1. Study Site

A variety of chestnut plantings using genetic materials provided by The American Chestnut Foundation research farm in Meadowview, Virginia, were studied at two sites in the southern Appalachians. These sites were planted to establish an ongoing research project to examine the growth, silvicultural effects, and blight resistance of different breeding generations of the American, Chinese, and hybrid chestnuts[2]. All chestnus were grown as one-year old bare root nursery seedlings at the Georgia Forestry Commission's Flint River Nursery, near Byromville, GA in 2008. Seedlings were then lifted from the nursery, and each seedling was assigned an individual number and tagged to maintain genetic identity throughout the study. In February, 2009, seedlings were planted in experimental plots within two different southern National Forests. The areas were well- drained, high-quality mesic to sub-mesic sites that had soils with less than 20% clay and at least 50% sand. Site index (base age 50 years) for northern red oak (*Quercus rubr*a) was >70ft. The presence of American chestnut sprouts and small trees at the sites indicated prior presence of American chestnuts. Soils from each site were tested for presence of root rot disease, *Phytophthora cinnamomi* Within a year prior to planting, each site was treated with a commercial even aged regeneration harvest, a shelter-wood with reserve [2] method that leaves approximately 10-20 square feet of basal area, and will provide some protection to regeneration from drought, frost, and wind damage. Trees were planted using an incomplete block design with single tree plots with a nested, factorial treatment arrangement to determine differences in performance among parental species/hybrid generations, families within parent species/generations, and seedling size classes within families.

This study was conducted during the 2012 summer at two of the above chestnut plantings in North Carolina (site NC) and, Tennessee (site TN). Two hundred twenty saplings from various pure (American, Chinese) and hybrid backcross generations (Table 1) were surveyed three times during the growing season: early (May), mid (July), and late (September).

Table 1. Number of trees surveyed by generation at sites TN and NC in 2012.

Generation	NC	TN
American Chestnut	20	20
Chinese Chestnut	20	20
BC_1F_3	20	20
BC_2F_3	20	20
BC3F2	NA	20
BC_3F_3	20	20
Total	**100**	**120**

2.2. Assess Defoliation

To assess the defoliation, two observers visually examined the foliage of each sapling and collected insects that were potential feeders seen resting or directly feeding on the tree. The observers then determined the percentage of all leaves occurring in each of the following 3 defoliation categories: <30% leaf area missing, 30-60% leaf area missing, >60% leaf area missing. Observers rated trees independently and then collaborated to assign a final, joint rating. The joint rating was used to calculate the defoliation index. Each of the three categories was given a weight: <30% leaf area missing x 1, 30-60% leaf area missing x 2, and >60% leaf area missing x 3. These described calculations were done to each tree's defoliation rating then summed to give the tree a number in-between 100-300. The defoliation index was averaged within each generation to achieve the final mean defoliation index.

2.3. Collecting Weevils

After visual examination of the tree, two 1 meter-square beat sheets were held beneath the lowest branches opposite one another. A stick was used to lightly strike the tree 10 times on each side to dislocate remaining insects that were missed during visual observation. All *C. castaneus* were collected from the sheets and at least one specimen of all other species was collected to be identified at a later date, and placed in vials with 95% ethanol to be preserved. Insects that are known to not be phytophagous were not collected. The vials were labeled with tree identification number, site, and date. Photographs were taken of all species in the family Lepidoptera, as they are preserved poorly in ethanol. All specimens will be identified to order and family, and to genus and species whenever possible.

2.4. Emergence Of *C. Castaneus*

To monitor the abundance and timing of emergence of the adult Asiatic oak weevil eighteen conical, wire mesh traps were placed on the forest floor at site TN. They were secured using bags of sand around the edges of each trap. A collection cup was secured at the top of each trap. Nine traps were placed under chestnut oak (*Quercus prinus*) canopy and nine under non-oak canopy (pine or open sky). The traps were checked approximately every week from May through October.

3. Results

3.1. Data Analysis

Means of each response variable were compared by generation, month, or canopy cover type using either a t-test or ANOVA and Tukey's HSD (α=0.05).

3.2. Defoliation Of Chestnuts

In all months, most leaves were classified in the <30% defoliation category and leaves with >60% defoliation were rare. The 30-60% category was most useful for showing differences between the generations. At site TN, the BC_2F_3 generation had more defoliation in the 30-60% category than Chinese chestnut in May (F=2.485;df=1,5;P=.036). In July there were no significant differences between defoliation in the 30-60% category (F=1.293;df=1,5;P=.279) (Figure.1). In September the BC_3F_2 generation had more defoliation than Chinese chestnuts (F=4.11;df=1,5;P=.002).

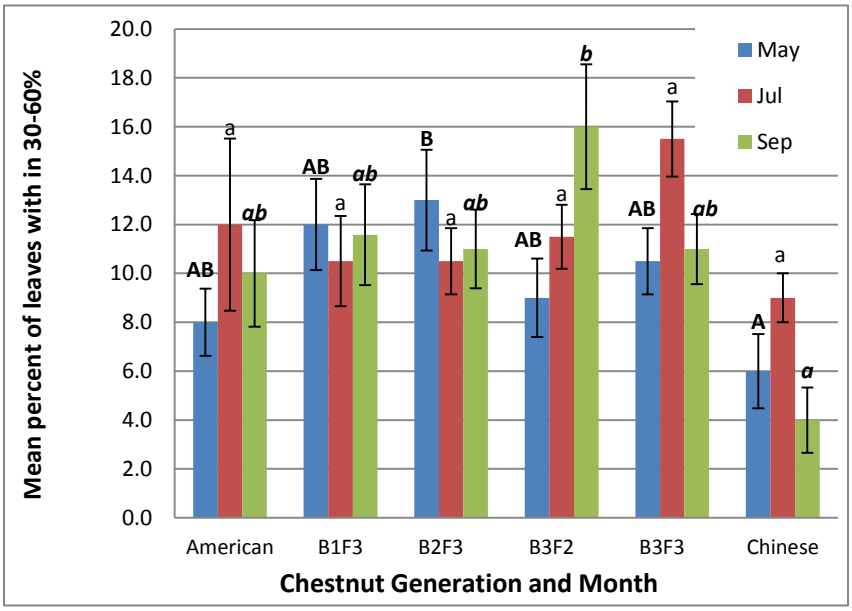

Figure. 1. Site TN 30-60% defoliation category, by generation and month.

Figure 1. Mean percentage of leaves in the 30-60% defoliation category, by generation and month, at site TN in 2012. Within month, bars labeled with the same letter are not significantly different.

At site NC, July and September exhibited no significant difference between generations defoliated within the 30-60 percent category. In May the BC_2F_3 and Chinese chestnut generations had more defoliation in the 30-60% category than the American and BC_3F_3 generations (F=7.16;df=1,4;P=.000) (Figure. 2).

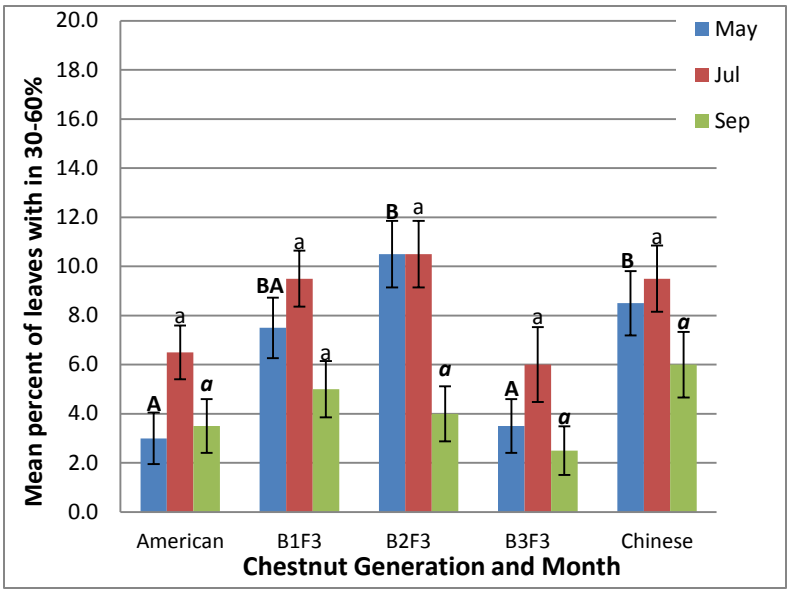

Figure. 2. Site NC 30-60% defoliation category, by generation and month.

Figure 2. Mean percentage of leaves in the 30-60% defoliation category, by generation and month, at site NC in 2012. Within month, bars labeled with the same letter are not significantly different.

The defoliation index at site NC (Figure 3) and TN (Figure 4) shows little variation between American, and Chinese species, and the different generations of hybrids.

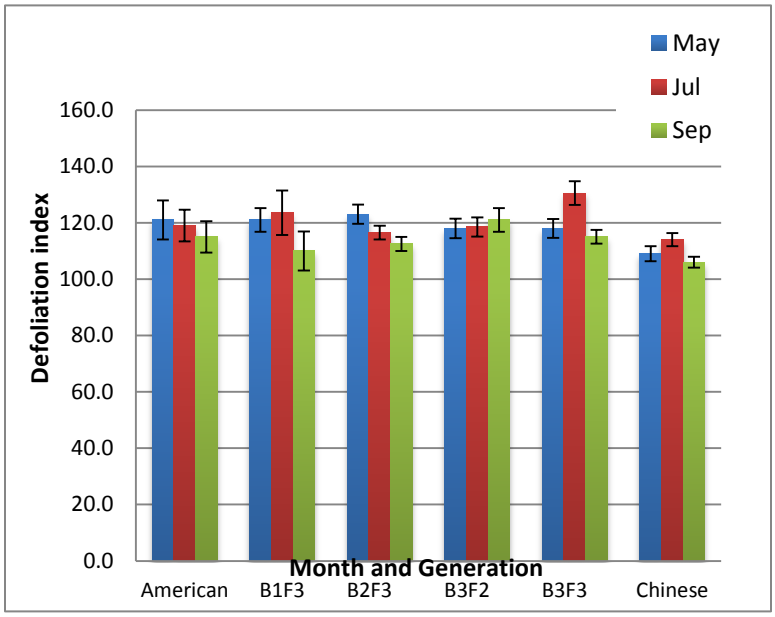

Figure. 3. Chestnut Defoliation at site TN

Figure 3. Calculated chestnut defoliation index, by month and generation, with at site TN in 2012 with error bars.

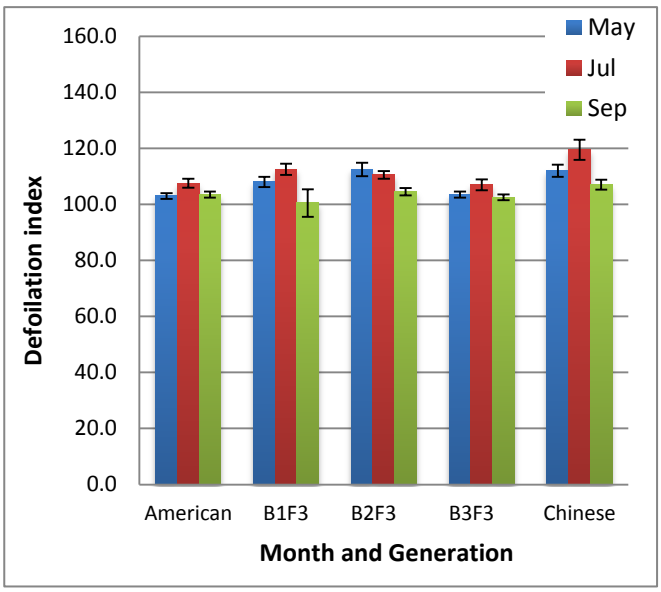

Figure 4. Chestnut Defoliation Index at site NC

Figure 4. Calculated chestnut defoliation index, by month and generation, with at site NC in 2012 with error bars.

3.3. Weevil Abundance

Significantly more *C. castaneus* adults were collected per seedling in July and September than in May at both sites (Figure 5). May collection at TN had no significant difference between American, Chinese, and hybrid chestnuts (F=0.638;df=1,5;P=.671). At site TN in July, fewer Asiatic oak weevils were collected from Chinese chestnuts than from BC_3F_2 and BC_3F_3 hybrids. At TN in September, fewer weevils were collected from Chinese chestnuts than from all other chestnut hybrids and American, except for the BC_1F_3 hybrid (F=4.077;df=1,5;P=.002)(Figure. 6).

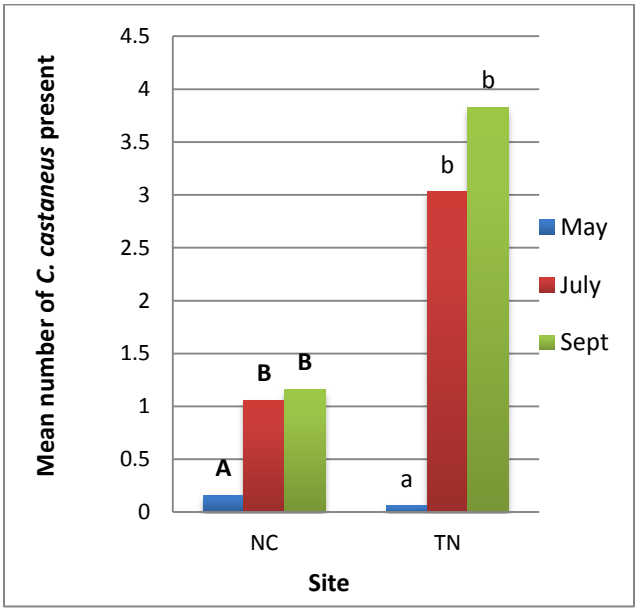

Figure 5. *Cyrtepistomus Castaneus* collected at sites by month.

Figure 5. Mean number of *C.* Castaneus present at NC and TN in early, mid, and late growing season. Within site, bars labeled with the same letter are not significantly different.

Fewer *C. castaneus* were collected at NC than at TN. In May at NC, fewer weevils were collected from Chinese than from American chestnuts (F=2.631;df=1,4;P=0.039). There was no significant difference (F=1.721;df=1,4;P=0.152) among generations in July at NC. In September, there was a greater abundance on American than on Chinese chestnuts (F=3.575;df=1,4;P=.009) (Figure 7).

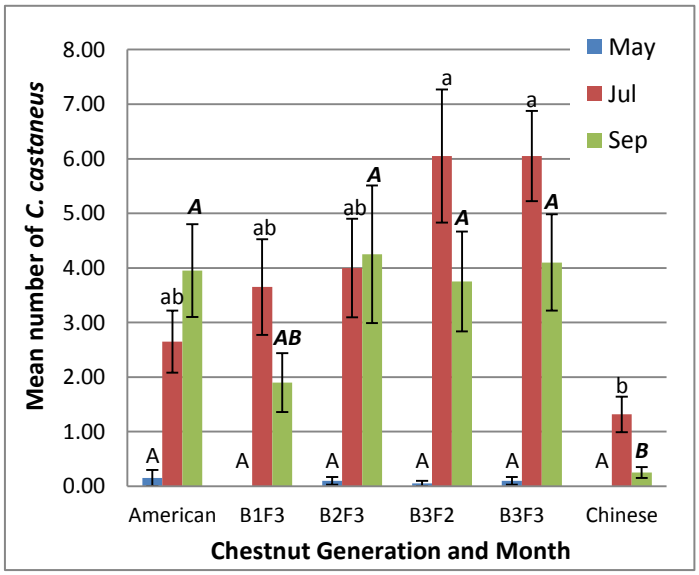

Fig. 6 Site TN mean of *C. castaneus* per tree by generation and month

Figure 6. Mean number of Asiatic oak weevils collected per tree, by generation and month, at site TN in 2012. Within month, bars labeled with the same letter are not significantly different.

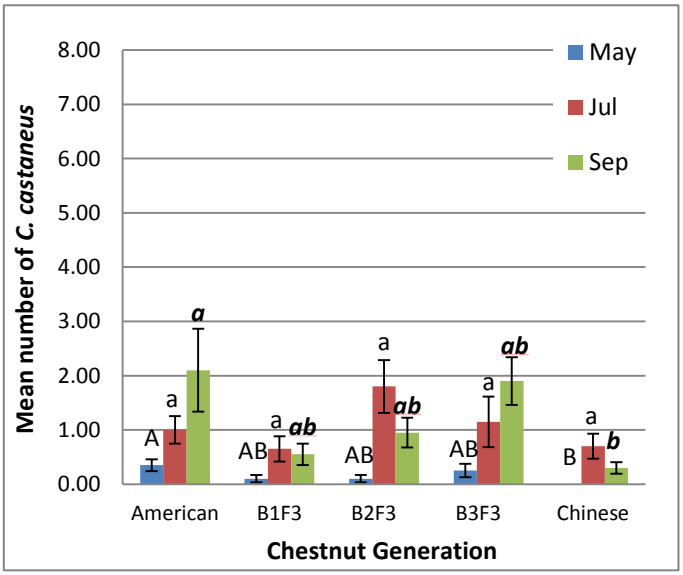

Figure. 7 Site TN mean of *C. castaneus* per tree by generation and month

Figure 7. Mean number of Asiatic oak weevils collected per tree, by generation and month, at site NC in 2012. Within month, bars labeled with the same letter are not significantly different.

3.4. Weevil Emergence

Emergence of adult *C. castaneus* at site TN began in late May, peaked between mid-June and mid-July, and continued to decrease into October (Fig. 8). Significantly more weevils emerged from traps located beneath chestnut oak (*Q. prinus*) canopy than beneath non-oak canopy (P=0.004) (Fig. 9).

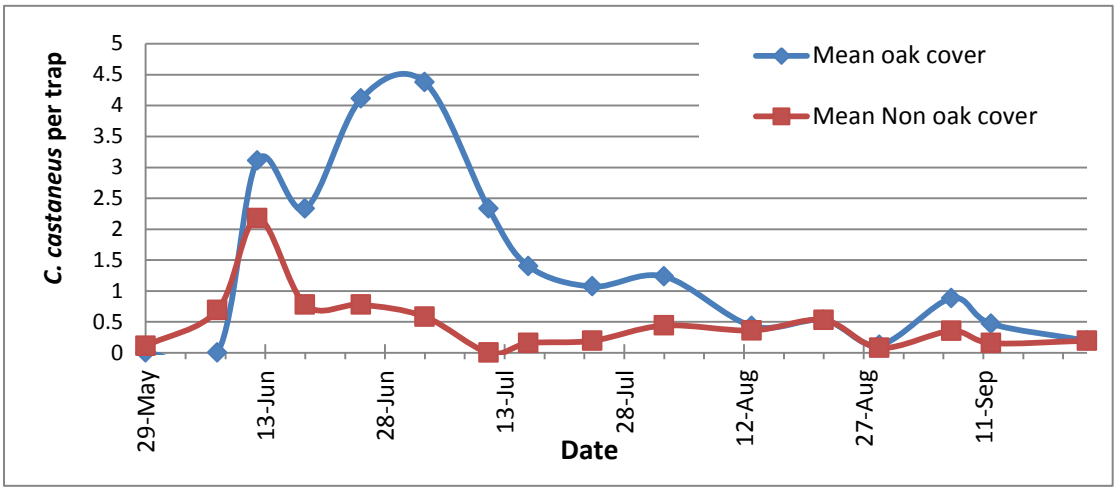

Figure.8. *C. Castaneus* per trap by week.

Figure 8. Mean numbers of *C. Castaneus* per trap per week, under oak and non-oak canopy at site TN 2012. All points represent dates of collection.

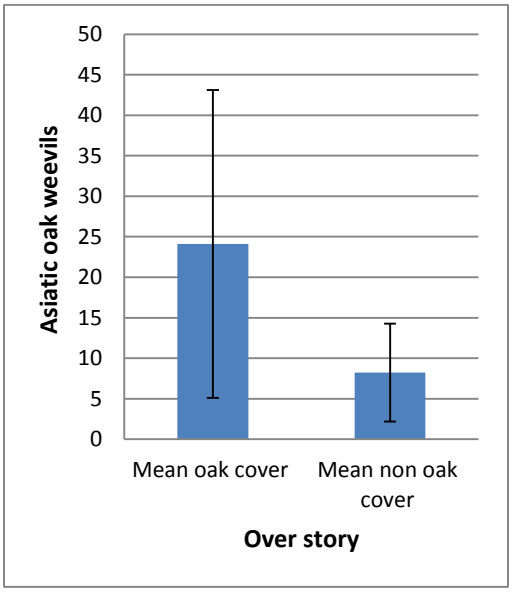

Figure. 9. *C. castaneus* collected below overstory.

Figure 9. Mean numbers of total *C. castaneus*, collected in traps which emerged under oak and non-oak canopy.

4. Discussion

The defoliation ratings do not support any trends suggesting that American, Chinese, or hybrid chestnuts have consistent differences in feeding throughout the growing season. During the growing season of 2012 when comparing the defoliation data with the abundance of Asiatic oak weevils, there appears to be subtle positive trends between mean numbers of *C. castaneus* and the amount of defoliation in the 30-60% category. For example, at site TN where Asiatic oak weevil densities were highest, Chinese chestnut seedlings sustained less defoliation and harbored fewer weevils than the hybrid chestnut generations (Figures 1,6). Further statistical analysis is needed to be certain of this correlation.

Chestnut defoliation at our sites was relatively low, despite our initial observations that defoliation was extreme. Additionally, we did not find significant differences between generations that were consistent among sites and throughout the growing season. However, at site TN, the results suggest that Chinese chestnuts may contain traits (e.g., leaf pubescence or secondary compounds) that make it less prone to colonization and herbivory by the Asiatic oak weevil, compared to American and hybrids. A significantly fewer mean number of Asiatic oak weevils were found on the Chinese chestnuts than on the American and hybrid chestnuts.

At site NC, there was less defoliation of all American, Chinese, and hybrid trees than at site TN. Although Chinese chestnut had the highest rate, in the mean percent of leaves within the 30-60 percent category, it did not exceed 10 percent. The mean number of Asiatic oak weevils also did not exceed one per tree throughout the growing season in the Chinese or BC_1F_3. The BC_1F_3 is the most genetically similar to the Chinese chestnut of the hybrids studied.

The study focused on *C. castaneus.* However, the defoliation ratings assessed all herbivore damage, with the exclusion of deer. To more clearly understand the threat of the Asiatic oak weevil, it would be important to devise a rating system that specifically rated weevil damage: a type of herbivory that tunnels between the veins of the leaf toward the midrib. It was also noted during the defoliation ratings that the majority of weevil defoliation occurred on the lower most foliage of the trees. This should be taken into consideration to formulate a new rating system.

Adult Asiatic oak weevils had a much higher emergence rate in traps with oak overstory than non-oak cover. Abundance of oak may imply an abundance of phytophagous insects that specialize in defoliating species in the Fagaceae family, making an area unfit for an abundance of chestnut saplings. This may have implications for site selection of new chestnut plantations. Additional research is needed to understand how much the Asiatic oak weevil or other defoliators impact growth of the various chestnut generations.

5. Summary and Conclusion

There is not a significant difference in defoliation between American, Chinese, and hybrid generations, although some of the data suggest less defoliation on Chinese chestnut. The greatest mean number of Asiatic oak weevils was found on the American and the BC_3F_3 hybrids. Fewer mean numbers of *C. castaneus* were found per tree on the BC_1F_3 and Chinese. The American and its most closely related hybrids were more prone to colonization by the Asiatic oak weevil. As blight resistant chestnuts are reintroduced, it will be important to understand and anticipate potential threats. The Asiatic oak weevil has become abundant in Tennessee. Triplehorn noted[10] the weevil was also abundant in New Jersey, Maryland, Delaware and adjacent states in 1955. It is my prediction that *C. castaneus* has become more prominent on the east coast than in the past and has the possibility of becoming a forest pest. The larval stage is unknown[10] but high emergence is found in close association to oaks. Future studies are suggested to understand the affect that *C. casteneus* and other phytophagous insects have on the growth and health of newly introduced chestnuts into their once native range.

6. Acknowledgements

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