Interactions among insect defoliation, insecticide treatments, and growth rate in American and hybrid chestnuts

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Dedication

I would like to dedicate this thesis to my Mother and Grandmother to whom instilled in me the value of education and the ability to pursue one.

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Abstract

The American chestnut, *Castanea dentata,* was once one of the most useful and abundant canopy trees in eastern North American forests. Over the last 200 years, the species has been decimated by two exotic pathogens, *Phytophthora cinnamomi* and *Cryphonectria parasitica,* killing millions of trees and reducing surviving *Castanea dentata* to short-lived sprouts. *Cryphonectria parasitica*-resistance breeding programs are currently producing advanced backcross generations, which are being compared with pure American chestnut in field tests of growth performance and *Cryphonectria parasitica* resistance. The Asiatic oak weevil, *Cyrtepistomus castaneus,* has been identified as a common defoliator of chestnut seedlings in these tests. A study was initiated in eastern Tennessee to quantify the impact of defoliation on growth of American and hybrid chestnut seedlings in 2013 and 2014 and northern red oak, *Quercus rubra,* seedlings in 2014. Defoliation ratings were used to evaluate the efficacy of the insecticides imidacloprid, acephate, and dinotefuran and a water control, applied via soil drench in June 2013. The abundance and emergence timing of *Cyrtepistomus castaneus* was monitored throughout the growing season. Defoliation was relatively low and mean percent defoliation ratings did not differ among American and hybrid chestnut or northern red oak seedlings. In most months, seedlings treated with dinotefuran or imidacloprid had the least amount of defoliation, suggesting both treatments were effective. However, seedlings given water control only became significantly different from dinotefuran and imidacloprid toward the end of the growing season. No strong correlations between two year growth and percent defoliation occurred implying the defoliation levels that were observed did not strongly impact early growth. *Cyrtepistomus castaneus* emergence began in early May, peaked in late July through mid-August, and ended by late November. No significant differences were found in spatial patterns of emergence of *Cyrtepistomus castaneus* suggesting relatively equal distribution of insects on the site.

Key words: defoliation, insecticide, pesticide, *Castanea dentata*, *Cyrtepistomus castaneus*

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Chapter 1. Introduction

The American chestnut [*Castanea dentata* (Marsh.) Brokh.], once one of the most useful and abundant canopy species in the eastern United States, occupied over 200 million acres of forest land from Maine to Mississippi (Wilson 1902, Berry 1980, Russell 1987). Its wood was valued for various products, was rot resistant, and had a high tannin content important to leather making. American chestnut mast was a reliable and an important food source for wildlife, livestock, and people (Wilson 1902, Burnham 1988, Schlarbaum et al. 1997). In the early 1900's, American chestnut was a highly competitive and fast growing species that grew well on a variety of soil types in the United States (Zon 1904, Hawley and Hawes 1912).

Over the last 200 years, American chestnut has been eliminated as a dominate canopy species by two exotic pathogens. *Phytophthora cinnamomi* (Rands) causes ink disease, a destructive root rot found on forest and nursery stock and is most prevalent at low elevations on wet or poorly drained sites and only in the southern United States (Crandall et al. 1945, Anagnostakis 1987). *Phytophthora cinnamomi* is thought to have been introduced to the southeastern United States as early as 1824 (Milburn and Gravatt 1932, Balci et al. 2013), although was not confirmed until the 1930's on a dying American chestnut (Crandall et al. 1945, Anagnostakis 2012). Chestnut blight [causual pathogen *Cryphonectria parasitica* (Murrill) Barr, formerly *Endothia parasitica* (Murr.), And. and And.] was noted in the early 1900's in the northeastern U.S. (Merkel 1906, Metcalf and Collins 1909, Schrenk and Spaulding 1909, Diller and Clapper 1965, Anagnostakis 1995). By the 1950's, *C. parasitica* had spread throughout nearly all of the native range. The resulting chestnut blight disease has been responsible for killing millions of trees and reducing chestnuts to short-lived understory sprouts (Crandall et al. 1945, Saucier 1973, Anagnostakis 1995, Paillet 2005, Anagnostakis 2012). Both of these pathogens still present a challenge for restoration efforts in forest and nursery settings (Anagnostakis 2012, Clark et al. 2014b).

In response to *C. parasitica*, multiple programs were initiated to restore a chestnut species to eastern forests (Graves 1950, Diller and Clapper 1965, Schlarbaum et al. 1994, Anagnostakis 2012). The American Chestnut Foundation (TACF) has primarily used Chinese chestnut

(*Castanea mollissima* Blume) to introduce *C. parasitica* resistance to American chestnut via a backcross breeding approach (Burnham 1988, Hebard et al. 2014). Seedlings from the third intercross of a third backcross generation, BC_3F_3 (hereafter referred to as hybrid chestnuts) are the most recent breeding generation. Hybrids are anticipated to have similar growth characteristics to American chestnut and to retain intermediate to high levels of *C. parasitica* resistance from Chinese chestnut (Hebard et al. 2014). Studies using parental and hybrid chestnuts are currently underway in anticipation of widespread planting and distribution (Rhoades et al. 2009, Clark et al. 2014b). Native and exotic insect damage and defoliation are thought to be one of the leading primary biotic factors that will influence reintroduction efforts (Anagnostakis 2012, Clark et al. 2014b).

Multiple studies have identified the Asiatic oak weevil [*Cyrtepistomus castaneus* (Roelofs)] as a harmful exotic pest that has the potential to negatively affect chestnut restoration (Connors et al. 2002, Anagnostakis 2012, Wang et al. 2013, Clark and Schlarbaum 2014a). *Cyrtepistomus castaneus*, indigenous to Japan, was first noted in the United States in 1935 and has since been observed in many eastern states (Triplehorn 1955, Johnson 1956, Roling 1979, Ferguson et al. 1992). The pest has been reported to cause up to 35% defoliation on Chinese chestnuts (Johnson 1956) and is one of the most common insects in association with trees in the Fagaceae family (Linit et al. 1986, Stanton 1994). The larva also impact roots and reproduction by feeding on fine root hairs and emerging radicals of acorns (Triplehorn 1955, Roling 1979).

Cyrtepistomus castaneus is just one of the many insects that are known to feed on chestnuts in the United States, although the insects' spatial distribution in emergence is poorly understood across geographic locations. Other native and exotic insects have been identified to severely impact chestnut. Native insects include: the two lined chestnut borer (*Agrilus bilineatus* Weber) (Chittenden 1897), chestnut timber worm *(Corthylus columbianus* Hopkins) (Ashe 1911), chestnut sawfly, *Craesus castaneae* Rohwer (Pinchot et al. 2011), small chestnut weevil, *Curculio sayi* Gyllenhal, and the large chestnut weevil *Curculio caryatrypes* L. (Payne et al. 1983). Exotic insects that have been acknowledged as a threat on chestnut include: Ambrosia beetles (*Xylosandrus crassiusculus* Mot. and *X. saxeseni* Blandford) (Oliver and Mannion 2001), Asian chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu,(Dixon et al. 1986, Clark et al.

2014b), and European gypsy moth (*Lymantria dispar* L.) (Mosher 1915). Multiple studies have noted severe defoliation by unknown insects, which have potentially affected overall seedling performance (McCament and McCarthy 2005, Rhoades et al. 2009, Clark and Schlarbaum 2014a). In general, the effects of insect herbivory on chestnut species in the U.S. is not well understood.

Knowledge of impacts from insect herbivory can influence success of chestnut restoration in eastern forests and horticultural settings. Identification of effective insecticides could provide managers with essential knowledge to efficiently protect trees against excessive herbivory in urban and seed orchard settings. However, no studies have been done to evaluate insecticides on herbivory of American or hybrid chestnut in the United States.

A northern red oak seedling seed orchard, on the Cherokee National Forest in northeastern Tennessee, was the subject of an insect diversity study in the 1990s of which *C. castaneus* comprised 25% of the total insect abundance (Stanton 1994). Due to the documented presence of this insect in high numbers, a study to evaluate the defoliation of American chestnut, hybrid chestnut, and northern red oak (*Quercus rubra* L.) seedlings throughout the growing season was initiated at this orchard. This study was designed to test efficacy of insecticide treatments on seedlings and to monitor emergence and spatial patterns of *C. castaneus*. The study had three primary objectives:

1. Evaluate species and insecticide treatment effects on defoliation severity and growth rates among American chestnut, hybrid chestnut and northern red oak seedlings.
    a. Determine family within species and insecticide treatment effects on defoliation severity and growth rates.
2. Compare visual and digital types of defoliation rating systems on American and hybrid chestnut seedlings.
3. Monitor the timing and emergence patterns of adult *Cyrtepistomus castaneus* around the perimeter of the orchard.

Chapter 2. Literature Review

American chestnut

The American chestnut, was once one of the most common overstory trees throughout hardwood forests in the eastern United States (Ashe 1911, Russell 1987). Its natural range included over 200 million acres of land from Ontario, Canada, and Maine to Mississippi; west through Tennessee and Ohio, with a few outlier populations in Michigan, Illinois and Iowa (Figure 2-1 ((Wilson 1902, Ashe 1911, Berrry 1959, Kuhlman 1978, Russell 1987, Anagnostakis 2012). American chestnut was prominent throughout its native range, growing between 18 to 37 meters in height and up to 2.5 meters in diameter. The species has been shown to regenerate from natural seeding, but was best regenerated through sprouting. It grows best on north and west slopes between elevations of 548 and 1066 meters (Zon 1904, Ashe 1911). American chestnut historically grew in a variety of conditions such as shallow soils on dry ridges and steep slopes (Wilson 1902, Ashe 1911), but also on more moist, bottomland sites (Crandall et al. 1945). On upland sites, American chestnut trees do best on loose soils mostly consisting of fine, sandy loam with a moderate amount of clay, well drained on the surface, and containing moderate moisture. Alkaline soils are the only soil type American chestnut has been found not growing well. Soil depth is also important as chestnuts are a deep rooted species, roots often spread 0.9 to 1.1 meters deep (Hough 1878, Ashe 1911, Hawley and Hawes 1912).

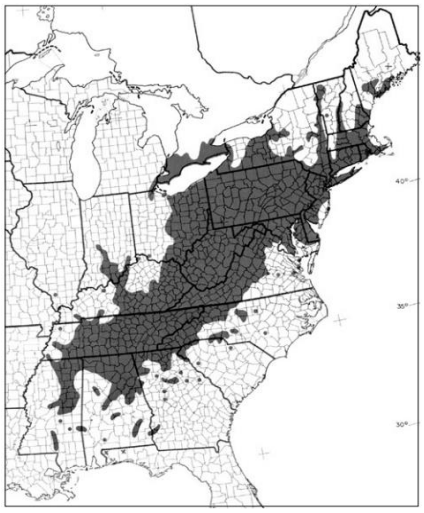


Figure 2-1 Native range of American chestnut, Castanea dentata, in the eastern United States (Saucier 1973)

Early growth.

Pre-*Cryphonectria parasitica* records reported American chestnut to be highly competitive and fast growing (Zon 1904, Ashe 1911, Hawley and Hawes 1912). Ashe (1911) reported that the average five year old American chestnut was between 4.6 to 5.5 meters tall. Others have reported chestnuts to have an even more rapid growth rate of 2.1 meters in the first year in the forest (Mattoon 1909, Hawley and Hawes 1912) and 64 cm in the second and third growing seasons(Mattoon 1909). Zon (1904) reported when sprouts arise from chestnut stumps they can reach heights up to 2.4 meters in the first year, although when grown from direct seed they seldom even reach 0.3 meters. More recent work examining early growth of the species have found pure American chestnuts diameter growth can average one cm per year and height increases close to one meter yearly for the first 20 years (Paillet 1989). Additionally, a study in a plantation setting in Wisconsin found that height increased an average of 84 cm per year in the first seven to eight growing seasons (Jacobs and Severeid 2004). After two growing seasons, studies have shown planted pure American chestnuts in eastern Kentucky averaged 94cm height growth per year (Rhoades et al. 2009). These more recent studies (Paillet 1989, Jacobs and Severeid 2004, Rhoades et al. 2009) have shown American chestnut growth rate to be similar to Ashe's observations, however none have shown the extremely low rates noted by Zon (1904) or extremely high rates reported by Hawley and Hawes (1912).

Utilization

American chestnut lumber was widely utilized in products, such as railroad ties, fence post, musical instruments and construction due to its rot resistance, durability, and abundance (Zon 1904, Ashe 1911, Anagnostakis 1987, Russell 1987). However, strength properties such as bending, maximum stress in compression parallel, and perpendicular to grain and shear strength of grain are relatively low in comparison to other hardwood species (Green et al. 1987). Chestnut was extremely important to the lumber industry, more than 71 million board feet were harvested in a single year in Tennessee in 1909 (Ashe 1911) and annual yields in southern Maryland were reported to be over 4.8 million board feet (Zon 1904).

In addition to its use in the lumber industry, American chestnut is a highly productive nut crop species, which is seldom damaged by frost as it flowers in the late May to early July (Zon 1904,

Paillet 2005, Wang et al. 2013). Bountiful mast production was reported to naturally occur every few years (Ashe 1911). The species is thought to have provided higher yields than any other hard mast species in eastern U.S. forest (Diamond et al. 2000, Burhans and Hebard 2012). Its mast production was utilized by wildlife, people, and sustained rural Appalachian communities through feeding of their livestock and selling or trading nuts for other goods (Thoreau 1854, Kuhlman 1978, Anagnostakis 2012). Furthermore, tannins were extracted from the bark and heartwood for use in tanning leather. Chestnut tannin extract was the main vegetable based contributor of tannin production in the United States until the 1930's (Burnham 1988, Davis 2006).

Major pathogen problems

While the American chestnut had no major native pathogens, the introduction of two exotic pathogens has nearly eradicated the species. The two diseases, ink disease and chestnut blight, were first confirmed in chestnut on southern and northern sites, respectively. *Phytophthora cinnamomi* causes root rot and widespread mortality in the southeastern and central United States to multiple species. *Phytophthora cinnamomi* mainly affected lowland chestnuts, but can also affect the species on poorly drained or clay soils in higher elevations in years with extreme rain (Milburn and Gravatt 1932, Balci et al. 2013). The pathogen is thought to have been introduced on plants imported from Europe as early as 1824 to 1838 (Milburn and Gravatt 1932, Crandall et al. 1945). *Phytophthora cinnamomi* was most likely described by Franklin Hough in 1878 as a pathogen that 'appears in the spring, according to location and weather…The malady has appeared on all kinds of soils but has proved most destructive upon those that are poor, wet, and sandy (Hough 1878). Confirmation of mortality from the pathogen *Phytophthora cinnamomi*, a soil-borne water mold, occurred in the 1930's, after it was isolated from a dying American chestnut (Crandall et al. 1945, Balci et al. 2013). Presently, *P. cinnamomi* remains a serious problem for chestnut seedlings in bare-root nurseries and prohibits successful establishment for restoration primarily on wet or poorly drained sites in the southeastern United States, but the disease can be virulent on any site given proper weather or soil conditions (Anagnostakis 2012, Clark et al. 2014b).

Cryphonectria parasitica is the causal pathogen for chestnut blight (chestnut bark disease), which causes cankers on all *Castanea* species including native American chestnut, Allegheny chinquapin [*Castanea pumila* (L.) Miller], and Ozark chinquapin [*Castanea ozarkensis*(L.) Miller]. This pathogen is thought to have been imported on Japanese chestnut (*Castanea crenata* Siebold & Zucc.) nursery stock (Anagnostakis 1995) and was first noted on dying chestnuts in the New York Bronx Zoo in 1904 (Merkel 1906). However, it is suspected that the *C. parasitica* was previously present in Newark, Delaware in 1902, labeled as a "bark disease" and in Bedford County VA in 1903 (Metcalf and Collins 1909, Anagnostakis 2012). The pathogens enters the tree through wounds or bark fissures and attacks the cambium by lowering the pH, inhibiting cell development. It extends in all directions until the branch or trunk is girdled leading to death above the infected area and eventually the tree (Schrenk and Spaulding 1909).

Post-blight distribution of American chestnut

By 1950, *Cryphonectria parasitica* was reported in nearly the entire native range of American chestnut. This resulted in American chestnut being ecologically extirpated as a dominant canopy species in eastern forests (Berrry 1959, Burnham 1988, Anagnostakis 1995). *Cryphonectria parasitica* pathogen, however, does not enter the xylem or live in the soil and therefore, the roots of American chestnut are not affected allowing trees to re-sprout vigorously from stumps (Mattoon 1909, Berrry 1959). This has resulted in many short-lived sprouts and multiple stemmed shrubs that can live for decades throughout chestnuts native range (Saucier 1973, Anagnostakis 1995, Paillet 2005). In the southern Appalachians, American chestnut is typically found in the understory, usually less than eight cm in diameter and densities greater than 1,000 trees per hectare (Griffin et al. 1983). A study in southern New England found 50 American chestnut sprouts per hectare in the understory of old growth forests (Paillet 1988). Large trees (>25cm DBH) have been found occurring in the southern Appalachians at frequencies from zero to seven trees per county (Griffin et al. 1983). The occasional large American chestnuts still persist today although "large trees" have been redefined to have DBH of ≥ 38cm and at least 75% of live crown (Griffin, personal communication 2015).

Native insect pests

While chestnut blight and ink disease continue to impact remnant populations of chestnut sprouts, insect damage are also expected to influence the success of American chestnut restoration (Anagnostakis 2012, Clark et al. 2014b). As early as 1893, the native two lined chestnut borer was reported responsible for the death of 75% of the chestnut trees in Fairfax County, Virginia (Chittenden 1897). This pest contributes to mortality of stressed trees by making galleries under the bark, which eventually combine to girdle the tree. The chestnut timber worm burrows through standing trees leaving holes and discoloration. American chestnut lumber that is harvested from insect damaged trees is referred to as "wormy chestnut" and compromises the integrity of the wood (Ashe 1911). Several other insect pests have been reported on American chestnut impacting both the leaves and the developing burs. Late-season defoliation on *Castanea* spp. caused by the chestnut sawfly has been noted ranging from moderate to severe on American, Chinese and hybrid chestnuts (Pinchot et al. 2011). Weevils such as the small chestnut weevil and the large chestnut weevil will lay their eggs in ripening burs and the larva will eat their way out of the nuts (Brooks and Cotton 1929, Payne et al. 1983, Anagnostakis 2012). Weevil damage often times leads to complete loss of the seed. When tested in a growth chamber, seeds damaged by weevils that germinated exhibit reduced growth by nearly 50 percent compared to undamaged seed (Dalgleish et al. 2012).

Exotic insect pests

In addition to the native pests, there are numerous exotic insect pests that impact the growth and survival of American chestnut. Ambrosia beetles (*Xyleborinus crassiusculus* Mot. and *X. saxeseni* Ratzeburg) bore through bark and into the sapwood, introducing a symbiotic fungus which serves as food for adults and larva (Oliver and Mannion 2001, Anagnostakis 2012). Ambrosia beetles often inoculate trees with additional harmful fungi or bacteria upon entry and can spread pathogens between trees easily and can cause decline and mortality (Oliver and Mannion 2001). The Asian chestnut gall wasp deposits eggs in developing seedlings, trees, leaves and flower buds. The resulting larvae forms galls, leading to suppressed shoot growth, reduced fruiting, deformation or death of the leaves or flowers (Dixon et al. 1986, Cooper and Rieske 2011). Severe gall wasp infestation on chestnuts can lead to 50-70 percent nut reduction and may result in the trees decline and death (Dixon et al. 1986, Graziosi 2015). Although

chestnut was considered resistant species to gypsy moth defoliation by Twery (1990) observations were made as early as 1915 of gypsy moth feeding aggressively on American chestnut (Mosher 1915). A study that assessed forest canopy noted severe defoliation from gypsy moth on American chestnut (Cooper et al. 1993). Rieske et al. (2003) enclosed gypsy moths on American chestnut and hybrid chestnut seedlings in growth chambers and found gypsy moths feed more on hybrid chestnuts than on pure American chestnut (Rieske et al. 2003), which has implications for restoration programs using Asian germplasm. Other silvicultural studies involving planted American chestnuts have noted moderate to severe defoliation by unidentified insect pests, potentially effecting overall seedling performance (McCament and McCarthy 2005, Rhoades et al. 2009, Clark and Schlarbaum 2014a).

Cyrtepistomus castaneus damage

The Asiatic oak weevil, *Cyrtepistomus castaneus*, has been reported as a harmful exotic pest to American chestnut restoration efforts (Connors et al. 2002, Anagnostakis 2012, Wang et al. 2013, Clark and Schlarbaum 2014a), although the USDA Forest Service classifies C. castaneus as a minor defoliator (Frederick and Gering 2006). This pest, indigenous to Japan, is primarily found on Quercus spp. and Castanea spp. (Triplehorn 1955, Evans 1959, Frederick and Gering 2006) and is suspected to be regulated by natural enemies in Japan (Frederick and Gering 2006). The weevil was first recorded in North America in New Jersey in 1935 (Triplehorn 1955, Roling 1979) and has since been observed in many eastern states (Figure 2-2) (Triplehorn 1955, Johnson 1956, Ferguson et al. 1992). Cyrtepistomus castaneus was found to be abundant on trees in the Fagaceae family (Triplehorn 1955) and more prevalent on chestnut trees than on oak trees (Johnson 1956).

Multiple studies have found *C. castaneus* occurring in high abundance in the mid and eastern United States (Triplehorn 1955, Johnson 1956, Linit et al. 1986, Stanton 1994, Case et al. 2012, Clark and Schlarbaum 2014a). In 1956, Asiatic oak weevils caused up to 35% defoliation on Chinese and hybrid chestnut trees in a Maryland orchard (Johnson 1956). Emergence traps at a northern red oak orchard, in the Missouri Ozarks, showed *C. castaneus* to be the most common insect encountered and contributed to poor oak regeneration (Linit et al. 1986). On northern red oaks, *C. castaneus* have been responsible for an average of 22% of total leaf area loss (Wright et

al. 1989). Another insect survey at a northern red oak orchard in eastern Tennessee found *C. castaneus* to be the most common beetle species, accounting for 25% of all insect abundance (Stanton 1994). *Cyrtepistomus castaneus* studied at two field plantings in the southern Appalachians have been found to occur in higher abundance on American and hybrid chestnut than on Chinese chestnut (Case et al. 2012). Most recently, *C. castaneus* was reported to cause defoliation on 10 - 36% of *Castanea* spp. on plots studied in the southern Appalachian mountains (Clark and Schlarbaum 2014a).

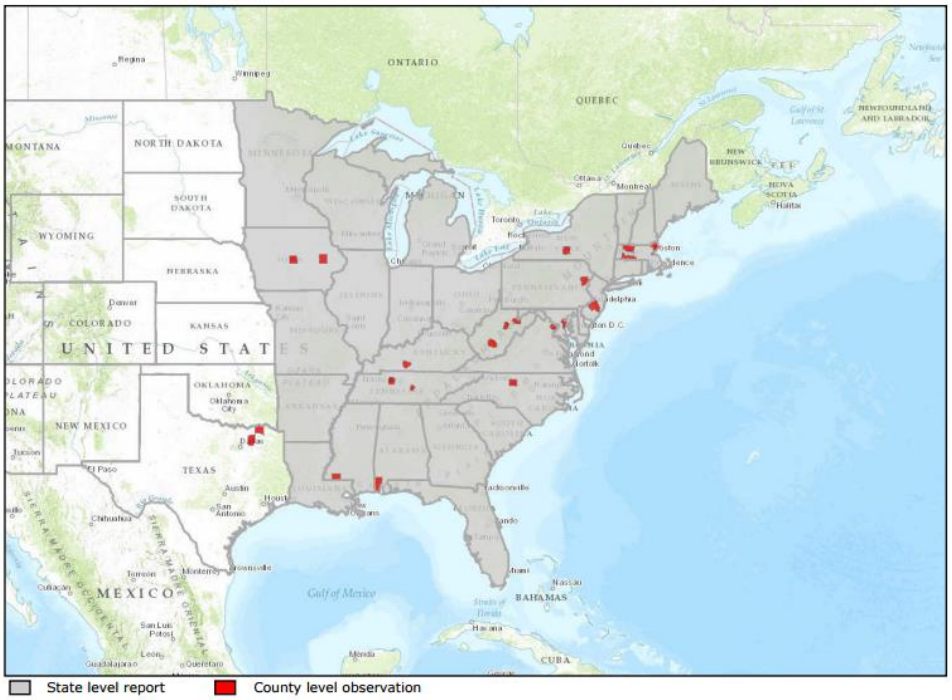


Figure 2-2. State and county level report map of the Asiatic oak weevil, Cyrtepistomus castaneus, in the eastern United States (USDA 2013).

Cyrtepistomus castaneus emergence

The life cycle of *C. castaneus* produces one generation per year undergoing egg, maturation, larva, pupa and the adult stage (Triplehorn 1955). A field study in Delaware noted adults beginning to emerge from leaf litter as early as May 10th with significant numbers of adults appearing by June 18th (Triplehorn 1955). A laboratory study by Roling (1979) in central

Missouri determined overlapping time periods of *C. castaneus* life stages, the larval stage is present in the soil from August until the following July. Pupa were found to persist in the upper five centimeters of the soil, in June and July, followed by adult emergence from approximately June until August (Roling 1979). Peak emergence was in July (Roling 1979). Larva are known to feed on fine root hairs of trees and emerging radicals of acorns, while adults feed primarily on foliage, preferably in the Fagaceae family (Triplehorn 1955, Roling 1979). Timing of peak defoliation has varied among geographic location. In Maryland, *C. castaneus* defoliation was reported in early August and becomes progressively more severe by the middle of September (Johnson 1956); whereas a study in Delaware reported most severe defoliation by *C. castaneus* at the end of June (Triplehorn 1955). Adults have been found as late as October 25 in Delaware (Triplehorn 1955) and in central Maryland, adults were found February 27 in the leaf litter (Evans 1959).

Insecticide treatments

Previous research concerning insecticide treatments on pure American chestnut in the United States is limited with more work being done in Asia and Europe. One study controlling gall wasps on American, Chinese and Japanese chestnut found systemic insecticides were ineffective, most likely due to the gall blocking translocation (Dixon et al. 1986). A Chinese chestnut orchard made recommendations to control weevil populations using a foliar insecticide after weevils emerged or the insecticide may be applied as a soil drench to treat larva (Payne et al. 1983). In a report concerned with high levels of defoliation on Chinese and hybrid chestnuts caused by *C. castaneus*, DDT (dichlorodiphenyltrichloroethane) spray or oil fog, was suggested for easy eradication (Johnson 1956), although DDT is now banned in the United States (EPA 1972). Chestnut orchards in Asia and Europe report losing 20-90 percent yearly nut crop yield due to insects (Speranza and Paparatti 2010, Yuan et al. 2011). A chestnut orchard in China used Thiacloprid DP to control the yellow peach moth (*Dichocrocis punctiferalis* Guenee) saw 16.4% less damage on treated trees than untreated trees (Yuan et al. 2011). A European chestnut (*Castanea sativa* Miller) orchard in central Italy also found Thiacloprid to be most effective to control chestnut gall wasp and chestnut weevils [*Curculio elephas* (Gyll.) and *C. propinquus* (Desbr.)] (Speranza and Paparatti 2010). A study in Ukraine has shown treating chestnuts with Imidacloprid to be effective as well (Fedorenko and Gamanova 2009).

Restoration

Multiple research programs have been initiated to restore American chestnut or a chestnut component back into north American forests (Diller and Clapper 1965). Breeding programs have focused on finding Asian chestnuts resistant to *C. parasitica*, within *Castanea* spp.), while other programs has studied the use of hypovirulent strains of *C. parasitica*. The availability of experimental materials has led to orchards producing chestnuts with varying levels of resistance. These seedlings are now being evaluated to define silivicultural parameters for best field planting practices.

The first scientist to attempt to breed a hybrid American chestnut was Van Fleet (1914). Prior to chestnut blight pathogen he conducted cross pollination experiments among European, Chinese and Japanese chestnuts from 1894-1911 in an attempt to improve nut quality. Most of Van Fleet's trees were producing nuts and averaged 7.6m tall, when they succumbed to chestnut blight in 1910 (Van Fleet 1914).

Testing of Asian chestnuts for resistance and replacement of American chestnut.

In the 1920's, the U. S. Department of Agriculture was in search of a *C. parasitica* resistant Asiatic chestnut that would grow well in eastern north American climates and could interbreed with American chestnut. Between 1936 and 1939, 21 plots of Asiatic chestnut orchards were established that consisted of approximately 22,000 trees (including Chinese, Henry, Japanese, Seguin species and hybrids). After 25 years of testing, one Chinese chestnut seed source from Nanking appeared to be better than the rest, resulting in its nuts exclusively utilized for years by the USDA Forest Service for wildlife-habitat-improvement programs and newly established seed orchards (Diller and Clapper 1965). An assessment of these plantings by Schlarbaum et al. (1994) showed that Asian chestnuts are not competitive in eastern forests without intensive management probable only on certain sites (Miller et al. 2014). Although this study indicates that Asian chestnuts cannot replace American chestnuts in respect to timber production, they could be a valuable hard mast crop for wildlife if managed (Schlarbaum et al. 1994) .

A program initiated by Dr. Arthur Graves in 1929, and later incorporated into the Connecticut Agricultural Experimental Station's (CAES) breeding program, was initially in search of a *Castanea* species that could replace the American chestnut in US forests (Graves 1950,

Anagnostakis 1987). Graves planted over 2,000 individual chestnut trees consisting of 12 species and 1,000 hybrids (Graves 1950). He rated their growth form and canker growth caused by *C. parasitica* concluding *C. mollissima* was the most resistant (Graves 1950). Starting in the late 1940's, Diller and Clapper planted 14 test plots with a mix of hybrid and Chinese chestnuts, comprised of over 12,000 trees (Diller and Clapper 1965, Berry 1980). Of the thousands of chestnuts produced, about three percent showed some degree of *Cryphonectria parasitica* resistance (Diller and Clapper 1965). When reevaluated in 1978, 50 trees (about 4%) were showing no signs of blight pathogen half of which were hybrids and the other were pure *C. mollissima* (Berry 1980). As of 1992, the orchards assembled by Clapper, Diller and Graves that were still in existence, survival ranged from 1.5 to 65.7 percent (Schlarbaum et al. 1994). Schlarbaum et al. (1994) hypothesize that with proper management a few of these orchards would to be able to produce *C. parasitica* resistant chestnuts adapted to eastern North America.

Hybrid chestnut breeding programs

In the early 1980's, a traditional backcross breeding approach was initiated, which has been successfully used in many agronomic and horticultural crops (Briggs 1938, Burnham 1988). Individuals of hybrid progeny F_1 generation were backcrossed with American, producing the BC_1F_1 generation. Continuous screening for *C. parasitica* resistance and desirable form (Burnham 1988) has most recently produced BC_3F_3, the third generation of backcross and three intercrosses (Hebard et al. 2014).

The CAES and TACF have utilized the backcross breeding approach (Anagnostakis 1987, Hebard 2001). American, Chinese and Japanese hybrid chestnuts are utilized to look for resistance to the pathogens that cause chestnut blight and ink disease by CAES (Burnham 1988, Anagnostakis 2012), but TACF primarily use the Nanking Chinese chestnut in their backcross breeding program in search of chestnut blight resistance (Burnham 1988, Hebard et al. 2014). Continuous breeding has led to a BC_3F_3 generation that is theoretically $15/16^{th}$ American chestnut and is reported to have intermediate to high levels of resistance (Hebard et al. 2014). Field testing of BC_3F_3 generations started in 2009 (Hebard et al. 2014, Clark et al. 2014b). Chestnut blight occurrence on BC_3F_3 was reported to be similar to that of Chinese chestnuts, which is lower than American (Clark et al. 2014b), but cankering severity was relatively low

overall. Field test plantings are still too young to make definitive inferences regarding *C. parasitica* resistance (Clark et al. 2014b), as natural infection occurs in five to 25 years after planting (Samman and Thor 1975).

Pure American chestnut breeding programs

A few programs have attempted to breed pure American chestnuts (Thor 1976, Griffin et al. 1983). Studies started in 1960 by Dr. Eyvind Thor, at The University of Tennessee's Tree Improvement Program, bred surviving American chestnuts that possessed low levels of *C. parasitica* resistance (Thor 1976). The hope was that this would increase resistance in progeny that would be greater than either parent (Samman and Thor 1975, Thor 1976). Thor's work is no longer active, due to total loss of progeny in the late 1970s and early 1980s (Schlarbaum, personal communication, 2015). The American Chestnut Cooperators' Foundation (ACCF), founded in 1984, also uses pure American chestnuts that have low levels of *C. parasitica* resistance (Griffin et al. 2006) and field tests the progeny in combination with hypovirulent strains of *C. parasitica* (Griffin et al. 1983, MacDonald and Double 2004). ACCF has collected scions off selected large American chestnut survivors and grafted them onto American chestnut root stock where they are able to test seedlings in selected forest settings (ACCF 2013). A long term field planting (20+ years) by ACCF has shown that the use of hypovirulence on pure American chestnut has been effective in controlling *C. parasitica* on a mesic site at low elevations (Griffin et al. 2006).

Use of hypovirulent strains

Different research programs have been examining the effectiveness of hypovirulent strains of *C. parasitica* on chestnuts as a biological control in the United States (Choi and Nuss 1992, Anagnostakis et al. 1998, Griffin et al. 2006). Hypovirulent stains contain a double-stranded RNA virus and if vegetatively compatible with a virulent strain of *C. parasitica*, are able to transform the virulent strain to a hypovirulent state. Hypovirulent strains usually produce smaller superficial cankers that eventually heal (Anagnostakis and Jaynes 1973, Choi and Nuss 1992). Due to success of hypovirulent strains of *C. parasitica* in Europe, it was anticipated to be a successful tool for management of chestnut blight in the United States (Anagnostakis and

Jaynes 1973, Anagnostakis et al. 1998) However, hypovirulence has not yet been shown to be effective on a large scale in the United States (MacDonald and Double 2004).

Irradiation

Others have attempted to use irradiation to induce mutation to obtain *C. parasitica* resistant American chestnuts (Thor 1976, Dietz et al. 1978). Irradiated seed have been planted as early as 1958, since several thousand seeds have been submitted to radiation (University of Tennessee Agricultural Experiment Station 1962). After planting, some trees have produced nuts but few get taller than 3m before being killed back by the *C. parasitica* (Thor 1976). Dietz et al. (1978) reported irradiated seed germination rates to be between two and 90% and seedling survival to be 42% after 12 years. Highly *C. parasitica* resistant trees were thought to come from succeeding progeny (Dietz et al. 1978). However, further research on irradiated chestnuts has not continued.

Silvicultural studies

Current silvicultural studies have evaluated seedlings from breeding programs, which are becoming available for general testing, or from pure American chestnut. Studies have found pure American chestnut seedlings grew better under shelterwood treatments than mid-story removal treatments (Clark et al. 2012a) and survival was negatively correlated with the amount of sand and course fragments in the soil(Rhoades et al. 2009). Other studies have found chestnuts grow most in height and root collar diameter (RCD) with greater light availability (McCament and McCarthy 2005, Pinchot 2011) but they can survive for many years in relatively low light environments(Rhoades et al. 2009, Clark et al. 2012a). In a shelterwood with reserves, height differences were observed among seedlings from different breeding generations, originating from TACF program. Chinese seedlings grew significantly less than American and hybrid chestnuts, and small seedlings initially grow significantly more than large seedlings (Clark et al. 2011). Multiple field studies have reported herbivory from deer to be one of the leading causes of seedling mortality in unprotected field plantings (Clark et al. 2011, Pinchot 2011, Anagnostakis 2012, Hebard et al. 2014, Clark et al. 2014b).

Chapter 3. Materials and methods

Experimental material

Seedlings from northern red oak, pure American chestnut, and hybrid chestnut (BC3F3) were used in the study. Seed were collected from known open-pollinated mother trees in orchards, and are therefore assumed to be half-sibling progeny with known female parentage and unknown male parentage. Three families of *Q. rubra*, one pure *C. dentata* (AP5) family, and two families of BC_3F_3 hybrid chestnuts, were used in this study.

Northern red oak corns were collected from three mother trees, 1-20-2418, 2-16-528 and 7-14-630, in the fall of 2011 at the orchard (LaFarge and Lewis 1987). Mother trees originated from western NC and eastern TN (Table 3-1). The acorns were processed by immersion in water with floating acorns discarded (Gribko and Jones 1995). Sunken acorns were visually inspected for pedigree purity, as indicated by shape and other acorn characteristics, and kept in cold storage until sowing at approximately 2°C. Acorns were sown at the East Tennessee State Nursery in mid-December, 2013 at a density of 74-76/m^2 (Kormanik et al. 2000), and the resulting seedlings were grown with periodic applications of nitrogen at the East Tennessee State Nursery located in Polk County, TN (Kormanik et al. 1993). After one growing season, the seedlings were lifted in early February, 2013 using a Fobro machine lifter and undercut at a depth of 25.4 cm. Trees were then sorted and visually selected to choose the highest-quality 72 trees for planting (Clark et al. 2000). Seedlings were stored in cold storage at 2°C until planted.

The American Chestnut Foundation provided nuts from BC_3F_3 hybrid chestnuts and also supplied the *C. dentata* nuts. Hybrid nuts were collected from two mother trees in an open-pollinated orchard in Meadowview, VA (Hebard 2012). *Castanea dentata* nuts (family AP5) were collected from one open pollinated orchard tree (Pryor) in western NC and stratified (McCament and McCarthy 2005). Progeny from each mother tree were considered to be putative half-siblings (Table 3-1). All chestnuts were germinated in a climate-controlled greenhouse in February, 2013. Individual nuts were sown in pots (2.83 liters volume: 10.2 cm x 10.2 cm x 35.6 cm from Stuewe & Sons, Inc.) containing ProMix©. Soil was topdressed with

Osmocote© (18-6-12) twice, once after nuts were sown and once in late March. Seedlings were watered as needed until selected, based on visual assessment of health, to be planted at the study site in April, 2013.

Northern red oaks and chestnut seedlings were transported to the Watauga Orchard in covered vehicles and planted in April of 2013. Chestnuts were transported in containers, holes were dug with augers, and seedlings were planted by hand. Bare root northern red oak seedlings were planted with JIM GEM® KBC bars (modified to increase bar width to 30 cm). The frequency of seedlings from each family within insecticide treatments are illustrated in Table 3-2.

Table 3-1.Origins of parental C. castanea, hybrid chestnuts and Q. rubra trees.

Family	Species	State	County	Elevation (m)	coordinates
1-20-2418	*Q. rubra*	NC	Buncombe	1066	35º 28’ N -82º 42’W
2-16-528	*Q. rubra*	TN	Morgan	609	36º 08’ N -84º 28’W
7-14-630	*Q. rubra*	TN	Campbell	365	36º 18’ N -84º 01’W
AP5	*C. castanea*	NC	Henderson	678	35° 23' N -82° 20’ W
D-3-862	Hybrid	VA	Washington	623	36° 44’ N -81° 51'W
W-4-938	Hybrid	VA	Washington	623	36° 44’ N -81° 51'W

Table 3-2. Number of trees planted in 2013 by family, species, and insecticide treatment at the Watauga orchard.

Family	Species	Imidacloprid	Acephate	Dinotefuran	Water	Total
1-20-2418	*Q. rubra*	18	18	18	18	72
2-16-528	*Q. rubra*	18	18	18	18	72
7-14-630	*Q. rubra*	18	18	17	18	71
AP5	*C. castanea*	18	20	20	20	78
D-3-862	Hybrid	18	16	16	15	65
W-4-938	Hybrid	18	18	19	19	74
Total		108	108	108	108	432

Site description

The study site is located in the southern Appalachian Mountains in eastern Tennessee on the Nolichucky (formerly Watauga) Ranger District of the Cherokee National Forest. This study was established around the perimeter of the Watauga Orchard, which occupies approximately 6.56 hectares surrounded by a 3.05m deer fence. The orchard was originally established in 1973 as an open-pollinated progeny test by the Tennessee Valley Authority in 1984 the orchard was thinned and converted to a seedling seed orchard (LaFarge and Lewis 1987). The site is located on broad ridgetops and upper slopes of low lying intermountain ridges with elevations ranging from 625 to of 692 meters (36º 33' N 81º 99'W). The orchards soil texture is a medium silty clay loam to clay loam that grades to clay. Slopes vary from two to 35 percent but most are between six and 20 percent (LaFarge and Lewis 1987). The orchard was also known to contain a high abundance of *C. castaneus*, observed in previous insect surveys (Stanton 1994).

Experimental design

In April, 2013, 432 seedlings were planted in two linear transects with 3.6m spacing. The main transect consists of 344 seedlings, which bordered the canopy drip line of the orchard trees. A partial second transect was established along the east side on the orchard, comprised of 88 seedlings at 3.6m spacing from the first row, as the amount of experimental material exceeded the perimeter of the orchard when planted at a 3.6m spacing. The spacing was used to avoid any cross contamination with the insecticide application and reduce drift between seedlings. Within transects, families were arranged in a balanced incomplete block design with a block size of four. This type of design was selected due to lack of a homogeneous environment throughout the study site. Insecticide treatments and water control were randomly assigned to seedlings within complete blocks.

Insecticide

Imidacloprid, dinotefuran and acephate were chosen for this study because of their common use against forest pests and have been shown to significantly reduce the number of weevil larva (Coleoptera: Curculionidae) in the soil as well as defoliation by adults (Rosetta et al. 2000, Reding and Persad 2009, Reding and Ranger 2011). The three insecticides are systemic

neonicotinoids commonly used in agriculture, horticulture and forestry practices; Imidacloprid is one of the most widely used insecticides in the world (Yamamoto and Casida 1999).

Within each block, each seedling randomly received one of the four insecticide treatments (Table 3-2) approximately one quart of mixed solution or tap water at the concentration recommended by the manufacturer, listed below:

1. Imidacloprid (Touchstone® 75 WSP, Bayer Environmental Science, Research Triangle Park, NC). Mixed 1 packet (1.6 oz) in 6 gal water (45.36 grams per 22.7 L) = 2.0 g/L
   0.946 L x 2.0 g/L = 1.89 g of product delivered to each seedling. Product is 75% imidacloprid, 1.89 x 0.75 = 1.42 g of active ingredient per seedling

2. Acephate (Orthene® Turf Tree and Ornamental WSP, AMVAC Chemical Corp., Los Angeles, CA). Mixed 1 packet (1/3 lb) in 33.3 gal water (1 lb per 100 gal, or 453.6 grams per 378.5 L) = 1.2 g/L 0.946 L x 1.2 g/L = 1.14 g of product delivered to each seedling
   Product is 75% acephate, so 1.14 x 0.75 = 0.86 g of active ingredient per seedling

3. Dinotefuran (Safari® 20 SG, Valent U.S.A. Corp., Walnut Creek, CA)
   Mixed 2.5 level teaspoons in 1 quart water (6 grams per .946 L) = 6.34 g/L
   0.946 L x 6.34 g/L = 6.0 g of product delivered to each seedling
   Product is 20% dinotefuran, so 6.0 x 0.2 = 1.2 g of active ingredient per seedling

4. Water control (0.946 L of tap water delivered to each seedling)

Treatments were administered by hand in late June 2013 via soil drench. The soil at the base of the seedling was disturbed with a hand-held garden rake to facilitate soil penetration and reduce the risk of run off.

Defoliation assessments

A visual-categorical method and a digital-numerical method were used in assessing defoliation of Castanea seedlings once a month from August through October in 2013.

Seedling Selections for visual and digital defoliation assessments in 2013.

After planting, many seedlings exhibited typical symptoms of transplanting shock showing signs of yellowing, browning, leaf curl and loss of leaves (Struve et al. 2000). By June 2013, the majority of the *Q. rubra* seedlings were experiencing extreme delayed bud break and subsequently removed from the 2013 defoliation study.

The health of *Castanea* seedlings was visually assessed by insecticide treatment using a rating of S, U1, U or D, explained below. 'Healthy leaves' are defined by showing little to no yellowing, browning or leaf curl:

S- Satisfactory: majority of leaves were healthy

U1- Mildly unsatisfactory: seedlings had greater than 50% of healthy leaves

U- Unsatisfactory: seedlings have less than 50% healthy leaves

D- Dissatisfactory: seedlings had no healthy leaves present, tree appears to be dead or nearing death

Trees given a rating of 'S' were optimal for defoliation assessments. Due to the low number of S class seedlings in the hybrid D-3-862 family, this family was dropped from the 2013 assessment (numbers of seedlings within each class are present in the appendix Table A-1). Ten trees from each family/insecticide treatment were randomly chosen from the 'S' class using a random number generator, with the exception of two seedlings from family W-4-938 randomly chosen from the U1 class. A total of 80 *Castanea* seedlings were assessed in the defoliation studies for 2013; ten from each respective insecticide treatment and family.

Visual defoliation assessment

Visual defoliation assessments were similar to previously established methods (Erdle and MacLean 1999, Johns et al. 2006, Case et al. 2012). Two observers visually examined the foliage of each seedling to determine the percentage of leaves occurring in the following six defoliation classes of leaf area missing: less than 5%, 6-20%, 21-40%, 41-60%, 61-80%, and 81-100% (henceforth these will be referred to as classes 1-6, respectively). Observers rounded the defoliation assessment to the nearest tenth percent in each category (e.g., observations were recorded as 10, 20, 30 etc.) to minimize differences in estimates between the two observers.

Observers rated trees independently and then deliberated to assign a Final Joint Rating (FJR) for each category. The FJR was used to calculate a single visual defoliation rating for each seedling.

The Visual Estimated Percent of Defoliation (VEPD) was calculated for each tree. The percent medians of the six defoliation classes were multiplied by FJR and added together to attain the estimated percent defoliation. The less than 5% category was assumed to have zero defoliation, therefore the median of FJR class one was zero, and was not used in the calculations of VEPD.

VEPD= (**FJR2***0.125)+(**FJR3***0.305)+(**FJR4***0.505)+(**FJR5***0.705)+(**FJR6***0.905)

Digital defoliation assessment

For an individual tree, leaf area loss from insects was estimated from Leaf Area Before Defoliation (LABD) minus the Photographed Leaf Area (PLA) (Linit et al. 1986). To calculate leaf area in a noninvasive manner, all leaves on each tree were counted, leaves that had at least 5% defoliation were placed between two pieces of Plexiglas© to show the full surface area and alongside a metric ruler for scale. A picture was taken and the petiole diameter was recorded with digital calipers to the nearest 0.1mm at the portion of the petiole closest to the leaf blade. Pictures were analyzed in Adobe Photoshop©, to calculate PLA. Leaf length from tip to base and width at the widest portion of each leaf was recorded when herbivory patterns allowed. The strongest variable that best predicted area using length, width, or petiole diameter was then used in a regression equation to calculate LABD

Regression equations were based on 102 undamaged leaves from 15 *C. dentata* trees (Family AP5) and 101undamaged leaves from 16 W-4-938 hybrid chestnuts taken in July of 2013. Trees were chosen based on having two or more 'undamaged leaves' identified by having less than 5% defoliation. All undamaged leaves were photographed to insure a wide range of leaf size would be included in the sample. The photographs, leaf length, width and petiole diameter were recorded in the same noninvasive method previously described. Variables from families AP5 and W-4-938 were significantly correlated to each other and therefore combined to calculate three polynomial regression equations. Analysis were run (SAS 2013) with a 5% significance level to calculate leaf area using dependent variables individually (petiole diameter, leaf length

and width), regression equations, R-values, and plotted figures are shown in Table-A-2 and Figure A-1 in appendix. The upper confidence levels of each polynomial regression equation were used to obtain LABD to ensure no negative values were calculated.

Six hundred forty-five photographs were taken of leaves having greater than 5% leaf area defoliated from the 80 trees sampled during the three sampling dates. After PLA was calculated it was subtracted from LABD then divided by the LABD and multiplied by 100 giving a percent of Digital Estimated Defoliated Area (DEDA).

$$DEDA = \frac{LABD - PLA}{LABD}(100)$$

Monthly Digital Defoliation (DD) for each tree was calculated by summing DEDA for each leaf on a tree and dividing by the number of leaves present on the tree (n).

$$DD = \frac{\sum_{i=1}^{n} DEDA}{n}$$

Seedling selection for visual defoliation assessment in 2014

The procedure Proc Power in Program SAS (SAS 2013) was used to determine an appropriate sample size per family/treatment. With 12 observations in each species/treatment to conduct visual defoliation assessments on Castanea and Q. rubra seedlings in 2014. With 12 observations in each species/treatment, the model was predicted to have 80% power in correctly rejecting the null hypothesis. Seedlings had recovered from planting shock, however, due to low seedling survival rates in the first growing season (Table A-3 in appendix) not all family/treatments were able to have the optimum number of seedlings assessed. To choose seedlings, all full incomplete blocks were chosen first. Extra trees were then randomly chosen with a random number generator to obtain a maximum of 12 trees in each family/treatment when possible. Due to mortality only nine seedlings from D-3-862 (water control), 11 seedlings (imidacloprid and dinotefuran), and 10 trees (water control) from *Q. rubra* 1-20-2418 were able to be assessed (Table 3-3). Two hundred eighty-one trees were visual assessed for defoliation in July, June, Aug and October of 2014 using the same methods as described in visual defoliation assessment in 2013.

Table 3-3. Number of trees by family and insecticide treatment assessed in 2014 defoliation study.

Family	Species	Imidacloprid	Acephate	Dinotefuran	Water	Total
1-20-2418	*Q. rubra*	11	12	11	10	44
2-16-528	*Q. rubra*	12	12	12	12	48
7-14-630	*Q. rubra*	12	12	12	12	48
AP5	*C. castanea*	12	12	12	12	48
D-3-862	Hybrid	12	12	12	9	45
W-4-938	Hybrid	12	12	12	12	48
Total		**71**	**72**	**71**	**67**	281

Growth

All living trees were measured for height and root collar diameter (RCD) in April 2013, April 2014 and November 2014. RCD was measured with digital calipers to the nearest 0.1mm. The RCD is located at the base of the seedling and identified by a partial swelling and slightly lighter color than the rest of the stem. A three meter measuring stick was used to measure height to the nearest cm. Total seedling height was measured from the base of the tree to the tallest live bud without the tree being physically straightened. Growth in 2013 and 2014 was calculated by subtracting previous year's growth from current year's growth.

Cyrtepistomus castaneus

To determine adult *C. castaneus* emergence, thirty-one cone emergence insect traps were used, modeled after pecan weevil traps (2013). To assemble the traps, a protective durable 0.32 cm steel wire mesh hardware screen was cut in to half circles at a diameter of 1.82m and a 1.3cm half circle was then cut out of the straight edge. The straight edges were then brought together and stabilized with an 81.3cm wooden slat and stapled. Trap tops, manufactured by IPM, also used for boll weevils, pecan weevils and citrus weevils, were secured to the top of each trap using Liquid Nails®. A second thinner mesh wiring 0.16cm was placed on the inside of each trap to limit insect escape.

The traps were placed approximately 40 meters apart from one another on the inside perimeter of the Watauga Orchard (Figure 3-1). Positioning of the traps was chosen to be beneath the drip

line of mature oaks and over the last growing season's leaf deposits. Traps were secured to the ground with four to six sod staples and identified with aluminum tags and bright orange flags labeled 'W-1 through W-31'.



Figure 3-1. Relative positioning of insect traps in 2013 and 2014 at the Watauga Orchard.

Insects were collected approximately every seven days in 2013 from mid-June though late November. To confirm emergence patterns, all traps were reestablished in late April, 2014. Traps were moved clockwise around the orchard approximately 3m from their previous location while remaining underneath the drip line and placed on top of the previous growing season's leaf deposits. In 2014 traps were collected approximately every 14 days from mid-May to mid-November. Discontinuing the collection of traps in each year corresponded with two consecutive collections obtaining no *C. castaneus.*

During collection of traps, all insects were retained to ensure all *C. castaneus* were properly identified. Due to some variation in the days between collecting traps the equation below was used to standardize the days between collections:

$$Insects\ per\ week = \frac{A}{(\mathrm{D} * 0.1428)}$$

where A is the number of *C. castaneus* collected and D is the number of days that had passed between collections.

Statistical analyses

All analyses were conducted using SAS 9.4 software (SAS 2013). Mixed model analysis of variance was used on continuous variables to test their fixed effects that are bulleted below. A significance level of less than or equal to 0.05 (Fisher 1925) was used to detect significant differences.

- Defoliation assessments in 2013 (digital and visual) and 2014 (visual only) were each analyzed together to compare techniques and separately to evaluate fixed effects: insecticide treatment, family, month and their interactions with family nested in species for 2014. Month was analyzed as a repeated measure, and we used an autoregressive covariance structure. Due to the location of trees that were available for defoliation ratings in 2013, pre-established blocks were unable to be used therefor post-facto blocking was used.
- Growth measurements (root collar diameter and height) were tested for fixed effects of family nested within species, insecticide treatment, month and their interactions. For growth measurements year was analyzed as a repeated measure, and we used an autoregressive covariance structure.
- *Cyrtepistomus castaneus* emergence rate was tested for fixed effects of trap location, time period (month/year) and their interaction. To identify spatial differences in emergence a spatial spherical model was used with x and y coordinates of each trap location to visually compare differences in density around the orchard.

Pearson correlation was used to evaluate the relationship between two year height growth vs. defoliation and two year RCD growth vs. defoliation, in individual months for living trees. The relationship of mean seedling defoliation to ratings in October of all treatments and by treatments separately were compared to closest insect traps average weekly emergence of *C. castaneus.*

For most analyses, mean comparisons were conducted for fixed effects that were significant using the least significant difference (LSD) test, which is commonly used in forestry and plant sciences. *Cyrtepistomus castaneus* emergence rate was analyzed using Tukey's HSD mean separation test. Tukey's was used due to its more common use in the entomology field and conservative nature, requiring a larger difference between means before stating a significant difference. Mean comparisons were only reported when *p*-values were significant ($p \leq 0.05$).

Normality and equal variance assumptions were tested for dependent variables. The square root transformation was used on weekly count of *C. castaneus* collected from traps to satisfy assumptions of equal variance. The square root transformation was chosen for its moderate effect on distribution shape and reducing right skewness.

Kenward-Roger was used to compute the denominator degrees of freedom, except for 2014 visual defoliation. Instead, the DDFM=BETWITHIN option was used to divide the residual degrees of freedom into between-subject and within-subject portions and compute the denominator degrees of freedom (Schluchter and Elashoff 1990). This option for calculating denominator degrees of freedom was used as Kenward- Roger failed to produce reasonable degrees of freedom.

Chapter 4. Results

Defoliation assessments

2013 Visual defoliation assessment

Overall defoliation was relatively low, less than 12% in most treatments and months on the two families assessed, American chestnut (AP5) and hybrid chestnut (W-4-938). Defoliation in 2013 was dependent on interactions of insecticide treatment, family, and month. Defoliation increased significantly after each month sampled, from August to October, and insecticide treatment effects varied by month (Table 4-1). Between families, mean defoliation was not significantly different (P=0.0885). The effect of the insecticide treatment did depend on families (Figure 4-1) with the main interaction occurring on seedlings treated with acephate, American seedlings were significantly higher defoliated than hybrid chestnut. Both families had the lowest defoliation on seedlings treated with dinotefuran.

The treatment effects were dependent on month and family, but very few differences were detected (Figure 4-2). No treatment effects were apparent for hybrid chestnuts until October. In October, seedlings treated with imidacloprid and water control showed a trend of more defoliation than seedlings treated with acephate and water control, however within month no treatments were significantly different than each other. For the American chestnut, no effects were apparent in August. In September, however, defoliation on American chestnut seedlings treated with acephate (mean= 11.9%) were higher than all treatments including the control. By October, the acephate treatment (mean=16.4%) was no different than the control, and both were higher than the imidacloprid and dinotefuran (Figure 4-2). Actual values are shown by treatment, month, and family in appendix Table A-4.

Table 4-1. ANOVA table of 2013 visual defoliation of family American (AP5) and hybrid (W-4-938) with degrees of freedom (DF), F-values and P-values of fixed effect and their interaction.

Effect	DF	*F*-Value	*P*-value
Treat	3	4.11	0.0079
Family	1	2.94	0.0885
Treat*Family	3	2.87	0.0403
Month	2	28.03	<.0001
Treat*Month	6	2.35	0.0358
Family*Month	2	0.17	0.8423
Treat*Family*Month	6	2.44	0.0297

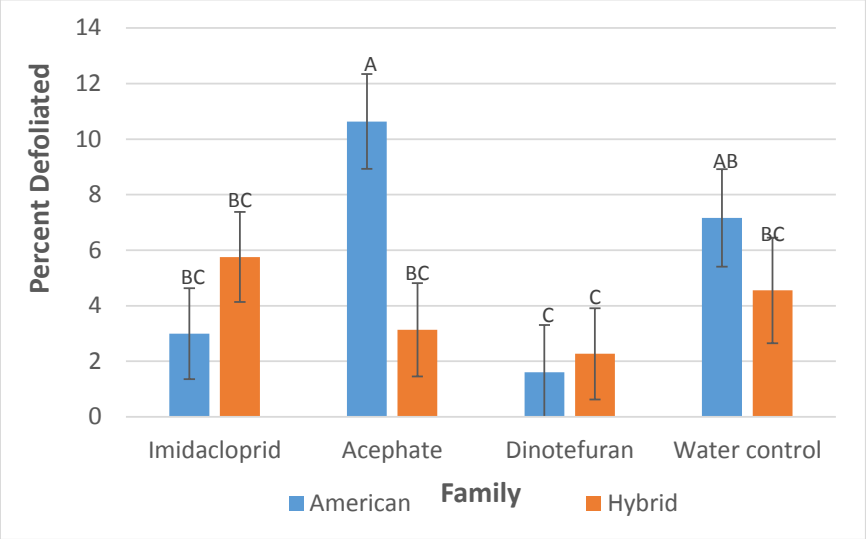


Figure 4-1. Mean visual defoliation in 2013 by generation and treatment. Treatment bars labeled with the same letter are not significantly different ($\alpha \leq 0.05$).

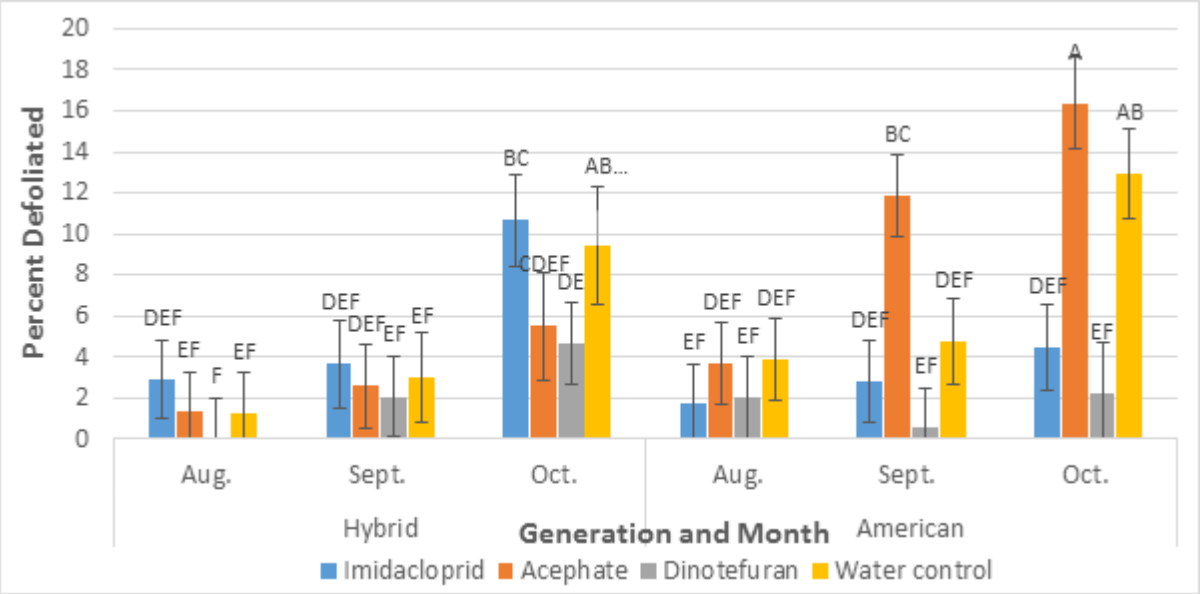


Figure 4-2. Mean visual defoliation in 2013 by generation, month and treatment. Treatment bars labeled with the same letter are not significantly different ($\alpha \leq 0.05$).

2013 Digital defoliation assessment

Digitally assessed defoliation was higher overall, in percentage, than visual methods. As with the visual method, each month mean defoliation significantly increased from August to October (Table 4-2). There was no significant effect of families on defoliation (P=0.3745), but there was an interaction between family and insecticide treatment (Figure 4-3). Mean defoliation on seedlings treated with acephate experienced more defoliation on American chestnut than on hybrid chestnut seedlings.

The treatment effects were dependent on month and family (Figure 4-4). Within hybrid chestnut, the no treatment effects were seen until October. In October, only seedlings treated with imidacloprid had significantly higher values of defoliation and did not differ from seedlings treated with dinotefuran in September. For American chestnut, no effects were apparent in August. In September, defoliation on seedlings treated with acephate (mean=29.1%) was higher than all treatments including the control. By October, the acephate was no different than the control and both, water control and acephate were higher than then dinotefuran family (Figure

4-4). Mean defoliation values by treatment, family, and month are listed in the appendix Table A-5.

Table 4-2. ANOVA table of 2013 digital defoliation of family AP5 and W-4-938 with degrees of freedom (DF), F-values and P-values of fixed effect and their interaction.

Effect	DF	*F*-Value	*P*-value
Treat	3	1.77	0.1576
Family	1	0.79	0.3745
Treat*Family	3	3.26	0.0256
Month	2	24.71	<.0001
Treat*Month	6	1.5	0.1835
Family*Month	2	0.99	0.3726
Treat*Family*Month	6	2.32	0.0365

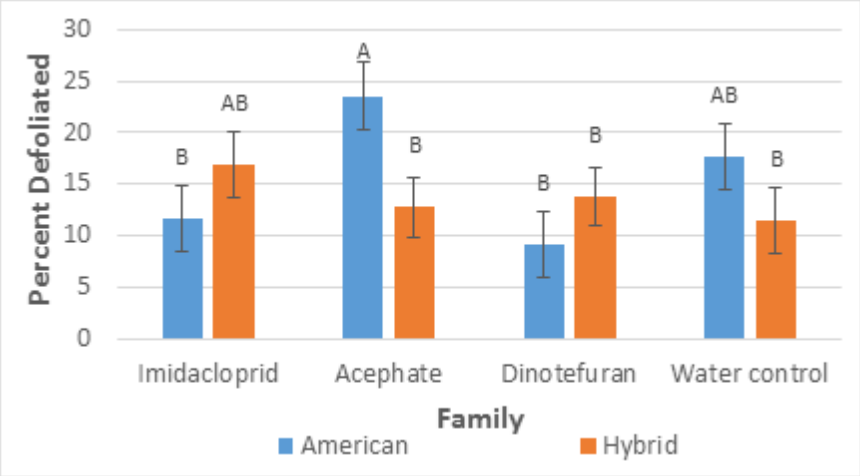


Figure 4-3. Mean digital defoliation in 2013 by family and insecticide treatment. Treatment bars labeled with the same letter are not significantly different (α ≤ 0.05).

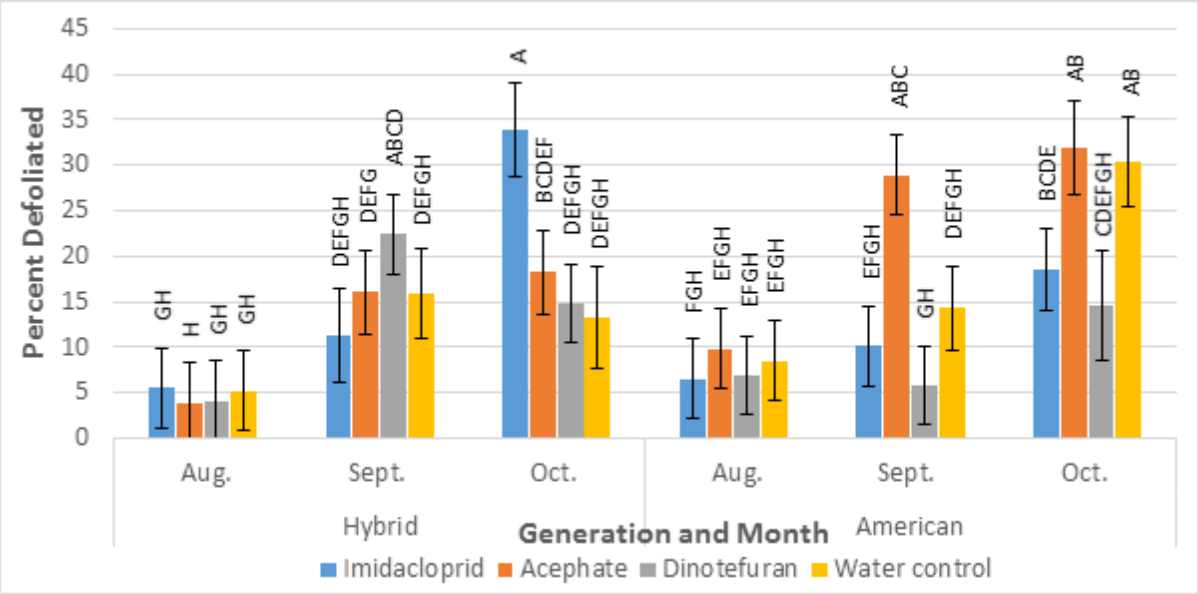


Figure 4-4. Mean digital defoliation in 2013 by month, treatment and generation. Treatment bars labeled with the same letter are not significantly different (α = 0.05).

Comparison of visual and digital defoliation methods

Percent of digital defoliation was significantly higher than visual defoliation. Visual defoliation mean averaged 4% whereas digital defoliation averaged over 14% (F=60.65; df=1; P<0.0001).

Visual defoliation in 2014

In 2014, all families planted were assessed (Table 3-2) for defoliation; most treatments and months were below 15%. Family within species had no significant effect on defoliation in 2014 and all interactions with family and species were also not significant (Table 4-3).

Seedlings treated with acephate and water control had a higher percent defoliation than imidacloprid and dinotefuran treatments (Figure 4-5). Each month that defoliation estimates were taken was significantly higher than the previous month, and treatment and month interacted in their effects on defoliation.

In June and July, defoliation was below 8% with few differences among treatments (Figure 4-6). Seedlings treated with the water control, in July, were slightly higher than seedlings treated with

imidacloprid and dinotefuran. In June and July, seedlings treated with acephate did not statistically differ from imidacloprid or dinotefuran (Figure 4-6). In August and October, seedlings treated with acephate and water control experienced significantly more defoliation than seedlings treated with imidacloprid and dinotefuran. In October, imidacloprid treatments had significantly less defoliation than in all other treatments.

Table 4-3. ANOVA table of 2014 visual defoliation ratings of all families with degrees of freedom (DF), F-values and P-values of fixed effect and their interaction.

Effect	DF	*F*-Value	*P*-value
Treat	3	10.02	<0.0001
Species(Family)	5	0.73	0.5999
Treat*species(Family)	15	0.57	0.8937
Month	3	143.54	<0.0001
Treat*Month	9	5.74	<0.0001
Month*Species (Family)	15	0.93	0.5341
Treat*Month*Species (Family)	45	1.27	0.1188

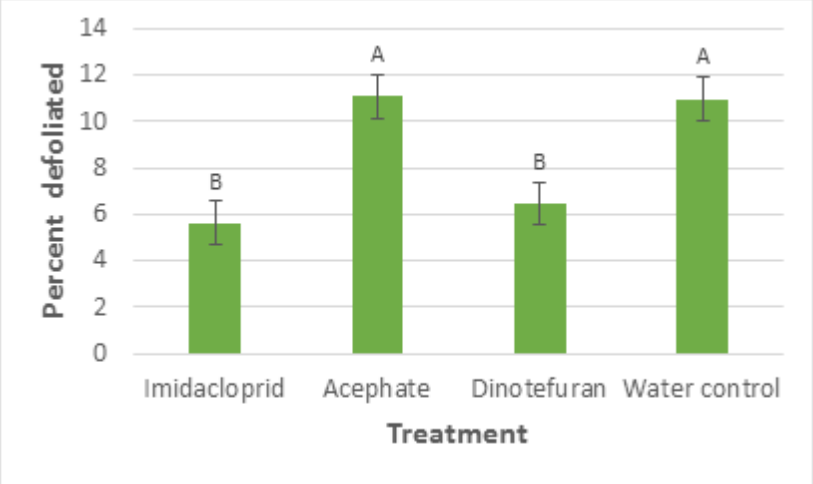


Figure 4-5. All seedlings mean percent defoliated in 2014 growing season. Treatment bars labeled with the same letter are not significantly different ($\alpha \leq 0.05$).

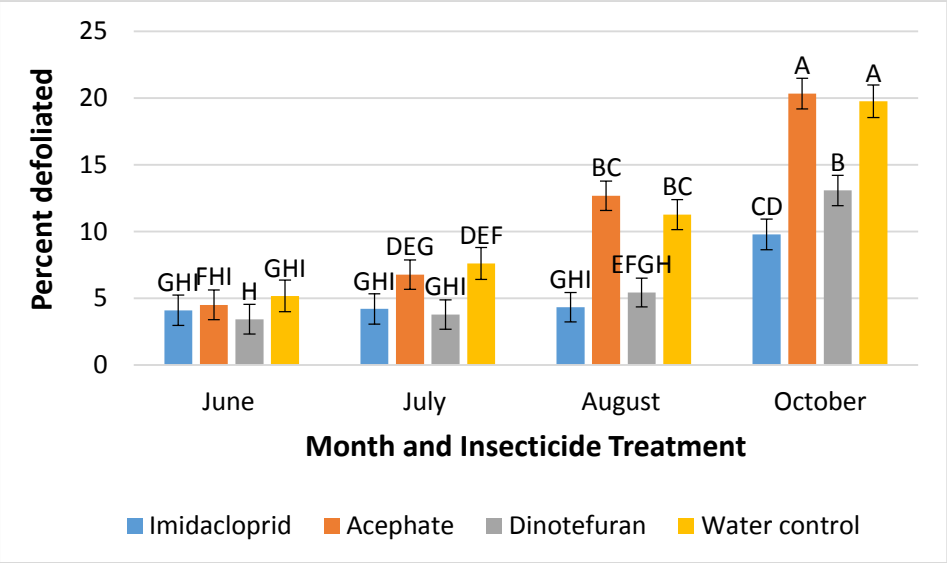


Figure 4-6. Mean visual defoliation in 2014 combined all families by month and treatment. Treatment bars labeled with the same letter are not significantly different ($\alpha \leq 0.05$).

Growth

Height

Insecticide treatment and all interactions including treatment did not affect mean growth over the two year measuring period (Table 4-4). Family within species had a significant effect on defoliation, there was greater growth of *Castanea* species than of *Quercus*. Mean growth in 2014 growing season was significantly higher than in 2013 growing. In 2013, *Castanea* families had similar growth to *Q. rubra* families, most ranging between -0.2cm to 10.8cm, with the exception of *Q. rubra* family 1-20-2024 which was significantly less than all families (mean= -28.1cm). This trend changed however in 2014, *Castanea* families' growth was significantly taller, ranging from 31.1-37.6cm, than *Q. rubra* families'. *Quercus rubra* growth increased by similar proportions in each growing season with the exception of family 1-20-2024, which was significantly greater in 2014 than 2013 (Figure 4-7)

Table 4-4.ANOVA table 2013 and 2014 mean height growth with degrees of freedom (DF), F-values and P-values of fixed effects: treatment, family year and their interaction of all families with in species

Effect	DF	*F*-Value	*P*-value
Treat	3	1.22	0.3013
Species(Family)	5	14.17	<0.0001
Treat*Species(Family)	15	0.28	0.9966
Year	1	95.62	<0.0001
Treat*Year	3	0.44	0.7229
Year*Species(Family)	5	11.3	<0.0001
Treat*Year*Species(Family)	15	1.54	0.0873

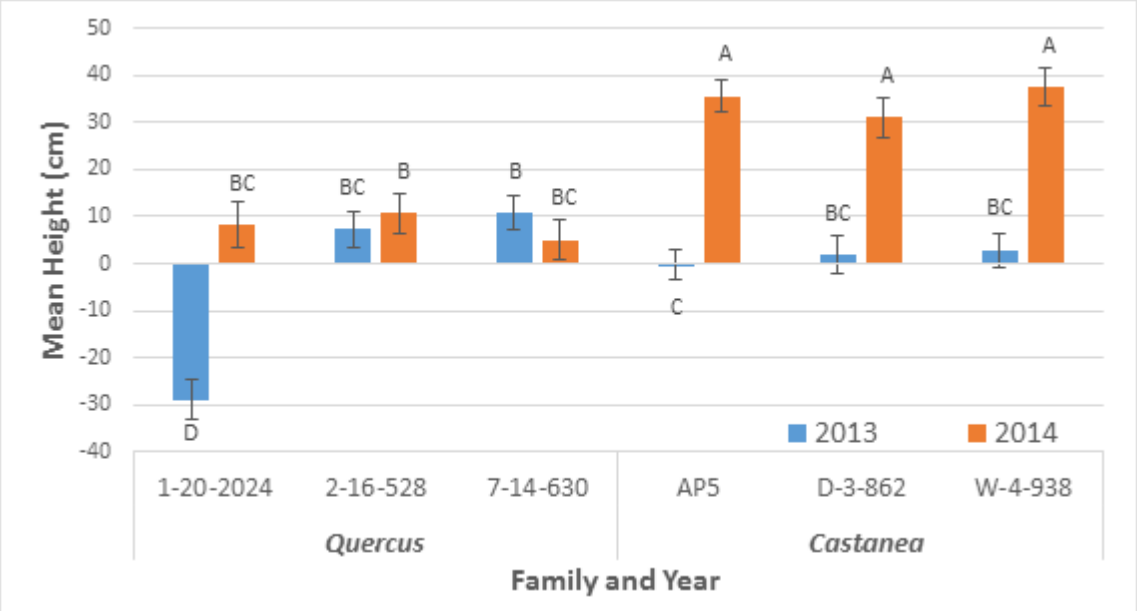


Figure 4-7. Mean height growth in 2013 and 2014 growing season with all families combined. Treatment bars labeled with the same letter are not significantly different ($\alpha \leq 0.05$).

RCD

Among families there was little amount of RCD growth in the first year with the majority of growth occurring in the second year (Table 4-5; Figure 4-8). In the 2013 growing season a mean reduction of 0.9 mm was reported on family 1-20-2024 and did not statistically differ from AP5 or W-4-938 in 2013. The greatest increase of RCD in 2013 occurred on family 2-16-528 (mean=0.5 mm) although did not statistically differ from 7-14-630 or D-3-862 (Figure 4-8). Oak families' RCD increased between 0.9 and 1.6 mm in the 2014 growing season whereas chestnut families had a significantly higher increase, ranging from 2.9 to 3.4 mm (Figure 4-8). The change in RCD was significantly higher on seedlings treated with dinotefuran (1.4 mm) than acephate and water control treatments (0.8 and 0.7 respectively), neither of which differed from seedlings treated with imidacloprid (Figure 4-9)

Table 4-5. ANOVA table 2013 and 2014 mean root collar diameter growth with degrees of freedom (DF), F-values and P-values of fixed effects: treatment, family year and their interaction of all families within species.

Effect	DF	*F*-Value	*P*-value
Treat	3	3.66	0.0123
Species(Family)	5	7.28	<0.0001
Treat*Species(Family)	15	1.19	0.2775
Year	1	202.75	<0.0001
Treat*Year	3	0.82	0.4827
Year*Species(Family)	5	8.16	<0.0001
Treat*Year*Species(Family)	15	0.96	0.4957

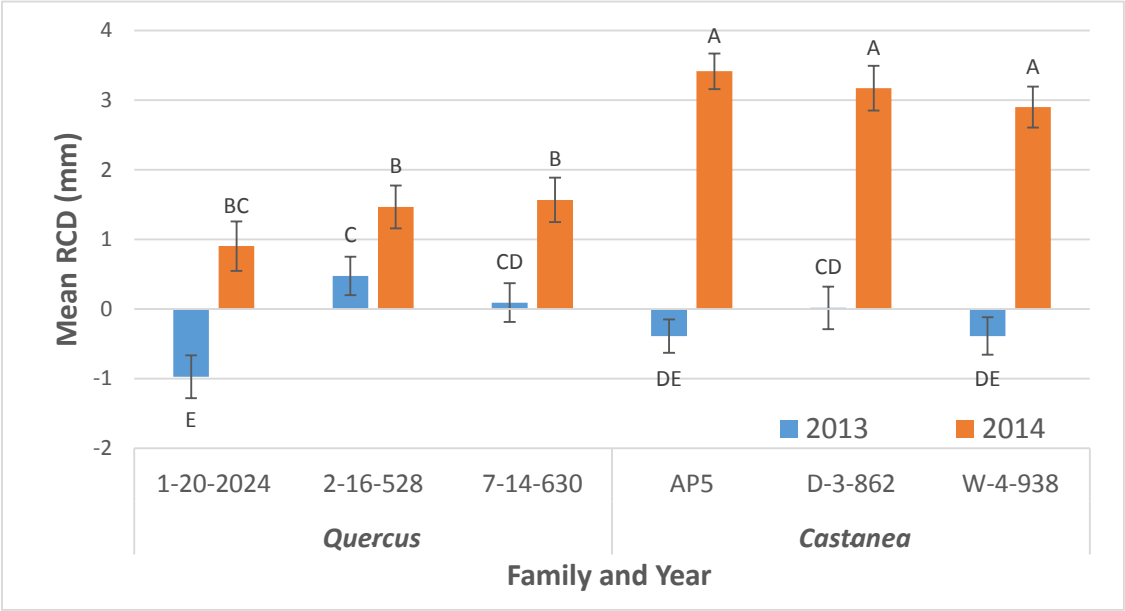


Figure 4-8. Mean root collar diameter growth in 2013 and 2014 growing season by family. Treatment bars labeled with the same letter are not significantly different ($\alpha \leq 0.05$).

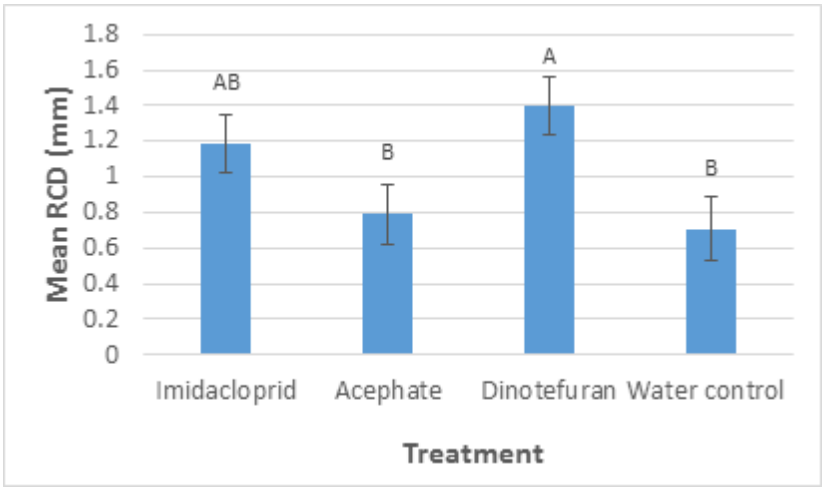


Figure 4-9. Mean root collar diameter change of two year growth by insecticide treatment. Treatment bars labeled with the same letter are not significantly different ($\alpha \leq 0.05$).

Growth Correlations

The majority of defoliation variables were not significantly correlated with change in height or RCD (Table 4-6). Defoliation in July 2014 was the only variable which had a significant correlation to two year height change with a negative weak relationship (r = -0.17). Defoliation assessments from July 2014 and October 2014 were significantly correlated with two year change in RCD although both were negative weak correlations (r = -0.22 and r= -0.17) (Table 4-6).

Table 4-6. Correlation estimates between two-year height growth, two year RCD growth, and defoliation assessments. Digital and visual methods in are labeled with a V or D for 2013 which includes AP5 and W-4-938 and 2014 includes all families.

Variable	N	Two year height change	N	Two year RCD change
2013 Aug V	73	-0.16 (*P*=0.8337)	73	-0.05 (*P*=0.6455)
2013 Sept V	71	0.03 (*P*=0.8337)	71	0.01 (*P*=0.9094)
2013 Oct V	62	0.05 (*P*=0.7123)	62	0.15 (*P*=0.2432)
2013 Aug D	73	-0.18 (*P*=0.1337)	73	-0.02 (*P*=0.834)
2013 Sept D	71	0.05 (*P*=0.6823)	71	0.05 (*P*=0.6874)
2013 Oct D	62	0.18 (*P*=0.1476)	62	0.06 (*P*=0.6159)
2014 June	274	0.05 (*P*=0.4075)	274	0.05 (*P*=0.4277)
2014 July	275	-0.17 (*P*=0.0092)*	275	-0.22 (*P*=0.0008)*
2014 Aug	275	0.03 (*P*=0.6473)	275	-0.08 (*P*=0.2055)
2014 Oct	236	-0.11 (*P*=0.1069)	236	-0.17 (*P*=0.0076)*

**indicates a significant correlation*

Cyrtepistomus castaneus

The average weekly emergence of *C. castaneus* was significantly higher in 2013 than in 2014 with respective weekly means of 2.5 and 1.8 (F=36.43; df=1; P<0.0001). The interaction of individual trap and year was approaching significance (F=1.85; df=30; P=0.051; Figure 4-10). Three: traps (2, 25 and 27), had significantly greater numbers of *C. castaneus* collected in 2013 than in 2014. Conversely, traps 8 and 11 had significantly higher collections in 2014 than in 2013 (Figure 4-10).

Temporal emergence in 2013 peaked in late July with a weekly collection of 128 *C. castaneus* (Figure 4-11). There was secondary peak in the mid-August before a sharp decrease in the beginning of September after which emergence steadily tapered off until ending in the middle of November. A similar trend was followed in 2014, although fewer *C. castaneus* were collected. Emergence in 2014 peaked in late July with an average of 50 *C. castaneus* collected per week and slowly decreased with a small rise in mid-October. Emergence once again ended in mid-November (Figure 4-11). Spatial spherical models using SAS showed no significant differences in density of emergence around the orchard. Although, when visually assessing mean weekly emergence there appears to be a higher number *C. castaneus* emerging from traps on the northern border (Figure 4-12) in 2013 and from the south eastern corner in 2014 (Figure 4-13). Furthermore there were few significant correlations between mean weekly emergence and the amount of defoliation on seedlings in October (Table 4-7). Digital defoliation assessed in 2013 had a moderate positive correlation on all treatments. When assessed by treatment, however, only the seedlings given the water control were significantly correlated. In 2014, there were moderate negative correlations on defoliation of seedlings given acephate and water control treatments to mean weekly emergence.

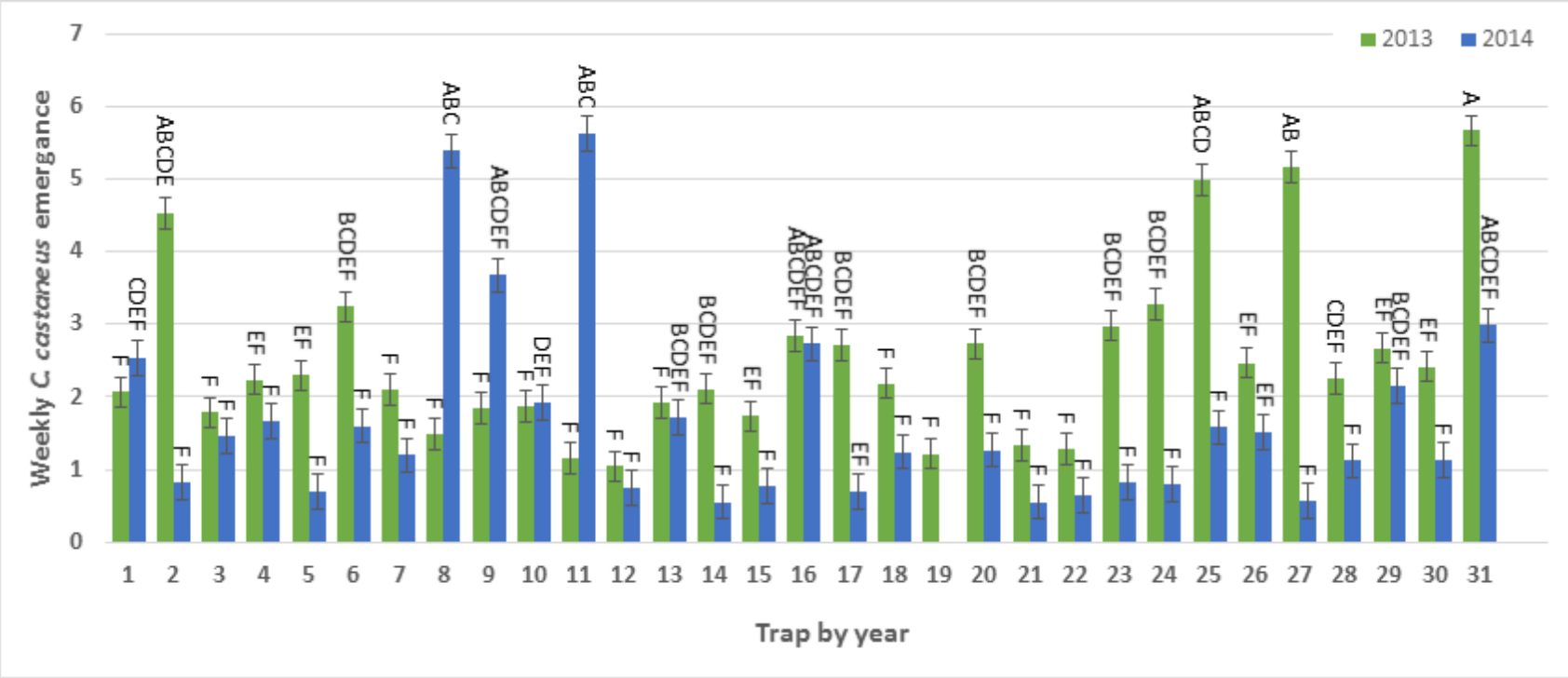


Figure 4-10. Mean weekly C. castaneus emergence from trap by year. Treatment bars labeled with the same letter are not significantly different ($\alpha \leq 0.05$).

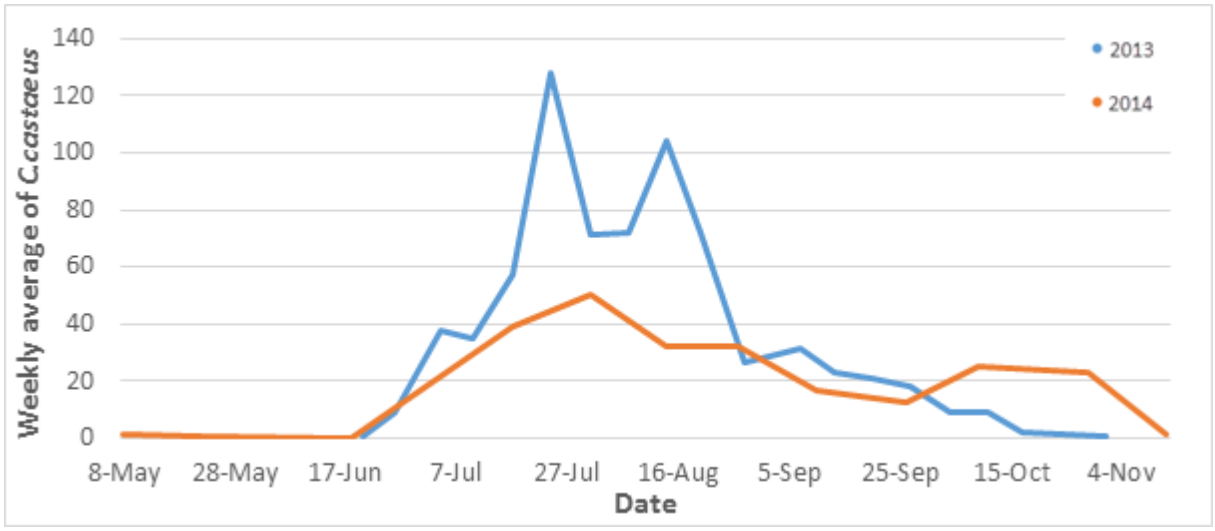


Figure 4-11. Weekly Cyrtepistomus castaneus collected from all traps by date in respective years.

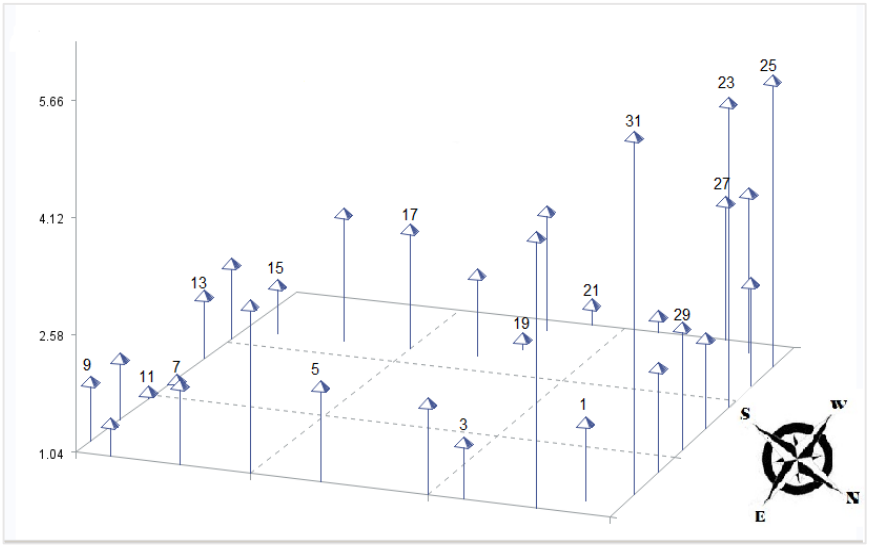


Figure 4-12. Mean weekly emergence in 2013 of Cyrtepistomus castaneus by trap location.

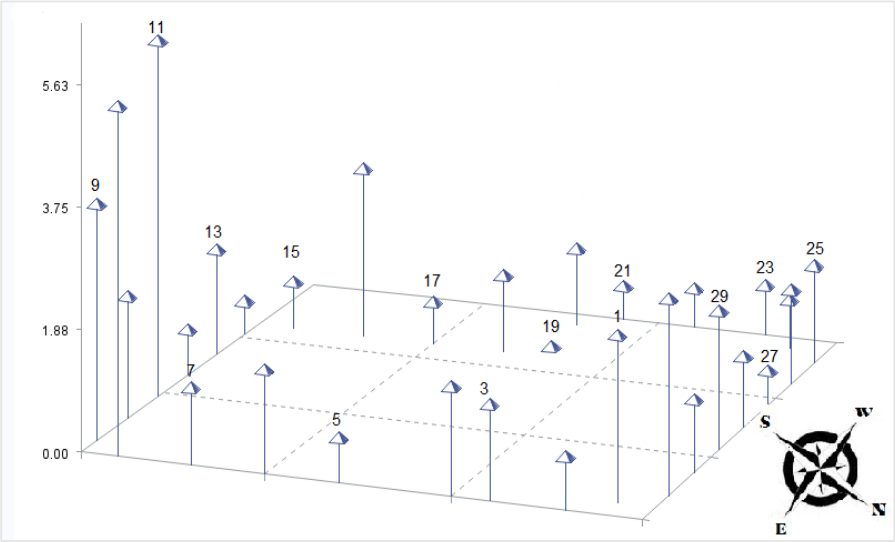


Figure 4-13. Mean weekly emergence in 2014 of Cyrtepistomus castaneus by trap location.

Table 4-7. Correlation estimates between 2013 (visual and digital) and 2014 (visual) mean defoliation assessments of seedlings, combined and by treatment, to closest trap to average weekly C. castaneus emergence.

Year (Method)	Treatment assessed	N	Average weekly *C. castaneus* emergence
2013 (Visual)			
	All treatments	28	0.02 (*P*=0.9203)
	Imidacloprid	12	0.41 (*P*=01.815)
	Acephate	14	-0.17 (*P*=0.5525)
	Dinotefuran	13	-0.43 (*P*=0.1425)
	Water control	14	0.30 (*P*=0.3016)
2013 (Digital)			
	All treatments	28	0.40 (*P*=0.0338)*
	Imidacloprid	12	-0.05 (*P*=0.8833)
	Acephate	14	-0.05 (*P*=0.87)
	Dinotefuran	13	0.01 (*P*=0.9899)
	Water control	14	0.61 (*P*=0.0259)*
2014 (Visual)			
	All treatments	31	-0.28 (*P*=0.1318)
	Imidacloprid	30	0.09 (*P*=0.6456)
	Acephate	30	-0.35 (*P*=0.0056)*
	Dinotefuran	28	-0.11 (*P*=0.5615)
	Water control	26	-0.45 (*P*=0.0226)*

**indicates a significant correlation*

Chapter 5. Discussion

Defoliation assessments

2013 and 2014 visual defoliations

A trend of slightly more defoliation was observed in 2013 on American chestnuts than hybrid chestnuts, although this difference was not significant. Unlike our study, a previous experiment, conducted in growth chambers, found differences in gypsy moth defoliation of American chestnut and hybrid chestnuts, potentially due to American chestnut having a higher concentration of carbohydrates and a lower concentration of tannins than hybrid chestnuts (Rieske et al. 2003). Field studies have found American and hybrid seedlings to have similar predation frequency by *C. castaneus* (Pinchot et al. 2011) and similar defoliation ratings throughout the growing season on hybrids most closely related to American chestnut (Case et al. 2015). A subtle preference of phytophagous insects between American or hybrid chestnuts might be better detected in a more controlled environment rather than in a field settings. In 2014, when all families of chestnuts and oaks were analyzed, families were similar in the amount of defoliation they received and no interactions including family by treatment and/or month was significant. The difference between 2013 and 2014 may be due to a more appropriate sample size in 2014 allowing more power to correctly fail to reject the null hypothesis. Differences between years may have also been influenced by the low health of trees and lack of leaves in 2013 opposed to 2014.

Visual and Digital methods

The 10%+ average difference between visual and digital defoliation assessments may be due to the methodology used for calculating digital defoliation. Using regression equations, upper most confidence levels potentially overestimated the amount of leaf area loss. We used the upper confidence levels in order to not underestimate and/or avoid calculating negative amounts of leaf area defoliated, but these methods could have inflated defoliation estimates. Visual defoliation estimates may have also been lowered by using the median of each category to generate a single number rather than a range to calculate the mean. Although there was a difference in defoliation estimates between the two methods, the trends of defoliation were similar with only one exception on W-4-938 seedlings in October. When comparing efficiencies of the two methods,

digital methods were more intensive in both the field and the lab than visual. Despite the differences, both rating methods led to the same conclusion for American chestnut that dinotefuran was the most effective treatment in the first year.

Insecticide treatments

Based on visual defoliation estimates Dinotefuran and imidacloprid treated-seedlings experienced the least amount of defoliation in both years. Seedlings treated with acephate had similar defoliation to the control group, potentially due to the low amount of defoliation that was observed in both years. Species and families experienced similar amounts of defoliation among treatments with a trend of seedlings treated with imidacloprid being least defoliated in the second year and dinotefuran treatments were lower in the first year. Although tree species metabolize insecticides differently (McCullough et al. 2009), previous studies have had similar results on eastern hemlocks (*Tsuga canadensis* (L.) Carrière) and *Fraxinus* L. species(Cowles and Lagalante 2009, McCullough et al. 2009). Cowles and Lagalante (2009) found imidacloprid to persist longer than dinotefuran. Imidacloprid titers peaked approximately 18-20 months after soil drench treatments and provided protection to trees for 5-7 years (Cowles and Lagalante 2009). Whereas dinotefuran has been found to have a higher initial defaunation than imidacloprid on *Fraxinus* species and on eastern hemlock (Cowles and Lagalante 2009, McCullough et al. 2009). Our results may suggest imidacloprid and dinotefuran metabolize in a similar way in chestnuts and northern red oaks as they do in eastern hemlocks and *Fraxinus* species. These results suggest imidacloprid is best utilized in long term protection from chronic infestations whereas dinotefuran is better utilized for protecting trees for one to two growing seasons.

Defoliation timing

In both years, the study found increased defoliation in every consecutive month sampled, which corresponds with *Cyrtepistomus castaneus* emergence. This finding parallels Johnson's (1956) observations in Maryland on chestnuts, noting defoliation getting progressively more severe throughout the growing season and peaking with the presence of *C. castaneus* in August (Johnson 1956). Not all studies have found analogous results in terms of *C. castaneus* defoliation and peak emergence. In Delaware, Triplehorn (1955) noted peak emergence and

most severe defoliation at the end of June and Wright (1989) saw observed peaks in mid-July in central Ohio. The differences in occurrence of the most severe defoliation among the studies is directly linked to highest emergence of *C. castaneus* (Triplehorn 1955, Johnson 1956, Wright et al. 1989). Emergence may be effected by yearly weather patterns, geographic location, or photoperiodism, as our study and others have found peak emergence to be between late June and early August in the eastern United States (Triplehorn 1955, Johnson 1956, Roling 1979, Case et al. 2012).

Growth

In the first year, most chestnut and oak families' height and RCD increased similar to one another with the exception of an oak family that experienced extreme dieback as well as the lowest survival rate. Chestnut growth was similar to previous silvicultural studies that found American and hybrid chestnuts height growth to between -3-18cm in first year field plantings (Clark et al. 2011) and 9-65cm in a second year field planting (Clark and Schlarbaum 2014a). Some families had a reduction in growth, which was linked to declining seedlings that eventually died. Reduced vigor of seedlings can lead to less water and nitrogen retention and to the reduction of chestnut seedling growth (Wang et al. 2012).

In the second field growing season, chestnuts added more growth and girth than northern red oak families. This may be due to the different methods that oaks (bare-root seedlings) and chestnuts (containerized) were propagated, as this factor along with harvesting and transplanting have been linked to affecting seedling establishment (Struve et al. 2000). The difference of growth between chestnut and oaks may also be due largely to species differences, as yearly growth of around 10cm on northern red oaks has been seen in other field plantings (Rebbeck et al. 2011).

The chestnut families in our study to grew similar to one another, which differed from Rieske et al. (2003), who observed American chestnut seedlings had greater height and RCD growth than BC_3F_1 hybrids in the first year. A study that was comparable to ours did not find differences between height or RCD growth of American and BC_2F_3 hybrid chestnuts in field plantings (Pinchot 2011), suggesting that more advanced hybrid generations exhibit similar growth patterns to American chestnuts.

Effects of defoliation on growth

The amount of defoliation observed within two growing seasons was not strongly correlated to seedling height or RCD growth, and defoliation in 2013 did not affect growth in 2014. A few negative weak correlations were significant between defoliation and both growth measurements in July 2014 and RCD in October of 2014. However, it is improbable that October 2014 defoliation negatively affected growth by November 2014, as the seedlings were in the process of going dormant. No research has been done to quantify the effects of defoliation on American or hybrid chestnut growth in field planting. Northern red oak seedlings subjected to artificial defoliation, in July found overwintering mortality to be significantly higher on seedlings that were 100% defoliated and significantly greater dieback on trees that were 50-75% defoliated (Wright et al. 1989). July was the only month in the study that showed a significant correlation. We ascribe this to mid-season defoliation, which has been found to have more of an effect on starch concentration in xylem and tree vigor (Gregory and Wargo 1986). July defoliation was less than 15%, leading to a weak correlation value.

Overall defoliation was low with control seedlings rarely having greater than 20% visual defoliation or 30% defoliation when calculated digitally. Consideration should be taken before the use of insecticides as to whether defoliation is severe enough to negatively affect trees. A previous study on oak and chestnut showed moderate defoliation caused decreased carbohydrate production and increased demand for carbohydrate reserve to re-foliate (Gottschalk 1990). Defoliation stress decreased competitive advantages for growing space, nutrients, and reduced seed production. Effects of severe defoliation can be seen in the same season with delayed re-foliation and bud-break in following seasons as compared to mild defoliation (Heichel and Turner 1983, Wright et al. 1989, Gottschalk 1990). Extreme late season defoliation has been seen to effect starch content and growth in oaks (Coffelt et al. 1993). The preceding four studies have been vague in what percent defoliation is considered to be moderate, severe, or extreme. Our results suggest defoliation of <30% in the late growing season did not negatively impact growth within two years. However, severe planting shock may have influenced the vigor and seedling growth.

Cyrtepistomus castaneus

Emergence

Peak adult emergence of *C. castaneus* occurred at our site in late July until mid-August in 2013 and 2014, which concurred with emergence studies in Delaware (Triplehorn 1955) and in central Missouri (Roling 1979). In contrast, a previous study, found peak emergence of *C. castaneus* occurring in late June and early July in a different site in Tennessee (Case et al. 2015). This is possibly due to variable microsite climates in the southern Appalachian Mountains and differences in general weather and temperature patterns between sites and years as similar methodologies were used. The study observed emergence tapering off in October which was also observed in other studies (Triplehorn 1955, Roling 1979, Case et al. 2015) corresponding to *Castanea* dormancy.

Insect traps collected high numbers of *C. castaneus*, which was to be expected as a previous canopy insect study at the Watauga Orchard found *C. castaneus* to be the most abundant of all insects collected from the canopy (Stanton 1994). More Asiatic oak weevils were found in emergence traps in 2013 than in 2014. This is may have been due to collections occurring bi-weekly in 2014 rather than weekly in 2013. The extra time the insects spent in the traps allowed for spider predation and subsequently decay beyond identification. This may have influenced mean emergence in 2014, as spider predation most likely lacked uniformity between traps. The higher number of *C. castaneus* in 2013 than in 2014 may have been a normal cyclical population pattern. More research is needed to confirm yearly cyclical densities of populations.

Cyrtepistomus castaneus emergence patterns in 2013 and 2014 did not have significantly different spatial densities in emergence around the orchard. This provides valuable insight to spatial dispersion patterns of *C. castaneus*. Our findings are similar to a study conducted in Boone county, Missouri assessing dispersion of *C. castaneus* that also found no significant difference in spatial distribution over the course of two years (Ferguson et al. 1992). Visually there was a trend, in 2013, of higher mean emergence on the uphill northern border and in 2014, there appeared to be greater emergence in the lower southeastern corner. Correlations between emergence and 2013 with digital defoliation in October when analyzed by treatment, only seedlings given the water control were significant and mostly caused the significant correlation

of all seedlings treated. In 2014 we observed negative correlations on seedlings treated with acephate and water control to average weekly *C. castaneus* emergence, which suggests that other insects were the primary defoliators in 2014. We still know little about what influences population densities of *C. castaneus*. Additionally there are many biological factors that could influence spatial dispersion of an insect population including: topography, oviposition behavior, mate behavior, spatial pattern of predator and parasite attack, and egg and larva density (Poole 1974, Ferguson et al. 1992).

It is also unclear how larval populations affect tree species vigor, as the larva feed on fine root hairs (Triplehorn 1955, Roling 1979) and may severely stress trees and impact crop yields, growth rate, and mortality (Frederick and Gering 2006). If insecticides are considered for the Watauga Orchard to combat weevil populations, a uniform distribution of treatment would probably be most effective due to the relatively even dispersion of *C. castaneus* populations.

Cyrtepistomus castaneus has been identified as an important defoliator of *Quercus* and *Castanea* species (Triplehorn 1955) and has previously been identified to more strongly prefer chestnuts than oaks (Johnson 1956). In this study, the amount of defoliation did not differ between species in 2014, and defoliation levels were not correlated to mean emergence from the closest insect trap. This may be due to the fact that there was a relatively low percent of defoliation, potentially a high number of other primary defoliators, and that this study did not differentiate *C. castaneus* defoliation on seedlings from other defoliation patterns. Additional research is needed to understand long term effects of defoliation on seed production, budbreak, starch content and growth to better understand the threats chestnut restoration will face.

Chapter 6. Conclusions

The lack of differences among families or species in defoliation amount suggests no strong insect preferences among families or species at the site. Digital defoliation methods are not recommended over visual methods, as they required considerably more time, but yielded similar result.

Use of dinotefuran is suggested when rapid defense is needed, but imidacloprid is recommended for long term protection in controlling harmful defoliating insect pests. Acephate is not recommended, as it provided no discernable protection with the low level of defoliation that was observed.

Families within species grew similar regardless of genetic origin and showed no initial growth differences between American and advanced hybrid chestnuts. The lack of strong correlations between defoliation on seedlings and growth indicates that defoliation below 15% in the mid growing season and 20% in the late growing season does not affect early growth of *C. dentata*, *Q. rubra* or hybrid chestnut seedlings. Although results were probably influenced by planting shock, growth differences may have been evident if the trees were well established.

Emergence of *Cyrtepistomus castaneus* showed a similar trend in both study years, which began in early May, peaked in late July through mid-August, and ended by late November. No significant patterns in densities of *C. castaneus* emergence in either study year suggests relatively uniform dispersion of *C. castaneus* populations. Defoliation levels increased throughout the growing season corresponding to *Cyrtepistomus castaneus* emergence. However, defoliation on seedlings did not differ when located near traps that had higher *C. castaneus* emergence, potentially due to the low percent of defoliation on seedlings, similar dispersion of *C. castaneus* and/or other primary defoliators.

As *C. parasitica* resistant chestnut are reintroduced, it will be important to understand and anticipate potential threats. Insect herbivory has been identified as one treat to American and hybrid chestnuts. The use of insecticides could initially aid in competitive abilities of seedlings post-planting and be a good management tool for protecting high valued trees.

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Appendix

Table A-1. Number of C. dentata and hybrid seedlings one month after planting ranked by suitability for the defoliation ratings (S- Satisfactory, U1- Mildly unsatisfactory, U- Unsatisfactory, D- Dissatisfactory) sorted by insecticide treatment.

	S	U1	U	D	Total
C. dentata	64	10	4	0	78
Imidacloprid	13	2	3	0	18
Acephate	16	3	1	0	20
Dinotefuran	19	1	0	0	20
Water control	16	4	0	0	20
D-3-862	28	15	17	5	65
Imidacloprid	10	3	2	3	18
Acephate	7	3	6	0	16
Dinotefuran	7	4	4	1	16
Water control	4	5	5	1	15
W-4-938	50	11	8	5	74
Imidacloprid	14	3	1	0	18
Acephate	8	5	1	4	18
Dinotefuran	14	2	3	0	19
Water control	14	1	3	1	19
Total	142	36	29	10	217

Table A-2. Percentage of seedling survival of Q. rubra and Castanea spp. by treatment at Watauga orchard in May of 2014

Family	Species	Imidacloprid	Acephate	Dinotefuran	Water	Total
1-20-2418	*Q. rubra*	90.3	93.1	90.3	88.9	62.5
2-16-528	*Q. rubra*	97.2	91.7	94.4	94.4	77.8
7-14-630	*Q. rubra*	95.8	95.8	93.0	93.0	77.5
AP5	*C. castanea*	97.4	98.7	100.0	98.7	94.9
D-3-862	BC_3F_3	93.8	93.8	93.8	90.8	72.3
W-4-938	BC_3F_3	94.6	91.9	95.9	97.3	79.7
Total		79.6	76.9	78.7	75.9	

Table A-3. Polynomial Regression equations for estimating area based on leaf length, leaf width, and petiole diameter of combined American and hybrid chestnut families with R2 values.

Variable	Equation	R-squared
Leaf length	$Y= 0.1686x^2 + 10.567x - 112.46$	0.9133
Leaf width	$y = -0.0512x^3 + 8.9266x^2 - 332.64x + 4708.8$	0.8733
Petiole diameter	$y = -1212.7x^3 + 5744.2x^2 - 4855.3x + 3068.2$	0.4585

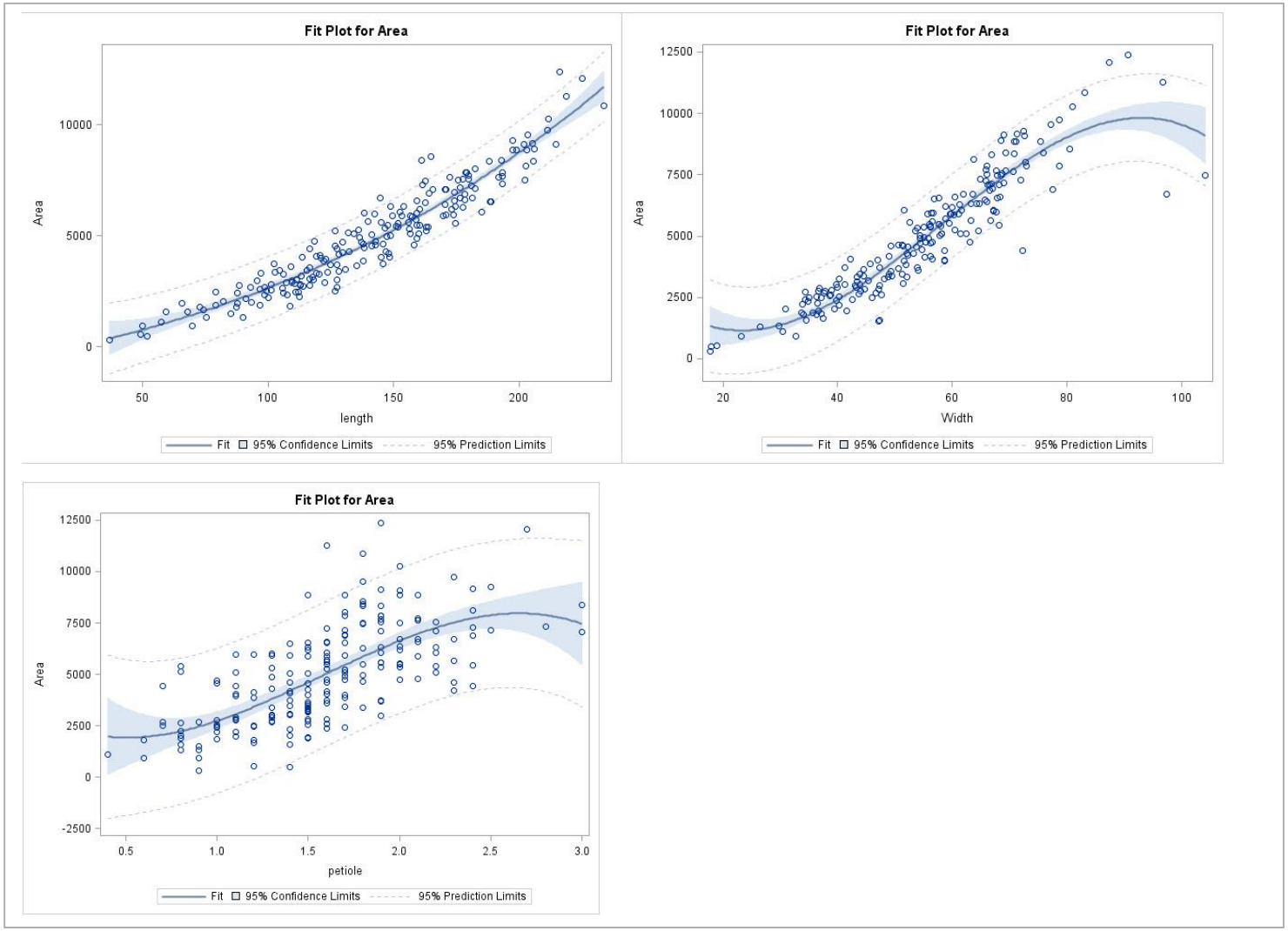


Figure A-1. Plotted leaf area regression equations to area by length (top right), width (top left) and petiole diameter (bottom left).

Table A-4. Means ± standard error of visual estimated defoliation in 2013 among treatments, family and month. Means with letters that are the same are not significantly different (α = 0.05).

	August		September		October	
	AP5	W-4-938	AP5	W-4-938	AP5	W-4-938
Imidacloprid	1.7 ef ± 1.9	3.0 def ± 1.9	2.8 def ± 2.0	3.6 def ± 2.1	4.5 def ± 2.1	10.7bc ± 2.2
Acephate	3.7 def ±2.0	1.3 ef ± 1.9	11.9 bc ± 2.0	2.6 def ± 2.0	16.4 a ± 2.2	5.5 cdef ± 2.6
Dinotefuran	2.0 ef ± 2.0	0.1f ± 1.3	0.5 ef ± 2.0	2.1 ef ± 2.0	2.2 ef ± 2.5	4.7 de ± 2.0
Water control	3.9 def ± 2.0	1.2 ef ± 2.0	4.7 def ± 2.1	3.0 ef ± 2.2	12.9 ab ± 2.2	9.4 abcd± 2.9

Table A-5. Means ± standard error of digital defoliation in 2013 among treatments, family and month. Means with letters that are the same are not significantly different (α = 0.05).

	August		September		October	
	AP5	W-4-938	AP5	W-4-938	AP5	W-4-938
Imidacloprid	6.5 fgh ± 4.4	5.5 gh ± 4.3	10.1 efgh ± 4.4	11.2 defgh ± 5.2	18.5 bcde ± 4.6	33.9 a ± 5.2
Acephate	9.8 efgh ± 4.4	3.9 h ± 4.4	28.9abc ± 4.4	16.0 defg ± 4.6	31.9 ab ± 5.2	18.3 bcdef ± 4.6
Dinotefuran	6.9 efgh ± 4.4	4.1 gh ± 4.4	5.8 gh ± 4.4	22.4 abcd ± 4.4	14.6 cdefgh ± 6.1	14.8 defgh ± 4.4
Water control	8.5 efgh ± 4.4	5.2gh ± 4.4	14.3 defgh ± 4.6	15.9 defgh ± 4.9	30.3 ab ± 4.9	13.2 defgh ± 5.6

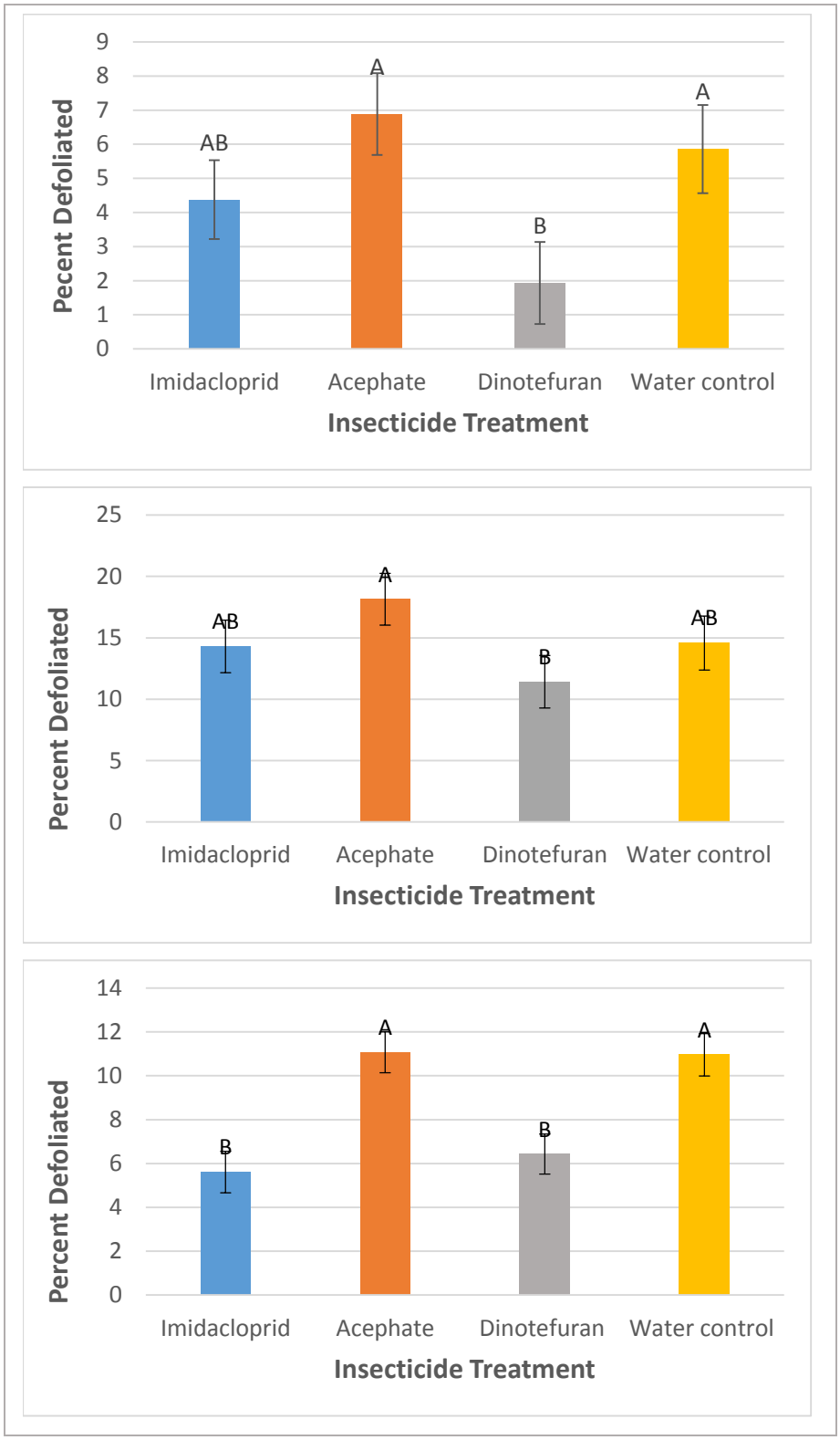


Figure A-2. Percent of defoliation on all seedlings assessed by treatment in 2013 using visual methods (top), 2013 digital methods (middle) and 2014 visual (bottom) Treatment bars labeled with the same letter are not significantly different (α = 0.05).

Vita

Ashley Elizabeth Case grew up in the mountains of western North Carolina where she enjoyed countless days of hiking, camping and swimming. It was in this time when her love for the outdoors developed. Her love for the environment has led her to pursue work with a sea turtle conservation project in Costa Rica, MAPS (Monitoring Avian Productivity and Survivorship) in the Great Smoky Mountains, and studying tropical ecosystems in Belize. In the fall of 2012, she received a Bachelor of Science degree with a focus in ecology from The University of North Carolina in Asheville (UNCA). During her time at UNCA she also had the opportunity to work with the USFS southern research station as a technician under Dr. Albert (Bud) Mayfield. It was this experience that led her to The University of Tennessee in Knoxville to pursue a Masters in Forestry which she earned in August 2015.