COMPENSATORY RESPONSE OF BLACK COTTONWOOD TO DEFOLIATION BY COTTONWOOD LEAF BEETLE

By

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COMPENSATORY RESPONSE OF BLACK COTTONWOOD TO

DEFOLIATION BY COTTONWOOD LEAF BEETLE

Abstract

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Many studies report that species of *Populus* respond to the negative effects of defoliation by cottonwood leaf beetle (CLB), *Chrysomela scripta* F. [Coleoptera:Chrysomelidae], through mechanisms of tolerance. Studies that report a physiological response to defoliation often fail to accurately simulate CLB feeding behavior in their experiments. During the summer of 2009, 1) we assessed the treatment techniques used in the literature and developed a set of protocols to simulate CLB feeding behavior and, 2) we measured the physiological and morphological response of *P. trichocarpa* to CLB and artificial defoliation treatments. We found that CLB avoided apical meristems, fed on young leaves (LPI 0-5) and avoided feeding on leaf midveins and primary lateral veins. To assess the response of *P. trichocarpa* to defoliation, we assigned two-year old trees a defoliation treatment (CLB or artificial) with individual branches defoliated at different levels (0, 25, 50, or 75% defoliation). Leaf and branch biomass were not different across different levels of defoliation and between types of defoliation due to increased leaf thickness and increased lateral branching. Branches that were defoliated by CLB had 22% lower leaf nitrogen than branches defoliated artificially. We concluded that 1) *P. trichocarpa*

completely compensated for low levels (<30% branch defoliation) by CLB through increased leaf thickness and increased lateral branching and 2) defoliation by CLB caused a decrease in leaf nitrogen content compared to artificial defoliation due to the selective removal of nitrogen rich interveinal tissue by the insects.

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INTRODUCTION

All plants must respond to the threat of herbivory. One form of defense against herbivory is to counteract the negative effects by compensating for tissue loss (Strauss and Agrawal 1999). This form of defense is termed "tolerance" and can be defined as the effect on plant fitness caused by herbivore damage relative to fitness for the undamaged controls (Strauss and Agrawal 1999). A second term, compensation, can be used to define the degree of tolerance that is exhibited by plants damaged by herbivory. Three patterns of compensation may occur: over-compensation, under-compensation, or full-compensation in which fitness is greater than, less than, or equal to an undamaged control, respectively.

A tolerance response has been shown in species of the genus *Populus* (Bassman and Dickmann 1982; Bassman and Zwier 1993; Reichenbacker et al. 1996; Coyle et al. 2008). *Populus* includes poplars, cottonwoods, and aspens that are widely distributed over the northern hemisphere. The trees are fast-growing, wind-pollinated and play a significant role in native ecosystems (Fischer et al. 2004). Due to their long life spans, large sizes, apparent lifestyles, and ecological dominance, poplars are subjected to numerous herbivores (Philippe and Bohlmann 2007). Jansson and Douglas (2007) estimated that *P. tremuloides* plays host to at least 300 species of insects. Furthermore *Populus* is attacked by moose (Chantal and Granstrom 2007), porcupine (Morin et al. 2005), and numerous pathogens (Coyle et al. 2006). The ability of *Populus* to withstand repeated attacks by herbivores is reflected in a diverse set of defense systems, one of which is the ability to compensate for tissue loss (Bassman and Dickmann 1982; Bassman and Zwier 1993; Reichenbacker et al. 1996; Coyle et al. 2008).

A major defoliating insect of *Populus* is the cottonwood leaf beetle (CLB), *Chrysomela scripta* F. [Coleoptera:Chrysomelidae], which occurs throughout most of the northern

hemisphere with the exception of the coastal regions of the Pacific Northwest (Mattson et al. 2001). CLB spend the majority of their life cycle on leaves of *Populus*. Adults aggregate on young leaves for feeding and mating and oviposite on the underside of immature foliage (Bingaman and Hart 1992). The larvae feed on leaf tissue through three instars until pupation. CLB may have multiple generations in a single season (Burkot and Benjamin 1979) and all will feed on leaves of *Populus*.

The compensatory response of *Populus* to defoliation by CLB has been studied by several researchers (Bassman and Dickmann 1982; Bassman and Zwier 1993; Reichenbacker et al. 1996; Coyle et al. 2008; Stevens et al. 2008). Studies that used natural populations of CLB focused on the morphological response (Stevens et al. 2008; Coyle et al. 2008), while reports that investigated the physiological mechanisms used artificial treatments (Bassman and Dickmann 1982; Bassman and Zwier 1993; Reichenbacker et al. 1996). *Populus* has been shown to respond differently to natural and artificial defoliation treatments (Arimura et al. 2004) thus the artificial treatments applied by the researchers may not have achieved accurate results.

Although the interaction between *Populus* and CLB has been studied in the past, the physiological response to defoliation remains unclear. In this study, we address this issue by 1) assessing the treatment techniques used in the literature and develop a set of protocols to simulate CLB feeding behavior and 2) measuring the physiological and morphological response of *P. trichocarpa* to defoliation.

In chapter 1 we report observations on the feeding behavior of CLB during a natural outbreak and assess the treatment techniques used in the literature to define a set of treatment protocols for our own research. We report that CLB rarely damage apical meristems, prefer to feed on young leaves, and avoid the midvein and primary lateral veins. We conclude that the

defoliation techniques used in the literature fail to accurately mimic CLB feeding behavior and that proper defoliation treatments must avoid meristem damage, damage leaves of LPI 0-5, and avoid damaging the midveins.

In chapter 2 we report the morphological and physiological response of *P. trichocarpa* branches to defoliation by CLB or artificial means. After a six week study period, the branches had no difference in biomass, despite significant removal of leaf tissue. We show that increased assimilation rates were not a mechanism of tolerance in *P. trichocarpa*, but increased leaf thickness and increased sylleptic branching were found to be associated with different levels of defoliation. CLB and artificial treatments differed in percent nitrogen of leaves and in the production of additional leaves and leaf area over the growing season. We conclude that 1) *P. trichocarpa* can fully-compensate for defoliation by CLB or artificial means, and 2) artificial defoliation techniques result in a different response compared to CLB defoliation in *P. trichocarpa*.

In this study, we show that reports using artificial treatments to measure the compensatory response of *Populus* to defoliation by CLB may not be accurate. We found that *P. trichocarpa* has a tolerance response to defoliation that is in agreement with previous reports. However, the differences between the treatment techniques used in this study and those used in previous literature may cause different degrees of tolerance by eliciting different physiological and morphological responses. In this study alone we found that branches defoliated with CLB had a different response than those defoliated by artificial means.

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CAPTER ONE

FEEDING BEHAVIOR OF COTTONWOOD LEAF BEETLE: ASSESSING ARTIFICIAL TREATMENT TECHNIQUES

1.1 ABSTRACT

Artificial treatment techniques have been used to study the physiological effects that defoliation by cottonwood leaf beetle (CLB), *Chrysomela scripta* F. [Coleoptera:Chrysomelidae], has on growth of *Populus* spp., but these techniques have been inconsistently applied by different researchers. The physiological response of plants is often dependent on the timing, duration, and physical application of defoliation. The purpose of this study was to assess the treatment techniques used in the literature and to develop a set of protocols to simulate CLB feeding behavior on *Populus* spp. by observing natural populations of CLB. We found that 98% of apical meristems remained intact during a natural outbreak of CLB. Leaves of LPI 0-2 were defoliated up to 100% when the larvae were allowed to move freely on the branch, percent defoliation decreased with increased leaf age, and leaves of LPI 6 or greater were not defoliated. The midveins of leaves had less than 1% of their total lengths removed by CLB when leaves were defoliated 25-50%. Lateral veins had less than 6% of their total lengths removed by CLB at 25% leaf defoliation and 20% removed at 50% leaf defoliation. The defoliation techniques we assessed failed to accurately simulate CLB feeding behavior because they removed apical meristem tissue, defoliated leaves perpendicular to the midveins, and defoliated leaves of LPI 6 or greater. We conclude that proper defoliation treatments must avoid meristem damage, damage leaves of LPI 0-5, and avoid damaging the midveins.

1.2 INTRODUCTION

Cottonwood leaf beetle (CLB), *Chrysomela scripta* F. [Coleoptera:Chrysomelidae], is a major pest species of hybrid poplars (Coyle et al. 2005) with a native range that overlaps with many species of *Populus*, including *P. trichocarpa* (Burkot and Benjamin 1979). CLB feed on leaves of *Populus* throughout the larval and adult stages of their life cycle and can have multiple generations in a single growing season (Brukot and Benjamin, 1979). CLB numbers can increase exponentially during the growing season resulting in severely defoliated trees (Harrel et al. 1982; Hart et al. 1996; Coyle et al. 2005) and a reduction in biomass and fitness (Reichenbacker et al. 1996; Coyle et al. 2008). The physiological effects that CLB has on growth of *Populus* have been studied but most research relied upon artificial defoliation rather than natural defoliation by CLB (Bassman and Zwier 1993; Reichenbacker et al. 1996; Tucker et al. 2004).

The artificial defoliation techniques used in many studies were not consistently applied between investigators. Bassman and Zwier (1993) removed leaf lamina parallel to the midvein during a single defoliation treatment. Reichenbacker et al. (1996) defoliated leaves by either scrapping the leaves to simulate early instar damage or removing parts of the lamina of juvenile and mature leaves parallel to the midvein during multiple treatments. Tucker et al. (2004) randomly removed leaf tissue during a single treatment. All treatment techniques varied by their timing, duration and physical application.

Differences in defoliation treatments elicit different responses in *Populus* (Arimura et al. 2004) so it is important to closely mimic CLB feeding behavior to achieve accurate results. The purpose of this study was to review previously used methods of artificial defoliation and to

develop a set of treatment protocols by observing the feeding behavior of CLB.

1.3 METHODS

A survey of apical meristem damage, evaluation of leaf preference and an assessment of leaf vein damage were conducted in June 2009 on the GreenWood Tree Farm near Boardman, OR (45.7797°N 119.5412°W, 188m elevation). Apical meristem damage was measured on June 12, 2009 during a natural outbreak of CLB that occurred on Plot 611-1 containing *P. trichocarpa* x *P. deltoides* hybrids (clone #PC-4). Trees were haphazardly chosen by blindly throwing an object and measuring the nearest tree to the object. Branches with more than 25% of the young leaves defoliated were chosen from each tree and scored for the presence of apical meristem damage.

Evaluations of leaf preference and assessment of leaf vein damage by CLB were conducted on *P. deltoides* x *P. nigra* hybrids (clone #BC-79) in Plot 18-4. The study area consisted of a 20 tree by 20 row plot with a 10 tree perimeter to act as a buffer from the roads. Leaf preference, recorded by leaf plastochron index (LPI *sensu* Larson and Isebrands, 1971), was assessed by two methods; netting whole branches, which allowed insects to choose a leaf, and a no choice treatment by netting individual leaves, to prevent insect movement between leaves. For the whole branch treatment, two branches were randomly selected from six randomly selected trees. The branches were netted and assigned two treatment levels; low, consisting of ~20 larvae/branch and high, consisting of ~40 larvae/branch. The treatment levels were assigned to the branches in a randomized complete block design. The larvae were allowed to feed for three days (June 13-16, 2009) before measurements of defoliation were made. In the second experiment, leaf preference was assessed by restricting 3 to 5 CLB larvae to a single leaf. Undamaged trees in the study area were selected and one branch was randomly selected from each tree. Leaves of increasing maturity (LPI 1, 3, 5, 7 and 9) were individually enclosed in a

net bag. Three larvae were enclosed within bags on leaves of LPI 1 and 3 and five larvae were enclosed within bags on leaves of LPI 5, 7, and 9. After two days the larvae were removed and the leaves were analyzed to determine percent defoliation.

Two branches of undamaged trees were randomly chosen in the study area to determine percent vein damage. A defoliation treatment of either 25% or 50% was randomly assigned to each branch in a randomized complete block design. Individual leaves were netted and larvae number was adjusted each day during the experiment to obtain desired defoliation levels. Insects were allowed to feed for five days before leaves were harvested for measurements.

Defoliation and vein damage was measured with a digital photograph of the leaves and analyzed in ImageJ (Abramoff et al. 2004). Leaf photographs were taken prior to and after treatments to measure percent defoliation and percent vein damage. Lateral veins were defined as leaf veins that branched directly from the midvein and did not include secondary lateral veins. Results were analyzed by a one-way ANOVA and a least significance difference test using SAS (SAS Statistics, Cary, NC).

1.4 RESULTS

CLB adults and larvae fed little on apical meristems and preferentially fed on leaves of low leaf plastochron index (LPI). The apical meristem survey showed only 2% of the meristems had tissue removed during a natural outbreak (n = 100). The leaf preference study indicated that high numbers of larvae (40 larvae/branch) completely defoliated leaves of LPI 0-2, while leaves of LPI 6 or greater were not defoliation (Figure 1). Lower numbers of larvae (20 larvae/branch) completely defoliated leaves of LPI 0, while those of LPI 6 or greater were not defoliated and between LPI 3 and LPI 6 defoliation decreased with increasing LPI. When larvae were confined to a single leaf they partially defoliated leaves of LPI 0-9 but the amount of defoliation decreased with increased leaf age (Figure 2). When larvae fed on leaves of LPI 1, each larva defoliated the leaf 14 ±6% but when larvae fed on leaves of LPI 7, each larva defoliated the leaf 2 ±1%. All leaves of LPI 5 and greater showed signs of skeletonization, a process where all leaf veins remain intact and not all layers of laminar tissue is removed.

Leaf midvein tissue was not removed by CLB and lateral vein damage increased significantly ($p < 0.05$) with increased leaf defoliation (Figure 3). Midveins had less than 1 ±0.2% of the length removed by CLB when the leaves were defoliated 25% to 50%. Lateral veins had 5 ±2% and 20 ±6% of their total lengths removed by CLB when the leaves were defoliated 25% and 50%, respectively. Lateral veins at the periphery of the leaves were damaged more by CLB than lateral veins near the midvein.

1.5 DISCUSSION

Damage to the apical meristem can cause increased lateral branching by the release of lateral buds from apical dominance (Strauss and Agrawal 1999; Haukioja and Koricheva 2000). Apical meristem damage has been reported to occur during CLB outbreaks (Burkot and Ben 1979) but we have no evidence of CLB feeding on apical meristems of *P. trichocarpa* hybrids. Because meristem damage can change a plant's morphology, it is important to consider this effect while designing artificial defoliation techniques. The techniques used by Tucker et al. (2004) blindly hooked and tore plant tissue which likely resulted in damage to the apical meristems. To mimic CLB defoliation caution must be used when defoliating leaf tissue to ensure that the process does not damage meristems.

CLB preferentially feed on young leaves (Bingaman and Hart 1992; this study). We found that CLB preferred leaves of LPI 0-5 and did not defoliate older leaves. Trees defoliated by CLB are left with photosynthetic material at the base of their branches that may supply photosynthate to promote growth (Bassman and Dickmann 1985). Artificial treatments that defoliate mature leaves remove tissue from the base of the branches and increase the negative effects of defoliation. Despite this, many studies have attempted to simulate CLB outbreaks by defoliating leaves greater than LPI 5 (Bassman and Zwier 1993; Reichenbacker et al. 1996; Tucker et al. 2004). Studies that artificially defoliated leaves of LPI greater than 5 may have results that are not applicable to natural conditions.

Midvein and lateral vein tissue was avoided by CLB. Leaf veins are important for the transport of water and nutrients to the leaf chloroplasts so disruption of the veins by herbivory can negatively affect photosynthetic production (Aldea et al. 2005). The treatments used by Bassman and Zwier (1993) and Reichenbacker et al. (1996) defoliated leaves parallel to the

midvein to closely simulate CLB feeding behavior. However, Tucker et al. (2004) defoliated leaves in a random fashion so likely caused midveins to be disrupted. Studies that used treatments that damaged midveins likely caused a greater reduction in photosynthesis than would occur with CLB defoliation.

In conclusion, researchers need to consider the timing, duration, and physical application of the defoliation treatment. The timing of defoliation should occur on leaves between LPI 0-5 because CLB have been shown to avoid feeding on the meristems and avoid defoliating mature leaves. The duration of the treatment application may occur throughout the growing season because CLB have multiple generations. The physical application of the treatment should avoid defoliating leaf midveins and avoid lateral veins because CLB preferentially feed on the interveinal material. A more accurate simulation of CLB defoliation may be achieved if the technique avoids removing any meristematic tissue and avoids the midvein while causing minimal damage to the primary lateral veins close to the midvein.

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1.7 FIGURE CAPTIONS

Figure 1.1. Leaf feeding preference of *Chrysomela scripta* when enclosed on a branch with a mesh net. Insects were allowed to move freely between leaves on a single branch and were allowed to feed for three days. Percent defoliation was measured with image processing software. Each point represents an average (n = 5) and error bars represent standard error.

Figure 1.2. Leaf feeding preference of *Chrysomela scripta* when enclosed on a single leaf with a leaf bag. Insects were allowed to feed on a single leaf during a two day period. Leaves of LPI 1 and 3 had three insects enclosed and leaves of LPI 5, 6, and 7 had five insects enclosed. Percent defoliation was measured with image processing software. Each point represents an average (n = 4) and error bars represent standard error.

Figure 1.3. Percent leaf vein length that was removed by *Chrysomela scripta* during a five day feeding period. Percent defoliation and leaf vein damage was measured with image processing software. Lateral veins were defined as leaf veins that branched directly from the midvein. Secondary branching of lateral veins was not considered. Each bar represents an average (n = 13 for 25% defoliated leaves; n = 10 for 50% defoliated leaves) and error bars represent standard error.

1.8 FIGURES

Figure 1.1

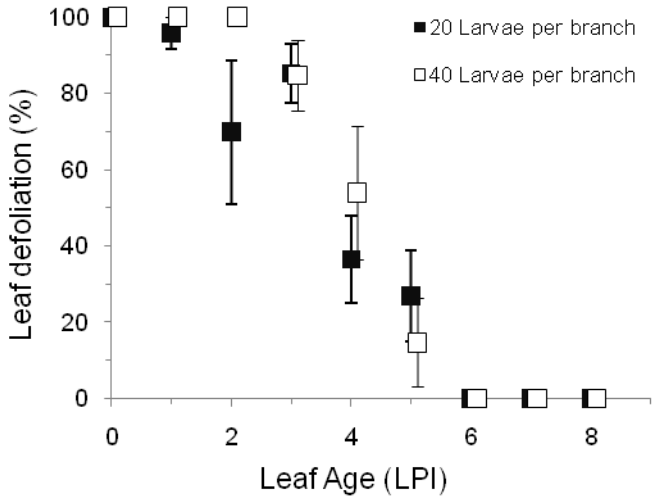


Figure 1.2

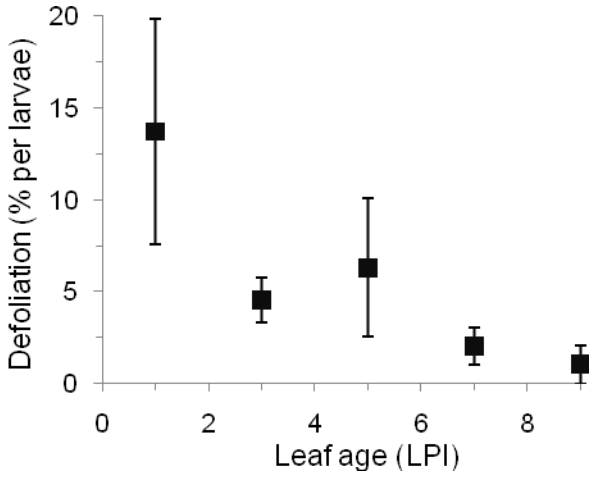
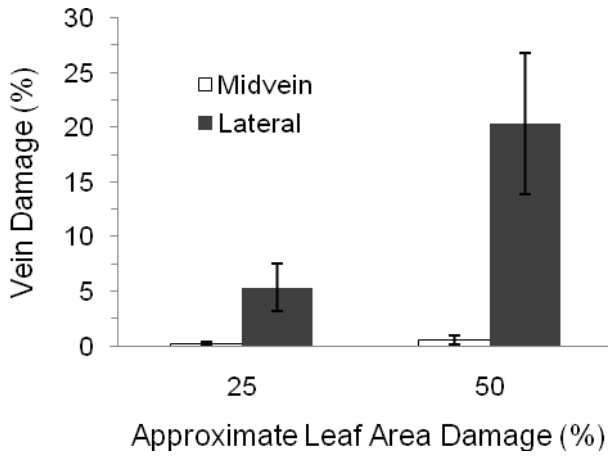


Figure 1.3


30
25
20
15
10
5
0
Vein Damage (%)
Midvein
Lateral
25
50
Approximate Leaf Area Damage (%)

CHAPTER TWO

COMPENSATORY RESPONSE OF BLACK COTTONWOOD TO NATURAL AND SIMULATED DEFOLIATION BY COTTONWOOD LEAF BEETLE

2.1 ABSTRACT

Plants may buffer the negative effects of herbivory through mechanisms of tolerance. Many studies report species of *Populus* tolerate herbivory, but fail to report the physiological mechanisms of tolerance. Studies that report a physiological mechanism often fail to accurately simulate insect feeding behavior in their experiments. During the summer of 2009, we measured the physiological and morphological response of *P. trichocarpa* to natural and simulated defoliation by cottonwood leaf beetle (CLB), *Chrysomela scripta* F. Two-year old trees were assigned to a defoliation treatment (CLB or artificial) with individual branches defoliated at different levels (0, 25, 50, or 75% defoliation) throughout the study period. At the end of the study, leaf and branch biomass were not different across defoliation type and between defoliation level due to increased leaf thickness and increased lateral branching. Specific leaf area decreased from 55 m^2 g^{-1} in control branches to 47 m^2 g^{-1} for branches with 30% defoliation. The number of sylleptic branches increased with increased defoliation and contributed to increased leaf area and leaf number. Branches that were defoliated by CLB had 22% lower leaf nitrogen than branches defoliated artificially. We conclude that 1) *P. trichocarpa* completely compensated for low levels (<30%) of defoliation by CLB through decreased specific leaf area and increased sylleptic branching and 2) defoliation by CLB caused a greater decrease in leaf

nitrogen content compared to artificial defoliation due to the selective removal of nitrogen rich interveinal tissue by the insects.

2.2 INTRODUCTION

Many plants fall prey to herbivory and have developed complex response systems. Plants may use combinations of resistance and tolerance to defend against attacks by herbivores (Ralph 2009). A resistant plant uses chemical or physical means to reduce the preference or performance of herbivores while a tolerant plant counteracts the negative effects of herbivory by compensating for tissue loss (Strauss and Agrawal 1999). The mechanisms and traits for resistance are well studied but the mechanisms describing tolerance are poorly understood (Lau and Tiffin 2009).

Mechanistic models of plant tolerance to herbivory have not been defined, thus tolerance is not readily studied within individuals and is often defined as the effect on plant fitness caused by herbivore damage relative to fitness for the undamaged controls (Strauss and Agrawal 1999; Núñez-Farfán et al. 2003). Compensation therefore, can be defined as the degree of tolerance that is exhibited by the damaged plant. A plant may show three patterns of compensation: over-compensation, under-compensation or full-compensation in which fitness is greater, less than, or equal to an undamaged control plant, respectively.

Populus have been shown to exhibit all three patterns of compensation. *P. deltoides* x *P. nigra* hybrids fully-compensate (Reichenbacker et al. 1996; Coyle et al. 2008), *P. nigra* x *P. maximimowiczii* hybrids have been shown to over-compensate (Coyle et al. 2008), and *P. trichocarpa, P.* x *euramericana* and *P. deltoides* were shown to under-compensate (Bassman and Dickmann 1982; Bassman and Zwier 1993; Coyle et al. 2008) when studies used biomass as an indicator of fitness. The compensatory response to defoliation by *Populus* apparently varies by species and genotype.

Many reports propose physiological or morphological mechanisms for tolerance in *Populus*. The proposed mechanisms include increased photosynthesis, increased branching, and increased leaf thickness. Stevens et al. (2008) reported leaves of *P. tremuloides* increased their photosynthetic rates following artificial defoliation. Similarly Bassman and Dickmann (1982) found that mature leaves below the zone of defoliation of *P.* x *euramericana* increased assimilation rates by up to 50% and leaves above the zone of defoliation had only a small increase in photosynthesis. Bassman and Zwier (1993), however, reported that *P. trichocarpa* did not respond to defoliation with increased assimilation rates.

Increased branch biomass through increased lateral branching is a commonly proposed mechanism of tolerance by *Populus*. Lateral branches grown from buds that were produced the same growing season are called sylleptic branches and has been reported in many species of *Populus*. Increased sylleptic branching following defoliation was found in *P.* x *euramericana* (Bassman and Dickmann 1982), *P. trichocarpa* (Bassman and Zwier 1993), and *P. deltoides*, *P. nigra*, and *P. maximowiczii* hybrids (Reichenbacker et al. 1996; Coyle et al. 2008). For these studies, the degree of compensation was reportedly different between species and genotypes but all reported increased branch biomass.

Populus has been shown to increase leaf thickness in response to defoliation but the response differs by species. *P. x euramerican* had increased leaf thickness of mature leaves below the zone of defoliation while leaves produced after the defoliation event decreased leaf thickness (Bassman and Dickmann 1982). The leaves of *P. trichocarpa* produced following defoliation, however, were thicker compared to controls (Bassman and Zwier 1993).

Research on the tolerance response of *Populus* has important commercial applications due to the use of hybrid poplars for their biomass, pulp and timber production. Pest outbreaks in

large poplar plantations can be a serious problem where genetically identical individuals occur in close proximity (Ranney et al. 1987). The cottonwood leaf beetle (CLB), *Chrysomela scripta* F. [Coleoptera:Chrysomelidae], is a defoliating insect shown to decrease biomass yield of *Populus* through the chronic removal of photosynthetic tissue (Reichenbacker et al. 1996; Coyle et al. 2008). The interaction between *Populus* and CLB has been studied by several researchers (Bassman and Dickmann, 1982; Bassman and Zwier, 1993; Reichenbacker et al. 1996; Coyle et al. 2008; see works cited) due to its economic importance.

Many reports on natural populations of CLB focused on the morphological mechanisms of compensation (Stevens et al. 2008; Coyle et al. 2008), while reports that investigated the physiological mechanisms of compensation used artificial treatments (Bassman and Dickmann 1982; Bassman and Zwier 1993; Reichenbacker et al. 1996; Tucker et al. 2004; Stevens et al. 2008). Studies that defoliated leaves through artificial treatments may have results that do not accurately represent the compensatory response of *Populus* to CLB (see Chapter 1). CLB has been found to avoid leaves older than LPI 5 and avoid damaging the midveins by feeding on interveinal lamina. Despite this, many studies defoliated leaves greater than LPI 5 (Bassman and Dickmann 1982; Bassman and Zwier 1993; Reichenbacker et al. 1996; Stevens et al. 2008) and defoliated leaves perpendicular to the midveins (Reichenbacker et al. 1996; Steven et al. 2008).

The physiological and morphological response of cottonwood to natural defoliation by CLB is unclear because 1) the genus *Populus* exhibits large variations in its compensatory response to defoliation, 2) studies that used natural populations of CLB neglected to identify a physiological mechanism of tolerance, and 3) studies that investigated the physiological tolerance response often used artificial treatments that may not accurately represent defoliation by CLB. To address the above issues, the objective of this study was to measure the

physiological and morphological compensatory response of *P. trichocarpa* to natural and simulated defoliation by CLB.

2.3 METHODS

The effect that natural and artificial cottonwood leaf beetle (CLB), *Chrysomela scripta* F. [Coleoptera:Chrysomelidae], defoliation had on poplar growth and physiology was experimentally assessed in Pullman, WA (45.73 N latitude, 117.17 W longitude; 732 m elevation) from July 7, 2009 to August 27, 2009. The study area consisted of four replicates of 15 *P. trichocarpa* genotypes that were planted in three rows during the summer of 2007 (See Appendix A for a list of genotypes used in the experiment). The clonal cuttings were collected previously along a west-east transect across Washington state (Sparks and Black 1999).

The experimental protocol consisted of the random selection of 20 trees that had more than four branches, were actively growing and had low insect herbivory. Ten trees were randomly assigned to the CLB treatment and 10 trees to an artificial treatment that simulated herbivory by CLB. For each tree, four branches were randomly assigned one of four defoliation levels of 0, 25, 50 and 75% defoliation. We were able to use branches as the experimental units because there is little carbon translocation that occurs between lateral branches of *P. trichocarpa* (Ceulemans and Isebrands 1996). The CLB treatment was applied by allowing CLB to feed on the foliage of experimental branches. CLB larvae were collected from a commercial poplar plantation operated by GreenWood Resources, Inc located near Boardman, OR and were reared in terrariums located in Pullman, WA. Additional larvae were collected on three separate occasions due to low numbers of feeding insects resulting from death of the individuals or pupation. Leaves were defoliated by placing larvae and adult beetles in a net bag surrounding a single leaf. The number of insects placed on a leaf varied based on estimated leaf area, insect availability and the life stage of the insects used. Treatment levels were achieved by varying the number of insects and the time that the insects were enclosed on the leaf. The artificial treatment

was applied by cutting the lamina parallel to the midvein with a pair of scissors. Defoliation treatments were applied to leaves of leaf plastochron index (Larson and Isebrands 1970) 1-5 throughout the study period at weekly intervals. Controls for CLB treated trees differed from controls of artificial treated trees. CLB treatments had a procedural control by placing a net bag over a leaf for 1-3 days to mimic the time it would take an insect to defoliate the leaf. Light penetration through the net bag was 80% ($R^2 = 0.95$, n = 27, y = 0.79x + 64.5).

Leaf demography, photosynthetic rates and stomatal conductances were measured at weekly intervals from July 7, 2009 to August 27, 2009. Leaf demography consisted of recording branch length, number of leaves and leaf abscission. Photosynthetic rates were measured with an LCPro+ Advanced Photosynthesis System (ADC BioScientific Ltd, Herts, UK) under saturating light intensities (Bassman and Zwier 1991), ambient temperatures and ambient CO_2 concentrations (Table 1). Stomatal conductances were measured with a Leaf Porometer (Decagon, Pullman, WA) under saturating light intensities, ambient temperatures and ambient CO_2 concentrations (Table 1). Leaves that were defoliated at LPI 1-5 were allowed to mature (Figure 1) before measuring photosynthetic rates and stomatal conductances at LPI 6-8. Gas exchange was measured between 11am and 3pm. After gas exchange measurements, sampled leaves were harvested, dried at 60° C for three days, and analyzed for carbon and nitrogen isotope composition. At the end of the study, branches were harvested, leaves separated, and dried at 60° C for three days for measurements of leaf and branch dry weight. Three leaf area measurements were made for each leaf; prior to treatment application, after treatment application, and at the end of the study. Leaf areas were measured using a digital photograph and analyzed in ImageJ (Abramoff et al. 2004). Specific leaf area was measured by dividing the total leaf area of a branch by total leaf biomass.

Data was analyzed in SAS (SAS Statistics, Cary, NC) as a split-plot experimental design. The whole plot consisted of a completely randomized design structure with trees acting as replicates and a one-way treatment structure (defoliation type: CLB or artificial). The sub-plot design structure was a randomized complete block design structure with a one-way treatment structure (defoliation level: 0%, 25%, 50% and 75%).

2.4 RESULTS

CLB and artificial leaf defoliation treatments had an effect on increased levels of branch defoliation (Figure 1). Branch defoliation was not significantly ($p = 0.92$) different between CLB and artificial treatments. The percent of leaf area removed from CLB treatment differed significantly ($p < 0.01$) from controls and between treatment levels. Treating the distal leaves on branches over the study with a 25, 50, or 75% CLB treatment resulted in total branch defoliation of 20, 21, and 30 ±2%, respectively. The percent of leaf area removed from artificial treatment branches differed significantly ($p < 0.01$) from controls and between treatment levels. Treating the distal leaves on branches with a 25, 50, or 75% artificial defoliation treatment resulted in total branch defoliation of 14, 21, and 29 ±2%, respectively. Total branch defoliation did not match the specified treatment level because branches retained undefoliated mature leaves at their base.

Leaf biomass and total biomass of branches were not affected by treatments (Figure 2). Controls for CLB treatment did not differ in leaf ($p = 0.23$) or total biomass ($p = 0.29$) from artificial treatment controls. Leaf biomass and total biomass of branches defoliated by CLB were not significantly ($p = 0.36$) different from CLB controls (6.3±0.8g and 12.2±1.7g for leaf and total biomass, respectively). Leaf biomass and total biomass of branches defoliated by artificial means were not significantly ($p = 0.7$) different from artificial controls (7.9±1g and 15.2±2g for leaf and total biomass, respectively).

Stomatal conductance increased with leaf age on untreated branches from LPI 0 to LPI 6 where it stabilized at an average of 650 mmol ±70 m^{-2} s^{-1} (Figure 3). Stomatal conductance values increased from 80 ±15 mmol m^{-2} s^{-1} in leaves of LPI 1 to 670 ±85 mmol m^{-2} s^{-1} in leaves of LPI 6.

Assimilation rates (Figure 4) and stomatal conductances (Figure 5) were not affected by defoliation treatments. Assimilation rates ($p = 0.38$) or stomatal conductances ($p = 0.34$) were not different between controls for CLB and artificial treatments. Assimilation rates ($p = 0.94$) and conductance values ($p = 0.75$) of CLB treatments were not significantly different from controls. Assimilation rates ($p = 0.30$) and conductance values ($p = 0.83$) of artificial treatments were not significantly different from their controls. The average assimilation rate was 16.0 ±0.8 μmol CO_2 m^{-2} s^{-1} and 16.4 ±1 μmol CO_2 m^{-2} s^{-1} for all CLB and artificial treatments, respectively. Average conductance values were 350 ±40 mmol CO_2 m^{-2} s^{-1} and 310 ±50 mmol CO_2 m^{-2} s^{-1} for all CLB and artificial treatments, respectively.

Estimated leaf production decreased with increased defoliation for both treatments (Figure 6). Estimated leaf production was not significantly ($p = 0.67$) different between controls for CLB and artificial treatments. Estimated leaf production was significantly ($p < 0.01$) different between CLB treatments and controls (0.035±0.003 μmol CO_2 $leaf^{-1}$ s^{-1}). Estimated leaf production for leaves defoliated 74% by CLB decreased 82% compared to controls. Estimated leaf production was significantly ($p < 0.01$) different between artificial treatments and controls (0.037±0.002 μmol CO_2 $leaf^{-1}$ s^{-1}). Estimated leaf production for leaves defoliated 61% by artificial means decreased 60% compared to controls.

Due to the continued growth of the branches over the eight week study, all branches had an increase in leaf area (Figure 7) and leaf number (Figure 8), but the amount varied by treatment. Controls for CLB and artificial treatments differed significantly ($p = 0.02$) by the measured increase in leaf area, but did not differ ($p = 0.2$) by their increase in leaf number. Increases in leaf area for CLB controls (70±10% increase) were 42% lower than the increases seen in artificial controls (120±15% increase). The increase in leaf area for CLB and artificial

controls did not result from sylleptic branching (data not shown). The increases in leaf area in CLB treatments were not significantly ($p = 0.82$) different from controls (Figure 7). Increases in leaf number for CLB treatments were significantly ($p = 0.049$) different than controls (70±10% increase) when sylleptic branches were included in the analysis (Figure 8). Branches defoliated 33% by CLB produced 42% more leaves than controls. The increases in leaf area for artificial treatments were significantly ($p = 0.02$) different from controls (Figure 7). Branches defoliated 32% by artificial means had a 62% reduction in increases of leaf area compared to controls. Increases in leaf number for artificial treatments did not differ significantly ($p = 0.86$) from controls (90±10% increase)(Figure 8). Sylleptic branching in artificial branches did not cause the difference in the leaf area between treatments. In summary, the increase in leaf number from sylleptic branching for CLB treatments resulted in no decrease in leaf area for branches, while leaf area decreased in artificial treatments.

Specific leaf area decreased with defoliation (Figure 9). Controls of CLB and artificial treatments did not differ significantly ($p = 0.92$) for specific leaf area. The specific leaf areas of controls for CLB and artificial treatment were 55 ± 1 m^2 g^{-1}. Specific leaf area was significantly different between CLB treatments and controls ($p = 0.02$). Leaves defoliated 30% by CLB had a 16% reduction in specific leaf area compared to CLB controls. Specific leaf area was significantly different between artificial treatments and controls ($p = 0.03$). Leaves defoliated 29% by artificial means had a 13% reduction in specific leaf area compared to the artificial treatment controls.

Leaf carbon (C), nitrogen (N) and C:N content varied by treatments (Figure 10). Controls of CLB and artificial treatments differed significantly by N ($p = 0.02$) and C ($p < 0.01$) but did not differ by C:N ($p = 0.28$). Leaf N was significantly lower ($p < 0.01$) in leaves

defoliated by CLB than in leaves defoliated by artificial means. Leaf N differed significantly ($p = 0.03$) from their controls (2.2±0.1% N) for CLB treatments, but leaf C:N did not differ significantly ($p = 0.13$) from controls (21±1). Leaf C differed significantly ($p = 0.04$) in artificial treatments from controls (50±1% C), but leaf C:N was not significantly ($p = 0.33$) different from controls (20±1).

The percent of leaf senescence, over an eight week study period, for artificial treatments was not significantly ($p = 0.67$) different from artificial controls (Figure 11). Leaf senescence for artificial control branches was 16 ±2%. Branches defoliated 29% by artificial means had 13 ±2% of their leaves senesce.

2.5 DISCUSSION

Defoliation by CLB or artificial means elicited various physiological and morphological responses in *Populus trichocarpa*. Stem and leaf biomass were similar for all defoliation treatments, suggesting that *P. trichocarpa* compensates for defoliation. Specific leaf area and sylleptic branching contributed to the lack of change in biomass among treatment levels. Assimilation rates did not increase with increased defoliation and did not appear to be a mechanism of tolerance. In CLB treatments, defoliation increased the number of leaves produced compared to controls. In artificial treatments, defoliation decreased the production of leaf area compared to controls. Defoliation by CLB decreased N content compared to artificial treatments.

Biomass is commonly used as an indicator of plant fitness and can be used to measure plant tolerance by comparing the biomass of a defoliated plant with controls. In the present study, biomass did not differ significantly between defoliated and undefoliated control branches despite a significant removal of leaf tissue and the accompanied reduction of estimated leaf production. No difference in branch biomass suggests that treatment branches of two-year old *P. trichocarpa* fully-compensated for low levels (< 30% of total branch leaf area) of natural and simulated CLB defoliation. Compensation to low levels of defoliation has been shown in other species of *Populus* (Bassman and Dickmann 1982; Bassman and Zwier 1993; Reichenbacker et al. 1996; Coyle et al. 2008).

Populus has been shown to compensate through increased photosynthetic rate in undefoliated tissue (Bassman and Dickman 1982). We found that photosynthetic rate was not significantly different across defoliation levels and is therefore not likely to be a mechanism of tolerance. These findings are consistent with a similar study on the compensatory response of *P.*

trichocarpa (Bassman and Zwier 1993). The photosynthetic rates we report are similar to rates measured in undefoliated plants (Bassman and Zwier 1993) suggesting that *P. trichocarpa* did not increase assimilation rates in response to defoliation. Although increased photosynthesis has been reported as a mechanism of tolerance in *P. deltoides* (Bassman and Dickmann 1982), we did not find increased photosynthesis in *P. trichocarpa*.

Secondary branching occurs in *Populus* due to the release of lateral buds from apical dominance following damage of the apical meristems (Burkot and Ben 1979; Fang and Hart 2000). CLB were shown in this study to avoid damaging the apical meristem and preferred leaf tissue not associated with buds that typically exude phenolic defensive compounds (English et al. 1991). Despite the lack of meristematic damage, we found that *P. trichocarpa* responded to defoliation through increased sylleptic branching. We report that sylleptic branching was responsible for a significant increase in leaf production at higher defoliation levels in branches treated with CLB. Increased syllpetic branching has been reported in *P. trichocarpa* in other studies (Bassman and Zwier 1993; Coyle et al. 2008) and the importance of sylleptic branching to maximize overall production and yield has been well studied (Ceulemans et al. 1990; Scarascia-Mugnozza 1991, Zeleznik 2007).

Decreased specific leaf area results from an increase in either leaf thickness, leaf density or both. Our results showed that specific leaf area decreased significantly with increased defoliation in CLB and artificial treatments. Previous studies have reported similar results in *P. trichocarpa* leaves produced following defoliation (Bassman and Dickmann 1982; Bassman and Zwier 1993) and correlations between leaf thickness and density and higher rates of photosynthesis have been reported (McClendon 1962; Mcmillen and Mcclendon 1983). Despite this, Bassman and Dickmann (1982) found that leaves of *P.* x *euramericana* produced after

defoliation had increased specific leaf area and increased assimilation rates. The differences between specific leaf area and assimilation rates in *P. trichocarpa* and *P. x euramericana* suggest that photosynthetic rates in *Populus* are not always correlated with a decrease in specific leaf area.

Decreased specific leaf area can also indicate increased leaf density as a result of the buildup of defensive secondary metabolites. Tsai et al. (2006) reported that phenolic glycosides, condensed tannins, and other flavonoids may constitute up to 35% of foliar dry weight in some *Populus* species. The sequestering of defensive compounds such as condensed tannins in *P. tremuloides* (Peters and Constabel 2002) and other secondary metabolites in hybrid poplars (Osier and Lindroth 2001; Kao et al. 2002; Tsai et al. 2006) has been shown as a rapid response to defoliation. Defensive secondary metabolites in *Populus* are predominantly carbon-based (Lou et al. 2008), thus a buildup of these compounds in the leaves should cause a shift in leaf C:N. We did not find that C:N levels changed which suggests that secondary metabolites did not increase and the reported reduction in specific leaf area was likely due to an increase in leaf thickness.

Leaf percent nitrogen in naturally defoliated branches was significantly less than percent nitrogen in leaves of control branches. Nitrogen is generally correlated with the amount of ribulous-1,5-bisphosphate carboxylase oxygenase and other proteins essential for carbon assimilation (Sage 1987). Although CLB defoliated leaves had less N, the assimilation rates remained unchanged. Thicker leaves produced following defoliation may be a mechanism to compensate for the reduced assimilation rates associated with a reduction in N from the removal of N-rich laminar tissue by insect grazing.

Branches treated with CLB had a greater increase in leaf number than controls while branches treated artificially did not differ from controls. The differences between the leaf production of CLB and artificial treatments could be explained by the differences in defoliation patterns. The CLB treatment branches had undefoliated leaves interspersed with defoliated leaves due to the inconsistent feeding behavior of CLB while artificial treatment branches had all leaves partially defoliated. Developing leaves in *Populus* transport carbon upward to other developing leaves (Dickson 1986). The occasional undefoliated leaves in CLB treatment branches could have provided greater photosynthate to the developing zone of the branch allowing greater leaf production for CLB treatments than occurred in artificial treatments.

Leaves defoliated by CLB had lower leaf N than artificially defoliated leaves. The difference may be a result of more precise removal of N-rich laminar tissue without the removal of carbon-rich structural tissue around leaf veins by CLB (Burkot and Benjamin 1979) compared to the unselective removal of leaf tissue by artificial means. Although branches defoliated by artificial treatments had the same degree of compensation as branches defoliated naturally, the difference in nitrogen may have long term effects.

CLB and artificial control branches differed by the number of additional leaves produced over the eight week study period and by leaf N content. The differences in the response of the controls may be a result of a procedural control used for the CLB treatments but this is unlikely because the net bags were placed on the leaves for a relatively short period of time (1-3 days), only 1-3 leaves were covered concurrently, and light penetration through the bags was 80%. Differences between the treatment controls could have resulted from systemic defensive responses to insect and mechanical wounding. Plants have been shown to detect specific insect herbivores (Howe and Jander 2008) and respond systemically (Ryan 2000). Reports suggest that

Populus is able to detect insect attack and elicit a different response than expected from mechanical damage alone (Arimura 2004; Major and Constabel 2006). Furthermore, *Populus* has been shown to exhibit a suite of complex systemic defensive systems (Constabel et al. 2000; Frost et al. 2007; Babst et al. 2009) in response to localized herbivory. The differences we report in the treatment controls may have resulted from the systemic responses elicited by insect and artificial defoliation.

In our study we measured the physiological and morphological responses of *P. trichocarpa* to defoliation by CLB and artificial treatments. We found that the physiological responses we measured did not act as tolerance mechanisms; photosynthesis and stomatal conductances were not influenced by defoliation treatments. We did, however, find that branches of *P. trichocarpa* displayed a morphological response to defoliation by increased leaf thickness and increased leaf number. We conclude that 1) defoliation treatment, artificial and CLB, had an effect on leaf production, leaf area, and leaf N and these differences should be considered when analyzing experimental results and 2) *P. trichocarpa* completely compensated for low (<30%) levels of branch defoliation by natural and simulated defoliation by CLB through a reduction in specific leaf area of leaves produced following defoliation and an increase in leaf area and leaf number from sylleptic branching in naturally defoliated leaves.

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2.7 TABLES

Table 2.1. Cuvette conditions during assimilation measurements. Defoliation category indicates the statistical categories; 0% defoliation, 1-25% defoliation, 26-50% defoliation and 51-75% defoliation.

Treatment	**Category** (%)	**PAR @ leaf surface** (μmol m^{-2} s^{-1})	**Leaf temp.** (°C)	**Reference CO_2** (μmol mol^{-1})
Natural	0	1330 ± 20	36 ± 1.0	374 ± 1
Natural	1-25	1270 ± 20	36 ± 1.1	372 ± 1
Natural	26-50	1290 ± 30	36 ± 1.2	373 ± 1
Natural	51-75	1270 ± 30	36 ± 1.3	374 ± 1
Artificial	0	1260 ± 30	37 ± 0.7	374 ± 1
Artificial	1-25	1160 ± 40	36 ± 0.9	376 ± 2
Artificial	26-50	1220 ± 30	37 ± 0.6	374 ± 1
Artificial	51-75	1230 ± 30	38 ± 0.7	375 ± 1

2.8 FIGURE CAPTIONS

Figure 2.1. Percent of leaf material removed from the entire branch by artificial and CLB treatments at different experimental defoliation levels. Each column represents an average and error bars are standard error. Treatment levels with different letters indicate a significant difference of ($p < 0.05$), absence of letters indicates no significant differences.

Figure 2.2. Leaf and total dry weight of branches harvested after the study period. Each point represents an average and error bars are standard error.

Figure 2.3. Stomatal conductance values (mmol m^{-2} s^{-1}) for different aged leaves of three genotypes of *Populus trichocarpa*. Leaf plastochron index was used to measure leaf age. Each point represents an average and error bars are standard error.

Figure 2.4. Assimilation rates (μmol m^{-2} s^{-1}) of leaves defoliated by *Chrysomela scripta* or through artificial means. Rates were measured in ambient CO_2, ambient temperatures and saturating light levels. Leaf defoliation (%) was calculated for average percent defoliation for each leaf in each of four defoliation categories (0, 1-25%, 26-50%, 51-80% leaf defoliation). Each point represents an average and error bars are standard error.

Figure 2.5. Stomatal conductance values (mmol m^{-2} s^{-1}) of leaves defoliated by *Chrysomela scripta* through artificial means. Rates were measured in ambient CO_2, ambient temperatures and saturating light levels. Leaf defoliation (%) was calculated for average percent defoliation for each leaf in each of four defoliation categories (0, 1-25%, 26-50%, 51-80% leaf defoliation). Each point represents an average and error bars are standard error.

Figure 2.6. Estimated leaf production for leaves (μmol CO_2 $leaf^{-1}$ s^{-1}) defoliated at different levels by either *Chrysomela scripta* or through artificial means. Leaf assimilation was calculated by multiplying remaining leaf area by assimilation rate (μmol CO_2 m^{-2} s^{-1}). Leaf defoliation (%)

was calculated for average percent defoliation for each leaf in each of four defoliation categories (0, 1-25%, 26-50%, 51-80% leaf defoliation). Each point represents an average and error bars are standard error. Treatment levels with different letters indicate a significant difference of ($p < 0.05$), absence of letters indicates no significant differences.

Figure 2.7. Percent increase in leaf area for branches defoliated by *Chrysomela scripta* or through artificial means. Percent increase in leaf area was calculated by the difference between leaf area at the start of the study and leaf area at harvest, an eight week period. Each point represents an average and error bars are standard error. Treatment levels with different letters indicate a significant difference of ($p < 0.05$), absence of letters indicates no significant differences.

Figure 2.8. Percent increase in the number of leaves on a branch defoliated by *Chrysomela scripta* or through artificial means. Percent increase in the number of leaves was calculated by the percent difference in leaf number at the start of the study and at harvest, an eight week period. Open squares (□) indicated leaf area of the primary branch and all sylleptic branches. Closed squares (■) indicated leaf area of primary branch only. Each point represents an average and error bars are standard error. Treatment levels with different letters indicate a significant difference of ($p < 0.05$), absence of letters indicates no significant differences.

Figure 2.9. Specific leaf area of leaves on treatment branches. Specific leaf area was calculated by dividing total leaf area of the branch by total leaf biomass. Each point represents an average and error bars are standard error. Treatment levels with different letters indicate a significant difference of ($p < 0.05$), absence of letters indicates no significant differences.

Figure 2.10. Leaf C content (%), leaf N content (%), and leaf C:N of leaves defoliated by *Chrysomela scripta* or artificial treatments. Leaf defoliation (%) was calculated for average

percent defoliation for each leaf in each of four defoliation categories (0, 1-25%, 26-50%, 51-80% leaf defoliation). Each point represents an average and error bars are standard error. Treatment levels with different letters indicate a significant difference of ($p < 0.05$), absence of letters indicates no significant differences.

Figure 2.11. Percent of leaves of a branch that senesced between the start and end of the study period, an eight week period. Each point represents an average and error bars are standard error.

2.9 FIGURES

Figure 2.1

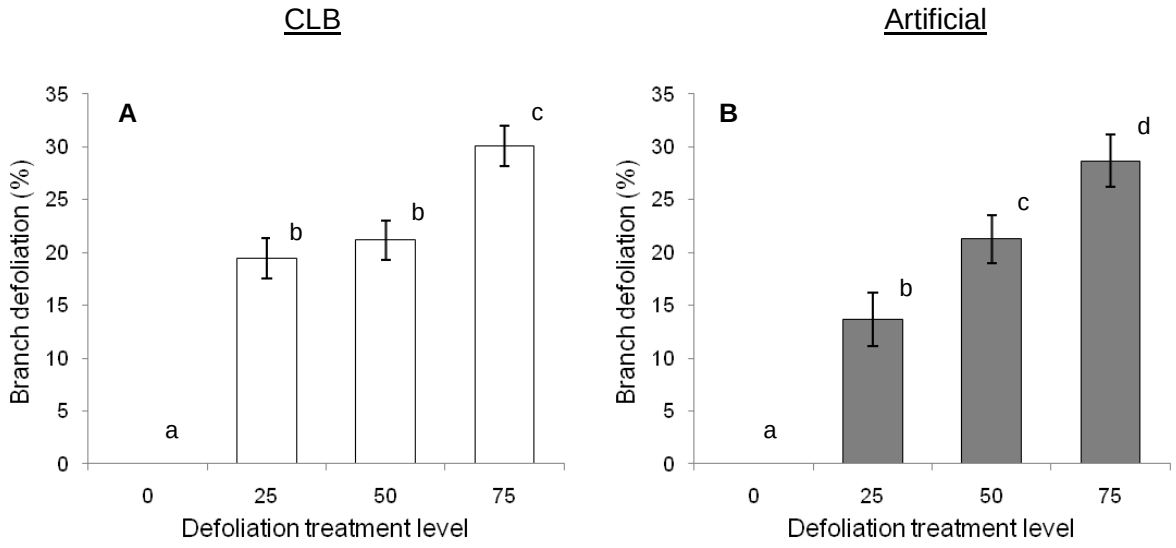


Figure 2.2

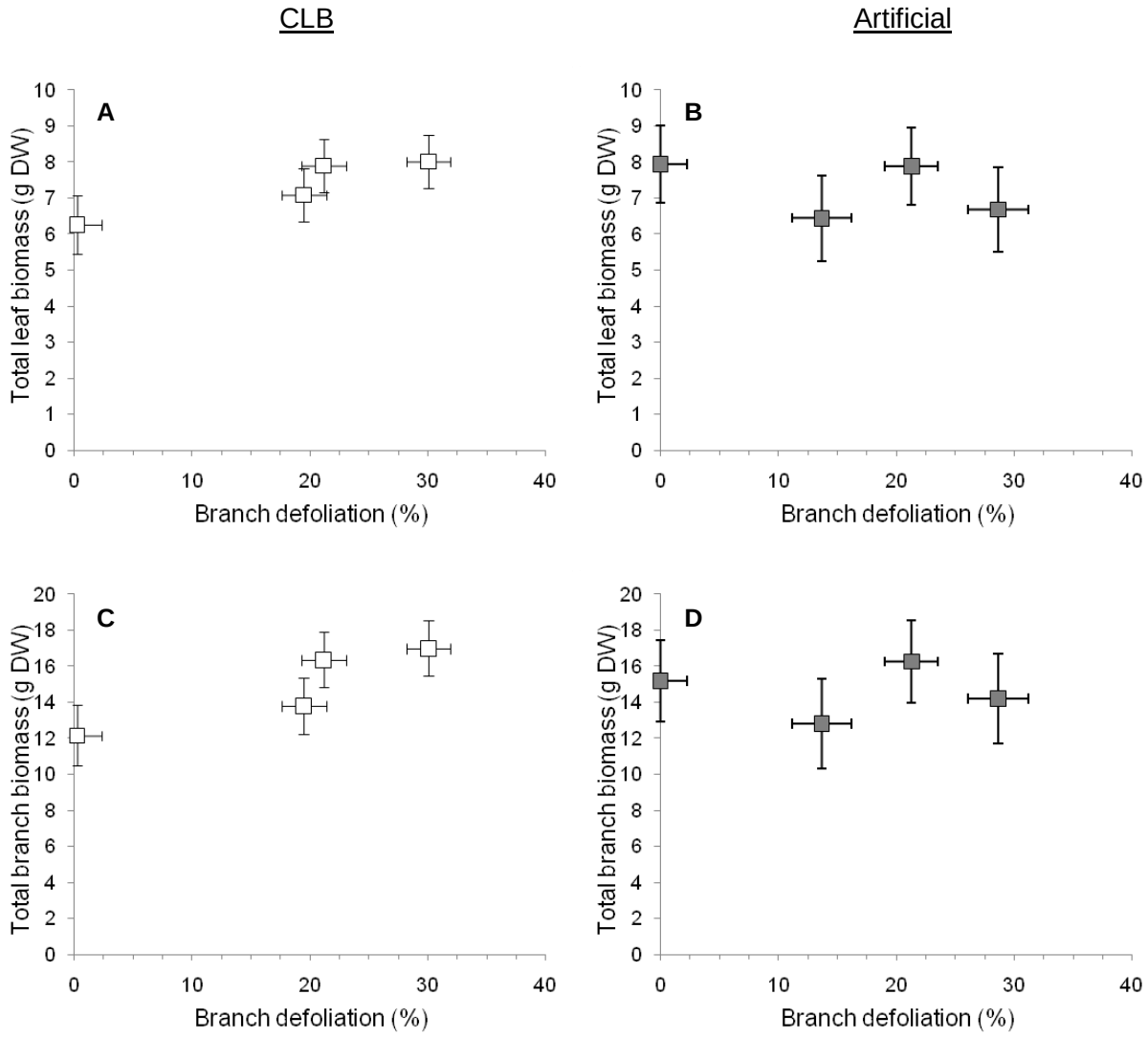

CLB
Artificial
A
B
C
D
Total leaf biomass (g DW)
Total branch biomass (g DW)
Branch defoliation (%)

Figure 2.3

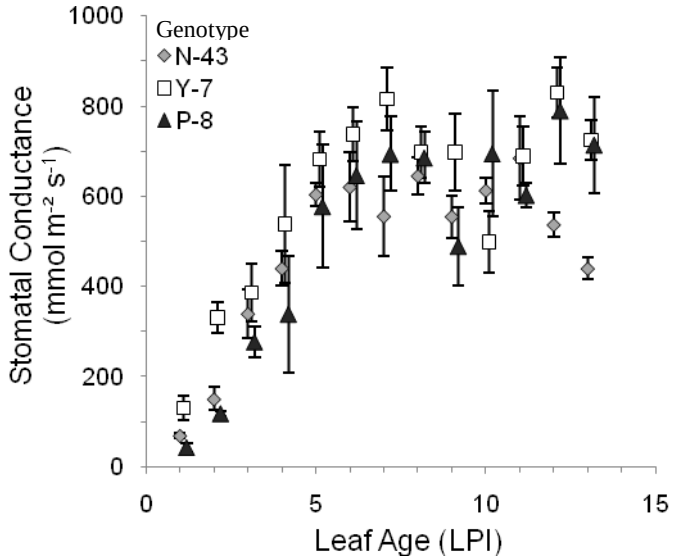

Genotype
N-43
Y-7
P-8
Stomatal Conductance (mmol m⁻² s⁻¹)
1000
800
600
400
200
0
0
5
10
15
Leaf Age (LPI)

Figure 2.4

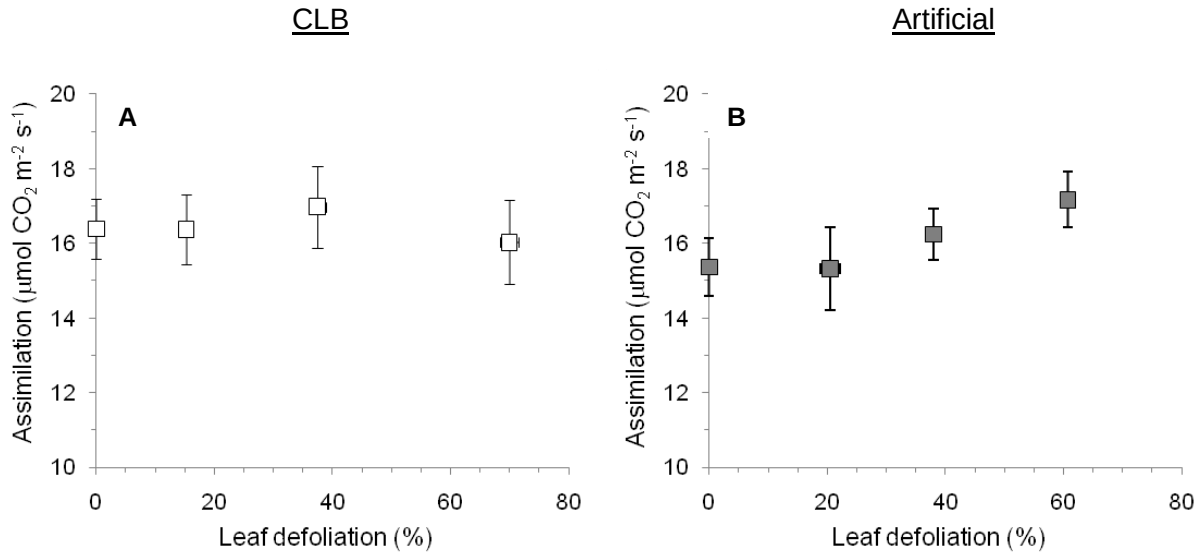

CLB
Artificial
A
B
Assimilation (µmol CO2 m-2 s-1)
Leaf defoliation (%)
10
12
14
16
18
20
0
20
40
60
80

Figure 2.5

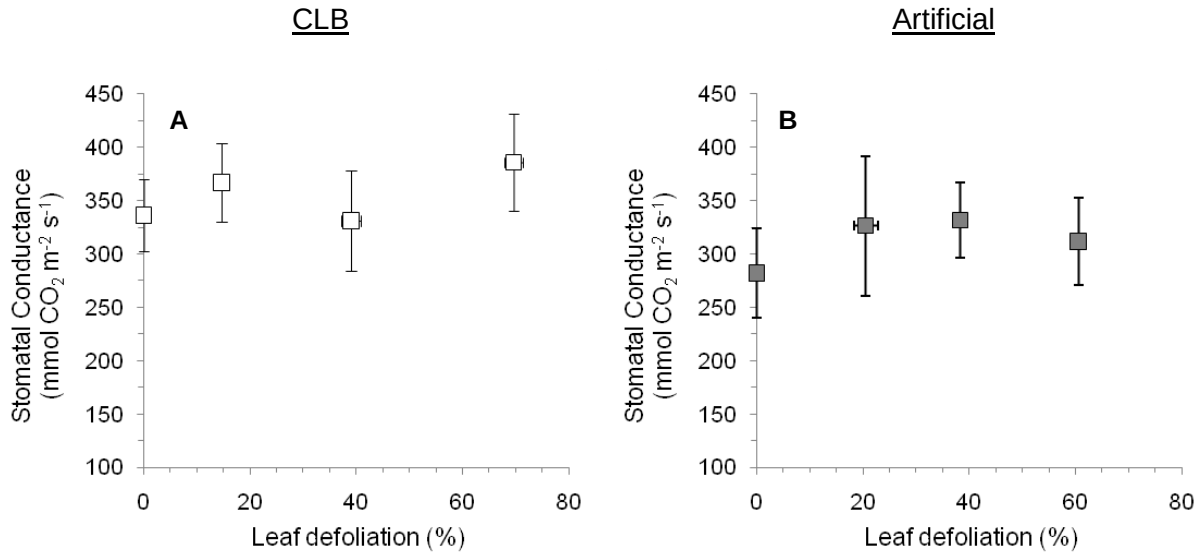

CLB
Artificial
A
B
Stomatal Conductance (mmol CO_2 m^{-2} s^{-1})
Leaf defoliation (%)
450
400
350
300
250
200
150
100
0
20
40
60
80

Figure 2.6

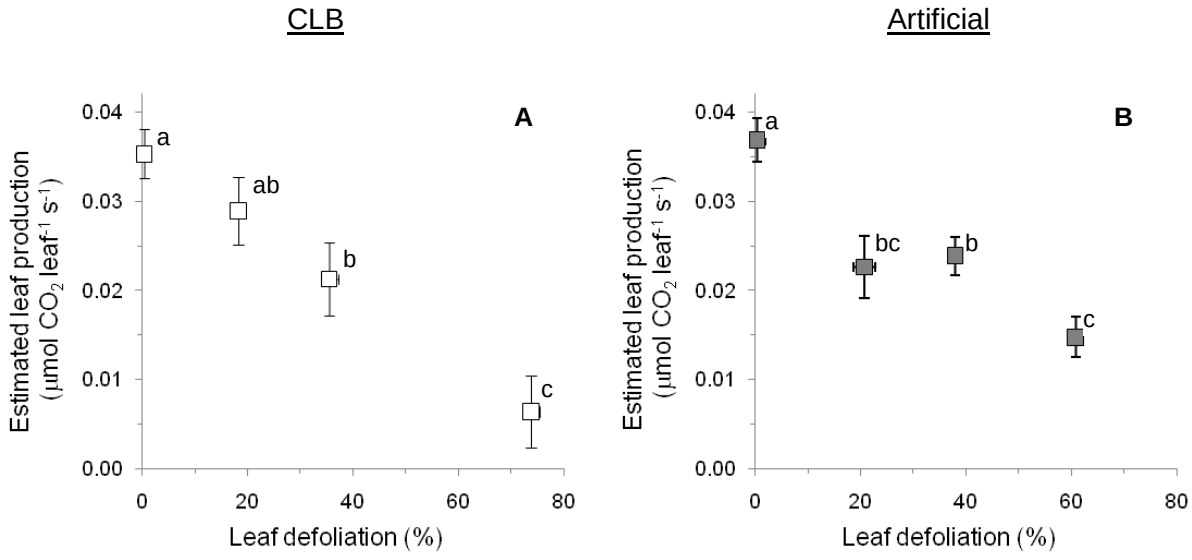

CLB
Artificial
A
B
Estimated leaf production (µmol CO2 leaf-1 s-1)
Leaf defoliation (%)
0.04
0.03
0.02
0.01
0.00
0
20
40
60
80
a
ab
b
c
a
bc
b
c

Figure 2.7

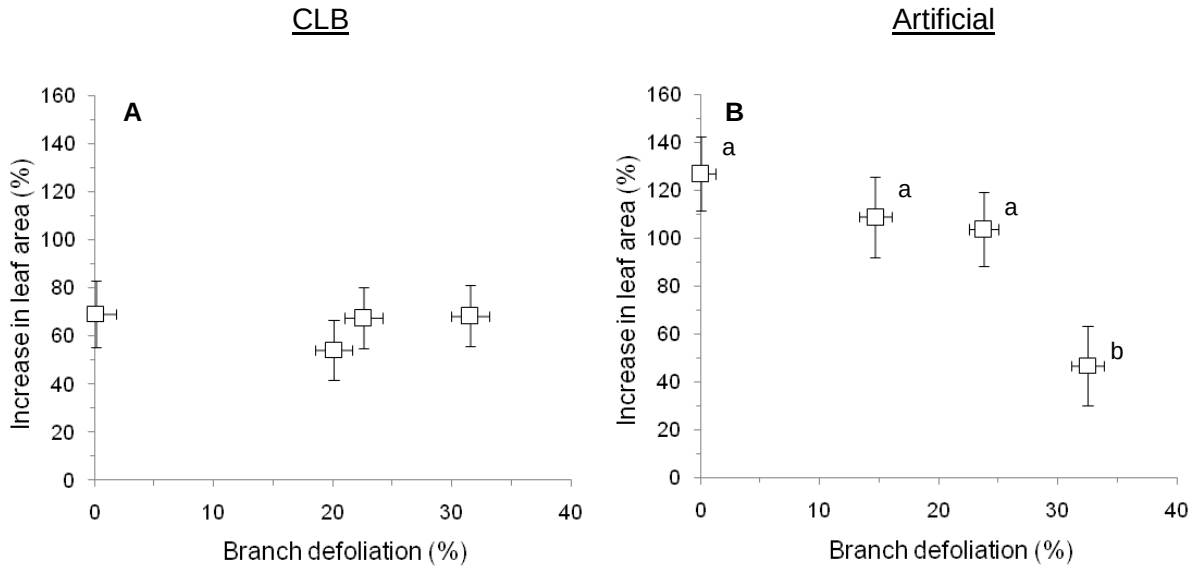

CLB
Artificial
A
B
Increase in leaf area (%)
Branch defoliation (%)
a
a
a
b
0
10
20
30
40
0
20
40
60
80
100
120
140
160

Figure 2.8

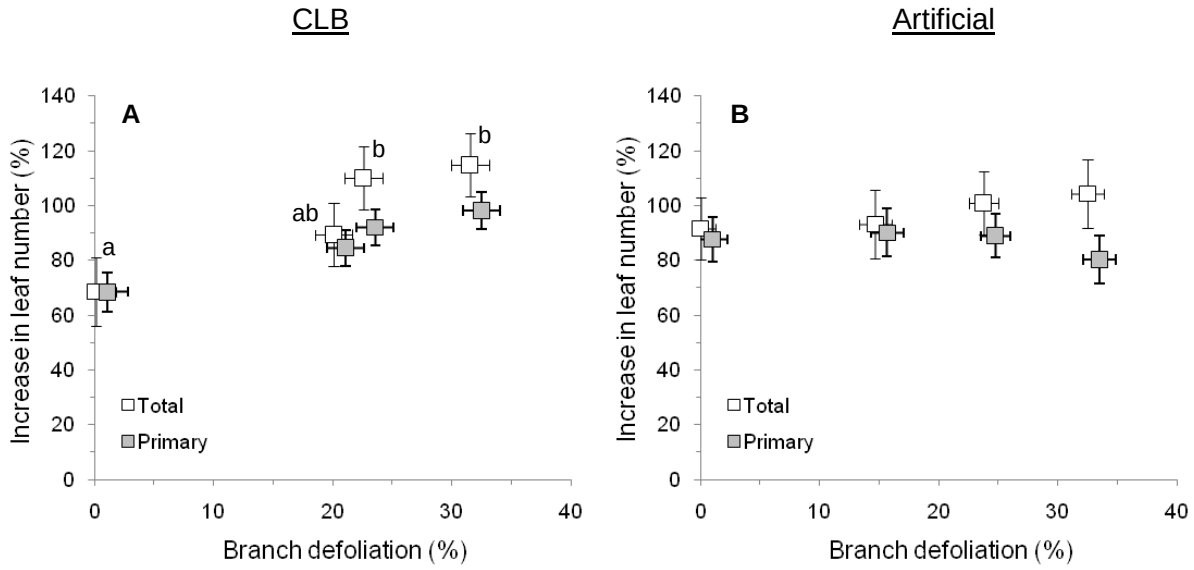

CLB
Artificial
A
B
a
ab
b
b
Total
Primary
Increase in leaf number (%)
Branch defoliation (%)
0
20
40
60
80
100
120
140
0
10
20
30
40

Figure 2.9

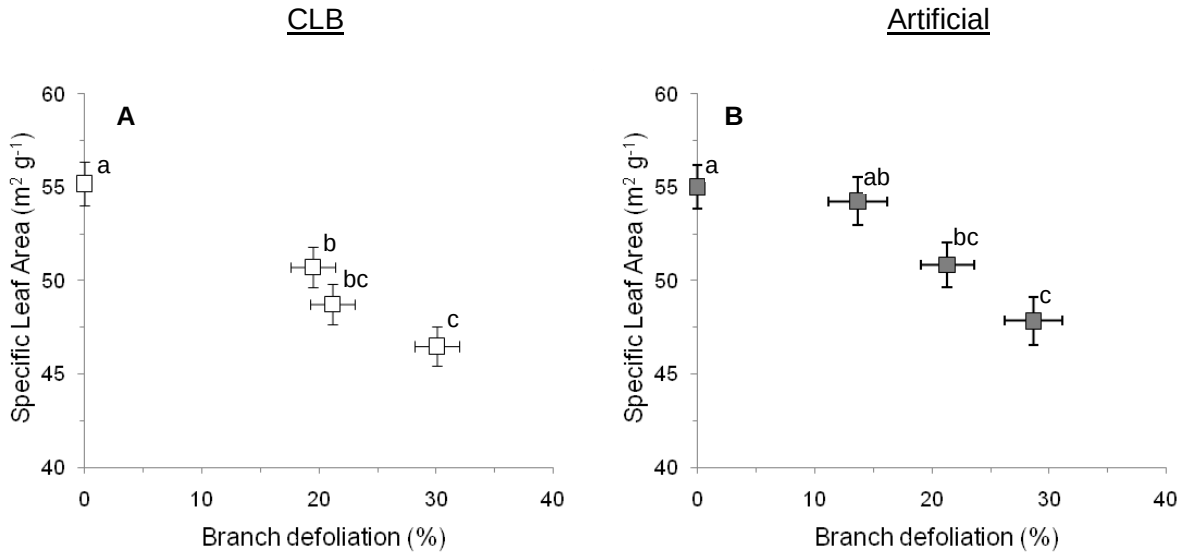

CLB
Artificial
A
B
Specific Leaf Area (m2 g-1)
Branch defoliation (%)
a
b
bc
c
ab
60
55
50
45
40
0
10
20
30
40

Figure 2.10

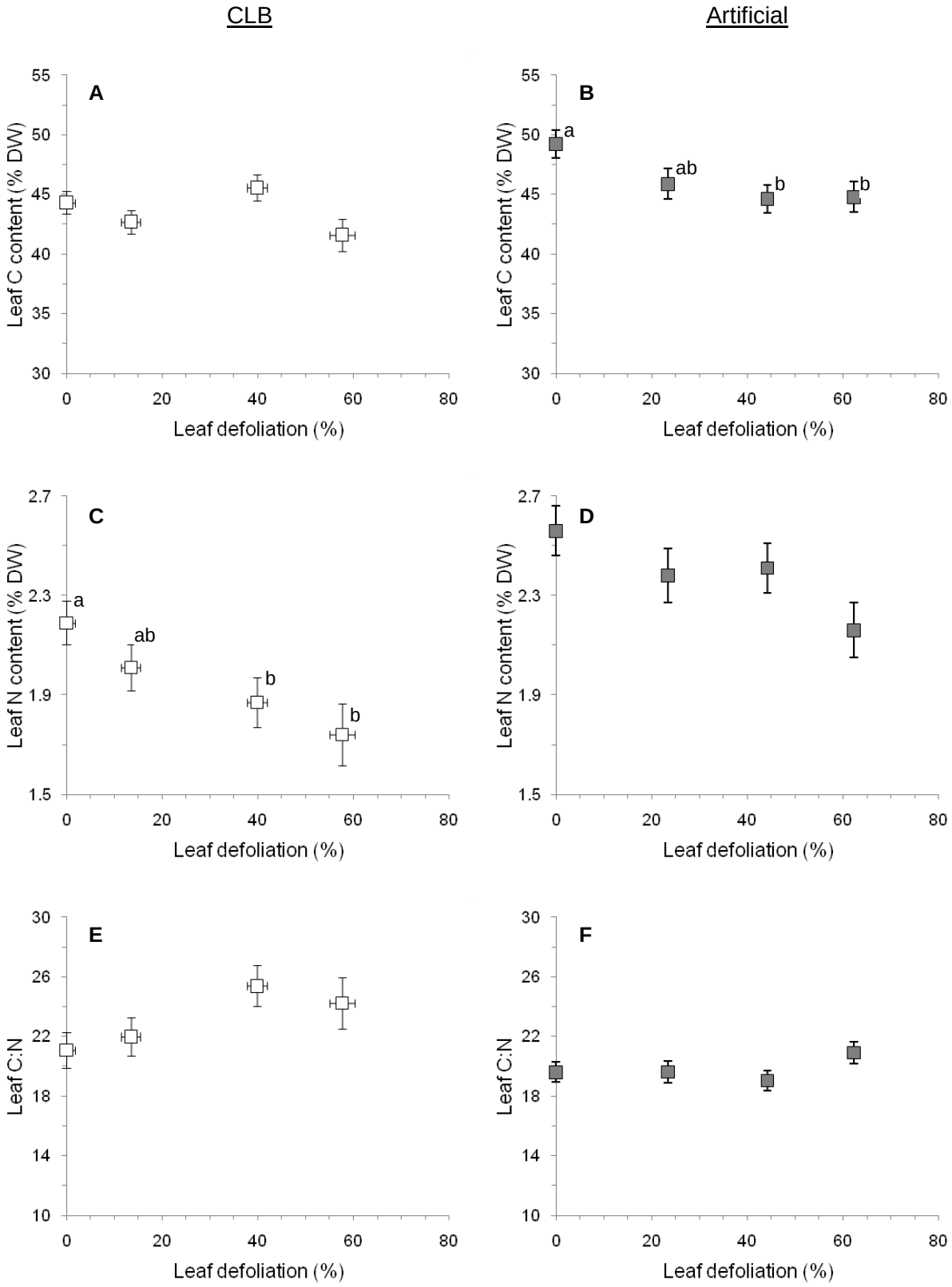
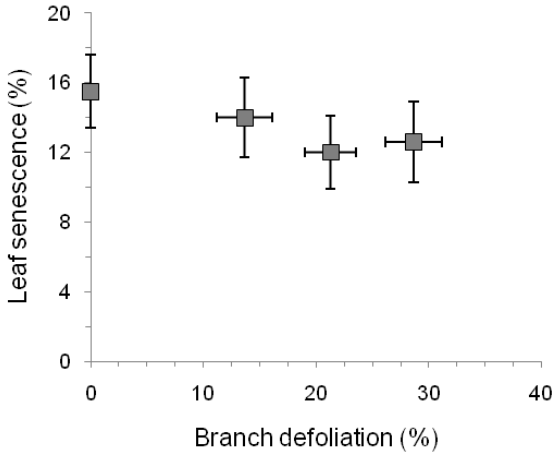

CLB
Artificial
A
B
C
D
E
F
Leaf C content (% DW)
Leaf N content (% DW)
Leaf C:N
Leaf defoliation (%)
a
ab
b
b

Figure 2.11



APPENDIX A.

Table A1. Clone population of treatment trees.

Nisqually River[1]

Tree ID	Clone	ID#	Location	Latitude	Longitude	Elev.
A10, N9	8-1040	N13	McKenna	47°01’48”	122°40’12”	60m
N8	8-1043	N43	La Grande	46°53’24”	122°19’48”	200m
A7, A8, N4	8-1020	N20	Alder	46°48	122°18	310m
N5	8-1049	N49	Big Creek Rd	46°45’	121°59’24”	550m
N7, A5	8-1029	N29	Kautz Creek	46°45’	121°51’	790m

Palouse River[2]

Tree ID	ID#	Location	Latitude	Longitude	Elev.
A4, N6	P8	1mi upstream golf course	46°55’18”	117°19’45”	610m
A1, N1, N2	P7	4mi upstream P8	46°56’15”	117°16’15”	634m
A9	P6	0.75mi upstream P7	46°56’45”	117°15’45”	640m

Yakima River[2]

Tree ID	ID#	Location	Latitude	Longitude	Elev.
N10, A6, A2	Y7	I82 Wapato Rd.	46°28’30”	120°23’45”	259m
N3, A3	Y12	Roza Rec site, Canyon Rd.	46°47’		366m

[1] Western transect populations, collected winter of 1984-1985 and maintained by Washington State University

[2] Eastern transect populations, collected on February, 1995 and maintained by Washington State University