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Developmental Contributions to Variation in Aspen Clones and the Influence of Pre-Fire Succession Status on Aspen Regeneration Success

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Developmental Contributions to Variation in Aspen Clones

and the Influence of Pre-Fire Succession Status

on Aspen Regeneration Success

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ABSTRACT

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This thesis includes two studies: The first examined developmental changes that take place in the physiology of aspen (*Populus tremuloides* Michx.) and to characterize developmental influences on patterns of phenotypic trait variation among different aged ramets within the aspen clones. We surveyed eight clones, each with 8 distinct age classes ranging from 1 to 170 yrs in age. Using regression analysis we examined the relationships between ramet age and expression of functional phenotypes. Eight of the phenotypic traits demonstrated a non-linear relationship in which large changes in phenotype occurred in the early stages of ramet development and stabilized thereafter. Water and nutrient concentration, leaf gas exchange and phenolic glycosides tended to decrease from early to late development, while sucrose and condensed tannin concentrations and water use efficiency increased with ramet age. We hypothesize that ontogenetically derived phenotypic variation leads to fitness differentials among different aged ramets, which may have important implications for clone fitness. Age-related increases in phenotypic diversity may partially underlie aspen's ability as a species to tolerate the large environmental gradients that span its broad geographical range.

Fire is an essential component of many forest ecosystems and fire exclusion policies and other anthropogenic factors have significantly altered disturbance regimes, which has lead to increased aspen succession to conifers. The second study examined how post-fire aspen regeneration success is influenced by increasing conifer abundance under longer fire return intervals. 66 sites were selected from the Sanford prescribed fire complex located in the Dixie National Forest. Slope, aspect, sucker regeneration heights, soil samples, and post and prefire stand densities were measured. Results from this study demonstrated that pre-disturbance conifer abundance and aspen densities are good predictors of aspen sucker regeneration success. Results also found that although conifer densities don't change across aspects, aspen densities are different on north facing slopes. We hypothesize the high levels of aspen regeneration came from a large disturbance size which overwhelmed the high levels of herbivores.

Keywords: aspen decline, condensed tannins, hydraulic limitation hypothesis, ontogeny, phenolic glycosides, quaking aspen, ramet, aspen, ecology, succession, prescribed burns, post-fire, regeneration

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TABLE OF CONTENTS

Chapter 1:

Developmental Contributions to Variation in Leaf Function, Morphology and Defense Chemistry within Quaking Aspen Clones

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Summary

There is a need to better understand how evolution by natural selection applies to clonal organisms. The objective of this study was to characterize developmental influences on patterns of phenotypic trait variation among different aged ramets within quaking aspen (*Populus tremuloides* Michx.) clones. We surveyed eight aspen clones, each with eight distinct age classes ranging from 1 to 170 yrs in age. Using regression analysis we examined the relationships between ramet age and expression of functional phenotypes. The data showed significant correlations between ramet age and 10 of the 12 phenotypic traits measured. Eight of the phenotypic traits demonstrated a non-linear relationship in which large changes in phenotype occurred in the early stages of ramet development and stabilized thereafter. Water and nutrient concentration, leaf gas exchange, and phenolic glycosides tended to decrease from early to late development, while sucrose and condensed tannin concentrations and water use efficiency increased with ramet age. We hypothesize that ontogenetically derived phenotypic variation leads to fitness differentials among different aged ramets, which may have important implications for clone fitness. Age-related increases in phenotypic diversity may partially underlie aspen's ability to tolerate the large environmental gradients that span its broad geographical range.

Keywords: aspen decline, condensed tannins, hydraulic limitation hypothesis, ontogeny, phenolic glycosides, quaking aspen, ramet

Introduction

The evolution of life is driven by natural selection acting on phenotypic trait variation among individuals in a population. The central dogma of biology outlines the close connection between an organism genotype and its expressed phenotype (Visscher et al. 2008). Morphological and physiological phenotypes also change as organisms acclimate to environmental heterogeneity (phenotypic plasticity) (Moriuchi and Winn, 2005, Magyar et al. 2007). Development related changes in phenotype (ontogeny) is a third but often overlooked factor influencing phenotypic variation (Coleman et al. 1994) and is particularly prevalent in plants because of their modular growth pattern (Allsopp 1967).

Asexual regeneration is a prevalent reproductive strategy in the plant kingdom (Zobel 2008). Among the most conspicuous and successful examples of a clonal species is quaking aspen (*Populus tremuloides* Michx.), the most widely distributed tree species in North America. Aspen reproduces using both sexual and asexual strategies (Mitton and Grant 1996), but in its western range it regenerates primarily through root suckering (Schier et al., 1985), resulting in clonal stands that can vary from 4-81 ha in size and have as many as 100,000 stems (Kemperman and Barnes 1976). A potential pitfall of clonal regeneration is that genetic uniformity among ramets may result in reduced phenotypic diversity resulting in clone-wide susceptibility to natural selection. Recent patterns of aspen decline in western North America highlights the need for a more complete understanding of the fitness implications associated with aspen's clonal nature (Worrall et al. 2008, St.Clair et al. 2010a).

Root suckering in aspen, commonly takes place following disturbance events such as fire (Frey et al. 2003). However, suckering can occur episodically in the absence of disturbance

resulting in genetically identical, multi-age classes within a single clone (Kurzel et al. 2007). Ontogenetically derived phenotypic variation among the developmentally staggered age classes, represents a potentially important source of phenotypic trait variation within clones. Little is known about how ontogenetic factors influence functional trait variation through the life stages of clonal plants in general, and aspen clones in particular.

An understanding of the morphological and physiological traits that allow plants to maintain nutrient, water and carbon homeostasis in response to abiotic and biotic stresses is a primary goal of stress physiologists and plant breeders. Aspen exhibits sensitivity to drought and temperature stress (Hogg et al. 2008, St.Clair et al. 2009), ungulate browsing, insect defoliation, and a host of leaf, stem and root pathogens (St.Clair et al. 2010a). Aspen produce two classes of phenolic-based allelochemicals from the shikimic acid pathway: condensed tannins and phenolic glycosides. Studies have shown that high foliar concentrations of phenolic glycosides in aspen leaves deter insect (Donaldson and Lindroth 2007) and mammal herbivores (Wooley et al. 2008). Emerging evidence suggest that condensed tannins may play a role in increasing resistance to microbial pathogens (Holeski et al. 2009). Investment in defense chemistry by young trees is likely to increase resistance to mammalian herbivores (Wooley et al. 2008). As canopies ascend above mammal browse pressure it may be more advantageous to shift to a strategy of tolerance, which conserves resources for compensatory growth following insect defoliations events. Developmental cues would likely be involved in this shift in strategy from resistance to tolerance.

The objective of this study was to characterize developmental influences on patterns of phenotypic trait variation among different aged ramets within quaking aspen clones. The following leaf traits were measured: photosynthesis, stomatal conductance, water use efficiency,

xylem water potential, nutrient and carbohydrate status, specific leaf area, and foliar concentrations of condensed tannins and phenolic glycosides. The following hypotheses were tested: 1) phenotypic trait variation is strongly influenced by ontogenetic factors within aspen clones; 2) aspen leaf physiology is reduced as ramets age; 3) allocation to defense chemistry decreases as ramets age as they shift from a strategy of resistance to tolerance as herbivore pressure changes.

Materials and Methods

Clone selection and sampling

Eight pure aspen clones (four in the Uinta National Forest and four in the Fishlake National Forest), located in northern and central Utah were included in the study. The geographical location of the clones on the Uinta National Forest (UNF) are: 40°25’42.63” N, 111°38’17.55” W; 40°25’27.94” N, 111°36’41.27” W; 40°25‘42.63” N, 111°36’41.27” W and 40°25’31.85” N, 111°36’26.16” W. The geographic locations of clones on the Fishlake National Forest (FLNF) are: 38°47’12.01” N, 111°38’17.55 W; 38°10’11.18” N, 111°35’40.07 W; 38°42’14.09” N, 111°33’44.22” W and 38°42’05.35” N, 111°32’19.64” W. Elevations at the sites ranged from 2307 to 3021 m. Eight age classes in each clone were chosen for sampling using stem diameters as an initial surrogate for ramet age. Ramets were generally selected from the edge of the clone to minimize shading effects of older age classes on younger age classes. Age classes were initially defined as current year suckers, or ramets with stem diameters of 1-2, 2-4, 5-7, 8-15, 16-25, 26-35, and 36+ cm until tree ring analysis was performed (description below). Three replicate ramets in each size class were selected in each clone with data

representing the average of the three replicates. The analysis and sample collection occurred from June 30 to July 9, 2008.

Leaf samples were taken (20+ leaves/tree) from two mid-canopy branches of each ramet. Depending on canopy height either a tree pruner or a shotgun was used to harvest branches. Leaf gas exchange and xylem water potential were measured immediately after branch excision (see description below). Only fully expanded, short shoot leaves were collected for analysis to avoid within year developmental differences in leaf traits. Leaf samples were placed in labeled freezer bags between blocks of dry ice in preparation for transport back to the lab, at which point they were stored in a -80°C freezer until further analysis was conducted. Estimates of tree heights were determined using a clinometer (Haglof HEC, Langsele, Sweden). For smaller age classes height was determined using a tape measure. Stem diameters were measured just above ground level with a hand caliper for younger ramets and a measuring tape was used to determine diameter at breast height (DBH) for larger ramets.

Leaf gas exchange and xylem water potential

Leaf gas exchange measurements were made on the two youngest fully expanded leaves, from each branch collected. Rates of photosynthesis (Amax) and stomatal conductance (g_s) were measured immediately after the branch was excised using a gas exchange system (LI-COR 6400, LI-COR Environmental Inc., Lincoln, NE, USA). Gas exchange measurements took between 60-90 s. We have found that stomatal responses of aspen branches are stable for ~five minutes following branch excision (unpublished data). Gas exchange was measured at a photosynthetic photon flux density (PPFD) of 2000 µmol $m^{-2}s^{-1}$ generated by a blue-red LED light source at

ambient temperature and humidity. Baseline leaf and reference chamber CO_2 concentrations of 385-µmol mol-1 were achieved using a CO_2 mixer. Measurements were initiated by sealing the leaf in the chamber. When CO_2 and water vapor concentrations in the leaf chamber reached steady state (60-90 s), rates of photosynthesis and stomatal conductance were logged. Following gas exchange measurements, the branch segment was measured for xylem water potential using a pressure chamber (PMS Instrument Company, Albany, OR, USA). All gas exchange and water potential measurements were taken between 10:30 and 17:00 h, a period in which gas exchange was found to be relatively uniform based on diurnal measurements. Water use efficiency was calculated as the ratio of CO_2 fixed per unit water lost through transpiration.

Determination of specific leaf area

Leaf area was determined using a leaf area meter (LI-COR 3000, LI-COR Environmental Inc., Lincoln, NE, USA). Leaves were then freeze dried for 48 hours and measured for dry mass using an analytical balance. Specific leaf area (SLA) was calculated according to cm^2 of leaf area per gram dry weight of leaf tissue.

Tree Ring Analysis

For ramets >3cm DBH, tree core samples were taken at a height of 34cm with an increment borer (Haglof HEC, Langsele, Sweden) as the first step in tree age determination. Aspen saplings (<3cm DBH) were cut at ground level and a basal disc was collected for age determination. The tree cores were dried and glued into grooved core mounts. While mounting, special attention was placed on orienting the cores properly so that the tracheids were vertical to

ensure that the cell structure of the annual rings was clear (Asherin and Mata 2001). Twisted cores were carefully broken and realigned so that the cells would be oriented properly for analysis. Samples were sanded by hand through progressively finer sheets of sandpaper ending with nine microns. Once sanded, the rings were clearly visible under a light microscope and no staining was required. Tree ages were determined by counting the annual rings of each core or disc using a binocular microscope (VanGuard 1275ZP and Fischer 12-562-3) (Elliott and Baker 2004). Cores that missed the pith were corrected according to the methods of Applequist (1958).

Phytochemical analysis

Phytochemical analysis was conducted on leaf samples that had been freeze dried to preserve the chemical integrity of the tissue (Lindroth and Koss 1996). Samples were ground (laminas only) and homogenized using a mixer mill (Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) and stored at -20°C until analysis was conducted.

Phenolic glycosides were extracted from leaf tissue in methanol. The samples were vortexed for five minutes at high speed. The liquid supernatant was then removed and placed in separate micro-centrifuge tubes. This procedure was repeated twice. Phenolic glycoside concentrations (salicortin and tremulacin) were quantified using high performance liquid chromatography (Agilent 1100 Series, Santa Clara, CA, USA) with a Luna 2, C18 column (150 x 4.6mm, 5um) at a flow rate of 1 ml/min. Compound peaks were detected using a UV lamp at a wavelength of 280 nm using purified salicortin and tremulacin standards isolated from aspen leaves (Lindroth et al. 1993).

Condensed tannins were extracted from leaf tissue using a 70% acetone-10 mM ascorbic acid solution. The sample was vortexed at high speed for 20 minutes at 4°C. The liquid supernatant was then removed and placed in separate micro-centrifuge tubes. This procedure was also repeated twice. Condensed tannin concentrations were measured with a spectrophotometer (SpectraMax Plus 384, MDS, Toronto, Canada) using the acid butanol method (Porter et al. 1986). Condensed tannins levels were quantified using a purified condensed tannin standard isolated from aspen leaves (Hagerman and Butler 1980).

Foliar N concentrations were determined with a nitrogen analyzer (TruSpec, CN Determinator, LECO Cooperation, St. Joseph, MI) using the combustion method (Campbell 1991). Phosphorus analysis was measured by placing approximately 20mg of leaf tissue in a 4 ml glass scintillation vial and ashing the sample in a muffle furnace at 495°C for 10 hours. The ashed samples were dissolved in 2 ml of 100 mM hydrochloric acid and analyzed according to the methods of Murphy and Riley (1962) using a spectrophotometer (SpectraMax Plus 384, MDS, Toronto, Canada).

Sucrose was extracted from leaf tissue according to the methods of (Hendrix 1993). Freeze-dried leaf sample was placed in screw cap micro-centrifuge tubes and then suspended in 0.67 ml of 80% ethanol. The samples were then extracted by placing them in a water bath at 80°C for 20 minutes. The supernatant was removed and placed in a separate 2 ml test tube. The ethanol extraction was repeated two more times for a final extract volume of 2 ml. Samples were placed in microplate wells and after evaporating off the ethanol at 55°C in a drying oven the samples were re-suspended in water. Sucrose in the samples were enzymatically hydrolyzed (at 37°C) using Invertase (Sigma, St. Louis, MO, USA) to yield glucose. Glucose reaction mix (GOPOD, Megazyme, Wicklow, Ireland) was added to all sample and standard wells. The

micro-plates were incubated for 20 minutes at 37°C after which, absorbance was read at 510 nm with a spectrophotometer (SpectraMax Plus 384, MDS, Toronto, Canada). Sucrose concentrations were determined after subtracting absorbance corresponding to native glucose in the leaf tissue. Sample concentrations were quantified using a purified glucose standard curve.

Starch was isolated from the sample tissues left over from the sucrose extraction. The tissue samples were extracted in 1 ml of water in screw cap microcentrifuge tubes that was autoclaved for 1 hour at 135°C. The autoclaved sample extracts were dried down and resuspended in 1 ml of a thermal stable alpha-amylase solution (Megazyme, Wicklow, Ireland) and boiled in a water bath for 20 minutes. Samples were mixed by inverting vigorously at 5, 10, 15, and 20 minute time points. Amyloglucosidase solution (15 µl) (Megazyme, Wicklow, Ireland) was added and samples were incubated in a shaking water bath at 50°C for 60 minutes. Sample volumes of 20 µl were placed in a 96 well plate. 200µl of the glucose reaction mix (GOPOD, Megazyme, Wicklow, Ireland) was added and samples were incubated at 37°C for 20 minutes. Absorbance was read at 510nm using a spectrophotometer (SpectraMax Plus 384, MDS, Toronto, Canada). Sample concentrations were quantified using a starch standard curve.

Genetic analysis

Simple sequence repeat (SSR) analysis was accomplished using leaf tissue samples to determine whether ramets within each putative clone were indeed genetically identical. DNA was extracted from the leaf tissue according to methods of Dellaporta et al. (1983) with modifications outlined by Todd and Vodkin (1996) using cesium chloride gradient centrifugation. DNA samples were analyzed for purity and quantification with a NanoDrop ND-

1000 spectrophotometer (NanoDrop, Wilmington, DE) and diluted with H_20 to approximately 20-50 ng/µl.

Polymerase chain reactions (PCR) were accomplished by amplifying a set of seven SSR loci using a DNA Engine Dyad Peltier Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). These seven SSR markers (WPMS15, WPMS14, WPMS20, GCPM970-1, PMGC2571, PMGC433, and PMGC576) were previously developed by Smulders et al. (2001) and Mock et al. (2008). We modified their protocol to visualize the PCR products on a Li-Cor 4300 DNA Analyzer (Li-Cor, Lincoln, NE) using M13 tailed primers on a 6.5% polyacrylamide gel according to Oetting et al. (1995). Specifically, each 10 µl reaction was composed of 7.76 µL H_2O, 1 µl 10 × PCR buffer with $MgCl_2$ (Sigma-Aldrich, Inc., St. Louis, MO), 0.5 µL template DNA, 0.4 µl dNTP (2 mM), 0.1 µl of both forward and reverse primers, and 0.14 µl JumpStart™ Taq DNA Polymerase (Sigma-Aldrich, Inc., St. Louis, MO). Additionally each reaction had 0.1 µl (0.01 µM) IRD700 or IRD800 Dye-labeled M13 primer (CACGACGTTGTAAAACGAC) (Biomers.net, Ulm, Germany). Thermocycling was initiated at 94°C for 3 min, followed by 38 cycles of 94°C for 45 sec, 45°C for 45 sec, 72°C for 2 min, then a final extension step of 72°C for 5 min.

Statistical analysis

Analysis of variance (ANOVA) was used to test the effect of clone genotype (independent variable) on response variables measured in the study. Within-clone error was estimated from measurements on ramets within each clone using a random effects model estimated using restricted maximum likelihood (Table 1). Simple regression using curve fitting

software (Sigmaplot 10, Systat Software Inc. Point Richmond, CA) was used to identify the best fit between ramet age and phenotypic leaf traits. However, data points for each genet were not statistically independent. To account for this, a random slopes and intercepts model was fit to the data to calculate appropriate P values for the regression models. Statistical significance was defined as $\alpha \leq 0.05$. Statistical analyses were performed using Sigmaplot 10 and SAS statistical software (SAS Institute, Cary, NC, USA).

Results

Genetic analysis

Utilizing the seven SSR markers clearly demonstrated that each clone had a unique "allelic fingerprint" (allelic variation data not presented). Furthermore, different aged ramets within each of the clones (~24 ramets per clone) were genetically identical with the exception of six ramets in the Uinta National forest. These six genetically distinct ramets were excluded from the statistical analysis.

Phenotypic variation between clones

There was significant phenotypic variation between clones for all traits measured (ranging from 1.2 to 5.1 fold differences) among the eight clones (Table 1).

Height growth

As expected tree height had a strong positive correlation with ramet age (Figure 1). Height growth increased exponentially in the first 80 years or so and then tended to stabilize thereafter (Figure 1).

Leaf gas exchange

Ramet age explained 23% of the variation in maximum rates of photosynthesis (Figure 2a). Newly emergent suckers had relatively low rates of photosynthesis, which increased two fold and reached a maximum by age 10-20. High rates of photosynthesis were maintained until approximately age 40 at which point photosynthesis decreased linearly with age. Stomatal conductance decreased linearly with ramet age (Figure 2b).

Leaf anatomy and water relations

Xylem water potential showed a strong non-linear correlation with ramet age. Xylem water potential decreased exponentially in the first 20 years, and from age 40 on stabilized at approximately -1.7 MPa (Figure 3a). Water use efficiency was not strongly correlated with ramet age but did tend to increase linearly as ramets aged (Figure 3b). Specific leaf area, which is strongly correlated with ramet age, decreased (thicker leaves) until approximately age 80 and stabilized thereafter (Figure 4).

Foliar nutrients and carbohydrates

A general pattern emerged in which N and P were accumulated in higher concentrations in the leaves of younger ramets (< 25yrs) (Figures 5). From age 40 on there were no observable changes in foliar nitrogen and phosphorus concentrations (Figure 5). Foliar starch concentrations were not significantly correlated with ramet age (Figure 6a). In contrast, sucrose concentrations show strong correlations with ramets age, increasing markedly in the first 10 years, with a gradual linear increase thereafter (Figure 6b).

Leaf defense chemistry

There was a strong exponential decrease in phenolic glycosides during the first 40 years of ramet development (Figure 7a). From age 60 on phenolic glycosides stabilized at approximately 7% dry weight (Figure 7a). In contrast, condensed tannins increased linearly with age but had a much lower coefficient of correlation (Figure 7b). Among all the phenotypic traits measured, defense chemistry showed the strongest changes (approximately five fold) over developmental time.

Discussion

Age-related phenotypic variation within aspen clones

Evolutionary studies have primarily focused on changes in populations of genetically variable and discrete organisms. There is a need to better understand how Darwin's model of evolution by natural selection applies to clonal organisms (Helgason and Fitter, 2009). Phenotypic diversity between aspen clones is among the largest observed in tree species (Barnes

1975). Significant trait variation between genotypes is observed in growth (Oksanen et al. 2001), leaf morphology (Barnes 1975), foliar chemistry (Hwang and Lindroth, 1997), and phenology (Yu et al. 2001). Results from this study demonstrate that genotype and environmental variables strongly influenced the expressed phenotypes we measured (Table 1). This is consistent with studies showing that both genetic and environmental factors are important sources of phenotypic variation in aspen (Barnes 1975, St.Clair et al. 2010b), which can account for differential clone success in response to natural selection (Berrang et al. 1991, Griffin et al. 1991).

Where our knowledge is more limited is in understanding the level of phenotypic variation that exists among genetically uniform ramets within clones. A clear pattern emerged from our data in which age-associated factors, with the confounding effects of genotype and environment controlled, showed strongly phenotypic patterns. Ramet age explained more than 40% of the variation in five of the phenotypes examined, and greater than 20% of the variation in four others (Figures 1-7). Starch and N concentrations were the only phenotypes that were not significantly correlated with ramet age. The data are clearly consistent with our first hypothesis that developmental factors significantly influence phenotypic variation within clones. We hypothesize that this ontogenetically derived phenotypic variation leads to fitness differentials among different aged ramets, which may have important influences on overall clone fitness in response to diverse selection pressures.

Age-related patterns in functional trait expression

In addition to the strong influence of ramet age on phenotypic expression, there were clear patterns of age based directionality to our data. Eight of the phenotypic traits measured

demonstrated a non-linear relationship in which large and/or rapid changes in phenotype occurred in the early stages of ramet development. As predicted in our second hypothesis a general pattern emerged in which physiological traits associated with soil resource (water and nutrients) acquisition and leaf gas exchange decreased beyond 20-40 years (Figures 2, 3a, 5). In contrast to this pattern, starch showed no relationship with age and sucrose tended to increase with age (Figure 6), which is consistent with other studies that have documented higher levels of foliar non-structural carbohydrates in older trees (Sala and Hoch 2009). Observed differences in leaf traits between age classes may be driven by programmed developmental differences (heteroblasty) inherent in the meristems that give rise to leaves (Poethig 1990, Day et al.2002).

An alternative explanation is that differences in the size and anatomy of roots and stems among different aged ramets, results in differential capacity to deliver water, nutrient and carbohydrate resources that are important determinants of leaf development and function (Bond et al. 2007). The anatomy of woody tissues has important influences on leaf functional traits via resource delivery (Zhang and Cao 2009). "The hydraulic limitation hypothesis proposes that increased path length (in roots, stems, and branches) decreases leaf-specific hydraulic conductance as trees grow in height" (Barnard and Ryan 2003). In order to regulate leaf water status, taller trees must lower stomatal conductance, which decreases net photosynthesis (Ryan et al. 2006). Aspen has been shown to lower stomatal conductance in response to increasing vapor pressure deficits (Dang et al. 1997) to avoid negative xylem water potentials that trigger cavitation (Sperry et al. 1994). Our results showing age-related reductions in xylem water potential and gas exchange (age 20-80) are consistent with these findings and suggest that increasing height may be a driving force behind reductions in leaf physiology as aspen ramets age.

Ewers et al. (2005) demonstrated that as an aspen increases in size, it reduces age-related constraints to water acquisition and carbon gain by increasing its sapwood to leaf area ratio (A_s:A_l) Increases in (A_s:A_l) and reductions in SLA (Figure 4) may partially explain the observed increases in water use efficiency with age (Figure 3b). Water use efficiency was negatively correlated ($r=-0.54$, $P < 0.0001$) with SLA (WUE tended to be greater in leaves that were thicker). Through these anatomical adjustments aspen may partially compensate for physiological constraints imposed by vertical growth. Interestingly, leaf carbohydrates were relatively stable, or increased in the case of sucrose, across age classes, suggesting that resource constraints in older ramets have little impact on leaf carbohydrate status. Since leaf carbohydrates represent only a fraction of a trees carbohydrate reserves it would be informative to examine age-related impacts on carbohydrates stored in stems and roots.

Studies on fire history in subalpine and boreal aspen forests, indicate that both climate conditions (Beaty and Taylor 2008) and fire suppression by humans (Van Wagner et al. 2006) has lengthened fire return intervals during the last century. It has been suggested that recent patterns of aspen decline in seral aspen communities (Rogers 2002) is partly being driven by the lengthening of fire return intervals that over time has reduced aspen cover, while increasing conifer dominance (Gallant et al. 2003, Smith and Smith 2005). Our data showing reductions in resource acquisition and physiological vigor as aspen clones age, suggest that aspen may be more competitive with conifers under shorter fire return intervals.

Age related defense strategies in aspen

The clones in our study invested heavily in defense chemistry, with phenolic glycosides and condensed tannins making up as much as 20% of leaf dry weight. As predicted in our third hypothesis phenolic glycosides concentrations were highest in young ramets, suggesting that younger age classes initially use a strategy of "resistance" to deter mammal herbivory (Wooley et al. 2008). It could be argued that induction of defense chemistry in response to browsing may have increased defense chemistry in younger ramets. However, we carefully examined each of the eight clones and found no evidence of active or past browsing on any of the ramets. Donaldson and Lindroth (2007) also found a negative correlation between phenolic glycoside concentrations and aspen ramet age in juvenile aspen clones in the Great Lakes region. They observed an exponential decrease (~50 %) in phenolic glycosides within the first five years in ramet development and stabilization in phenolic glycoside concentrations thereafter (Donaldson et al. 2006). The largest reduction in phenolic glycosides in our study occurred between ages 10-20 (Figure 7), which corresponds to the time point when ramets grew above the mammal browse line (>2 m) (Figure 1). These results support the interpretation that a strategy of tolerance is relaxed as ramets age. However, in contrast to Donaldson and Lindroth's (2007) findings in eastern aspen, we did not observe stabilization of phenolic glycosides until approximately 50-60 years of age (Figure 7). This suggests that there may be some adaptive value in retaining higher levels of phenolic glycosides in aspen growing in its western range. Western aspen clones in our study certainly invested much less in condensed tannins (Figure 7) than eastern clones (~3% vs. ~12%) (Donaldson et al. 2006), which may allow the sustained allocation to phenolic glycosides we observed.

In the western US, several insect species can be important defoliators of aspen including western tent caterpillars *(Malacosoma californicum*), forest tent caterpillars *(Malacosoma disstria),* and the large aspen tortrix *(Choristoneura conflictana)* (Jones, DeByle and Bowers 1985). There is little evidence that condensed tannins have an important role in deterring insect herbivory in aspen (Hwang and Lindroth 1997, Donaldson et al. 2006). Phenolic glycosides have been shown to negatively impact forest tent caterpillar performance (Hwang and Lindroth 1997), which could be one benefit of reducing levels of phenolic glycosides more gradually over time.

In its western range, aspen is host to a large number of disease-causing pathogens that are particularly common in aging aspen stands (St.Clair et al. 2010a). Very little research has explored whether defense compounds in aspen possess anti-microbial properties. A recent study examining *Venturia* shoot blight in aspen, suggests that condensed tannins may be more important than phenolic glycosides in reducing disease incidence (Holeski et al. 2009). Interestingly, condensed tannin concentrations in our study increased with ramet age (Figure 7), a pattern that is consistent with results from Donaldson and Lindroth (2007). We hypothesize that the more gradual reductions in phenolic glycosides and/or increases in condensed tannins observed in our study may be linked to greater resistance to foliar pathogens as aspen age. Future research efforts should focus on testing the putative role of defense chemistry in conferring resistance to the major pathogens that affect aspen.

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Tables and Figures

Clone	A_{max} ($\mu mol\ m^{-2}s^{-1}$)	g_s ($mmol\ m^{-2}s^{-1}$)	Xylem H_20 pot. (MPa)	WUE ($\mu mol\ mol^{-1}$)	SLA ($cm^2\ g^{-1}$)	N (% dry wt.)	P ($mg\ g^{-1}$)	Sucrose ($mg\ g^{-1}$)	Starch ($mg\ g^{-1}$)	Tannins (% dry wt.)	Phenolics (% dry wt.)
1	13.3 ± 0.6	167 ± 12	-1.59 ± 0.06	3.5 ± 0.16	127 ± 4.5	2.3 ± 0.09	1.15 ± 0.08	1.07 ± 0.07	2.25 ± 0.17	1.11 ± 0.20	15.0 ± 0.9
2	10.7 ± 0.5	123 ± 10	-1.68 ± 0.04	4.0 ± 0.20	110 ± 3.8	2.1 ± 0.09	0.94 ± 0.05	1.35 ± 0.09	1.80 ± 0.12	3.32 ± 0.44	8.2 ± 1.1
3	10.0 ± 0.4	109 ± 10	-1.66 ± 0.03	3.3 ± 0.11	115 ± 2.9	2.0 ± 0.09	0.78 ± 0.02	1.48 ± 0.08	1.75 ± 0.10	2.82 ± 0.22	15.1 ± 2.0
4	11.2 ± 0.7	131 ± 13	-1.51 ± 0.07	2.7 ± 0.08	122 ± 6.5	2.6 ± 0.09	1.04 ± 0.07	1.38 ± 0.08	1.29 ± 0.08	5.64 ± 1.13	10.2 ± 1.0
5	11.9 ± 0.5	167 ± 11	-	2.0 ± 0.08	126 ± 5.0	2.0 ± 0.09	0.80 ± 0.04	1.02 ± 0.04	1.23 ± 0.07	2.33 ± 0.27	10.6 ± 1.2
6	12.5 ± 0.7	196 ± 19	-1.47 ± 0.07	1.9 ± 0.07	143 ± 4.0	2.4 ± 0.09	1.21 ± 0.05	1.33 ± 0.12	0.78 ± 0.11	1.10 ± 0.15	9.3 ± 1.8
7	15.6 ± 0.6	231 ± 11	-	2.9 ± 0.11	139 ± 5.9	2.3 ± 0.09	1.21 ± 0.06	0.89 ± 0.07	0.89 ± 0.05	1.48 ± 0.29	7.9 ± 0.8
8	12.6 ± 0.9	172 ± 16	-	3.0 ± 0.11	117 ± 4.6	1.8 ± 0.09	0.97 ± 0.04	1.37 ± 0.07	0.81 ± 0.06	3.61 ± 0.65	12.0 ± 1.7
F-statistic	7.05	9.31	2.53	29.4	5.94	11.50	8.57	5.53	25.1	8.50	3.97
P-value	<0.0001	<0.0001	0.044	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0005

Table 1. Means and standard errors for phenotypic traits of all eight aspen clones in the study. F statistics and P values testing for significant phenotypic differences among clones were calculated using ANOVA.

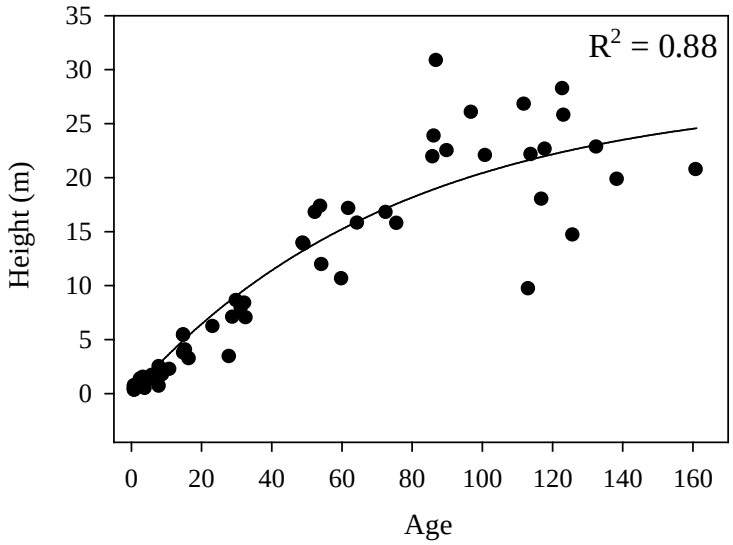

Figure 1. Age-related variation in height for the eight aspen clones. Exponential rise to maximum non-linear regression provided the best fit for height ($P < 0.0001$) data.

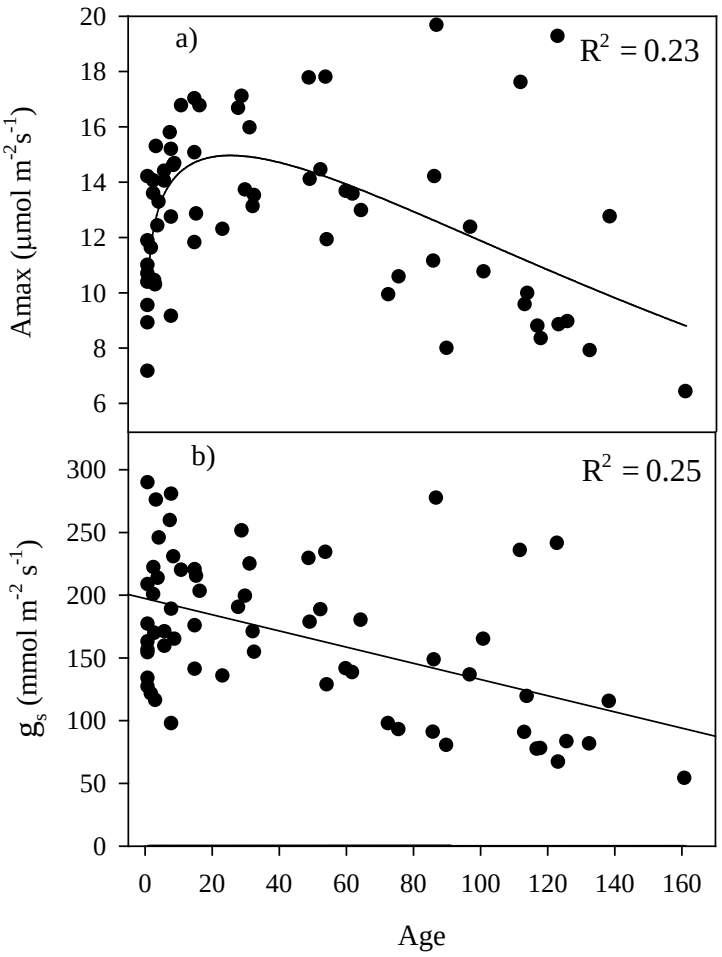

Figure 2. Age-related variation in leaf photosynthesis and stomatal conductance for the eight aspen clones. Weibull and linear regression provided the best fit for (a) photosynthesis (P = 0.019) and (b) stomatal conductance (P = 0.006) data.

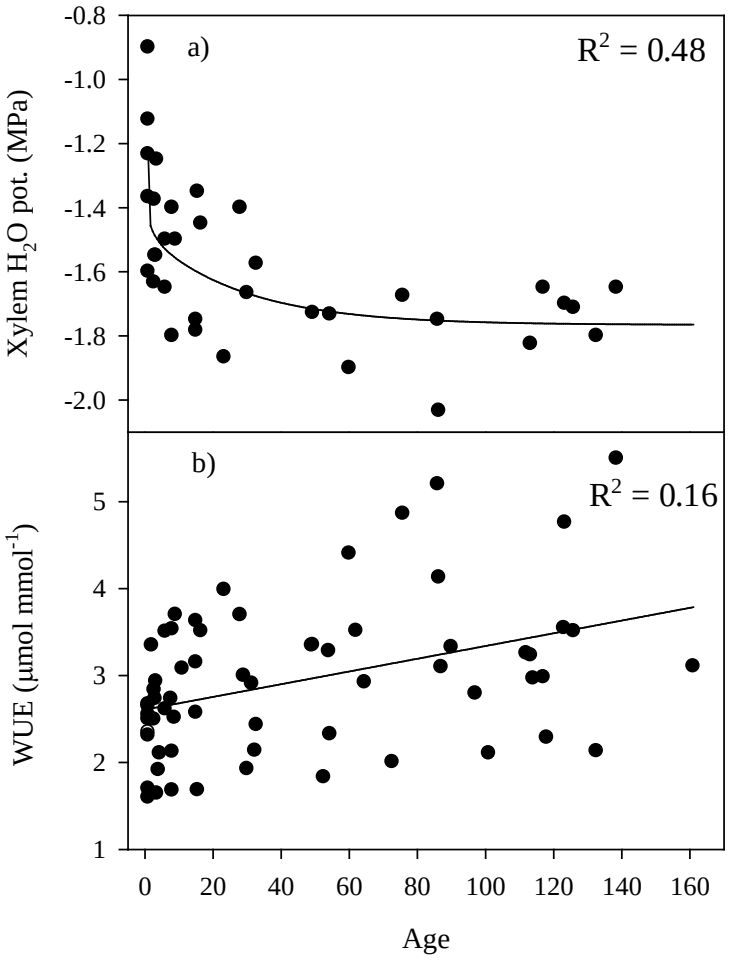

Figure 3**.** Age-related variation in xylem water potential and water use efficiency for the eight aspen clones. Exponential decay and linear regression provided the best fit for (a) xylem water potential ($P < 0.0001$) and (b) water use efficiency ($P = 0.004$) data.

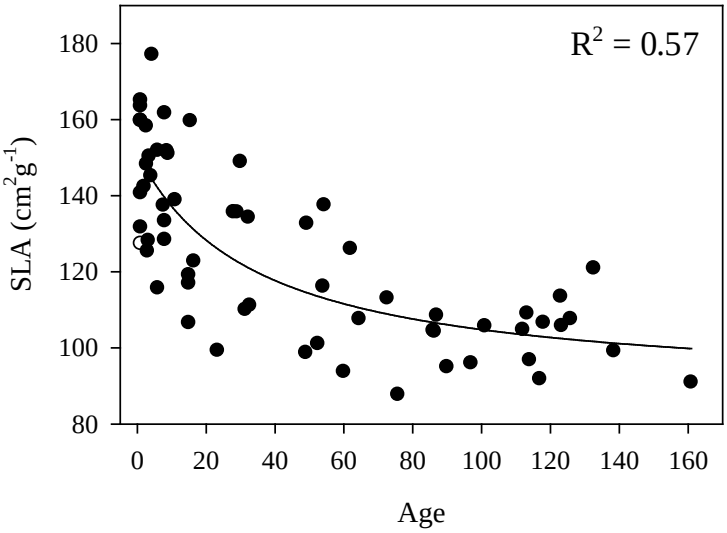

Figure 4. Age-related variation in specific leaf area for the eight aspen clones. Rational regression provided the best fit for specific leaf area data ($P < 0.0001$).

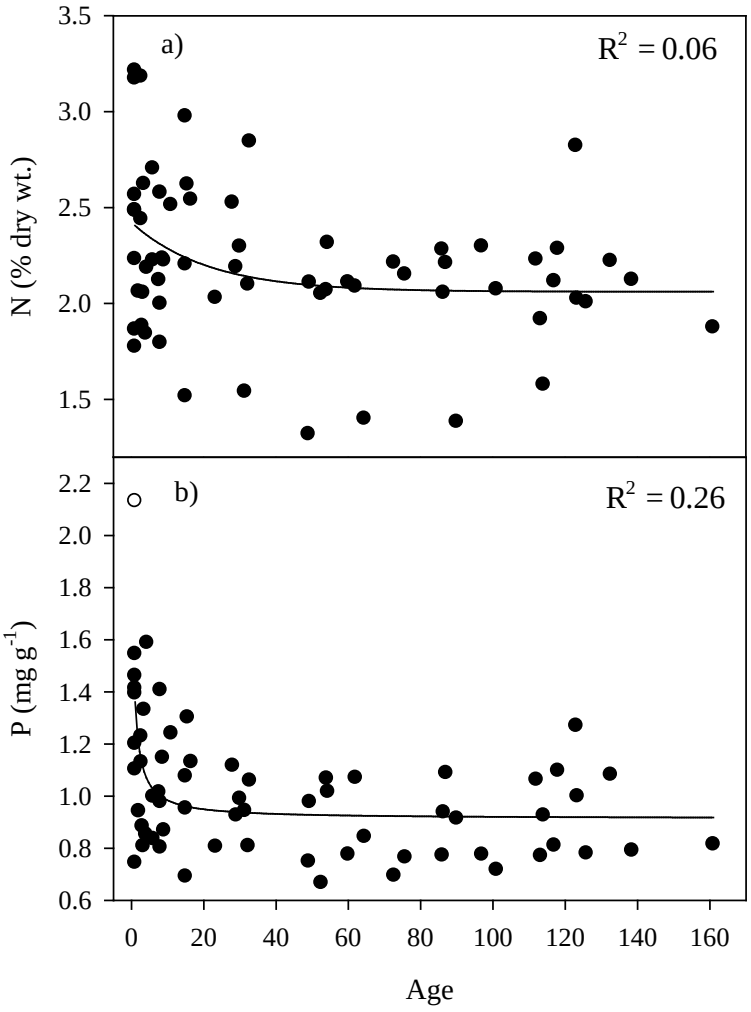

Figure 5. Age-related variation in foliar N and P for the eight aspen clones. Exponential decay and rational regression provided the best fit for foliar (a) nitrogen (P = 0.155) and (b) phosphorus (P = 0.0002) data.

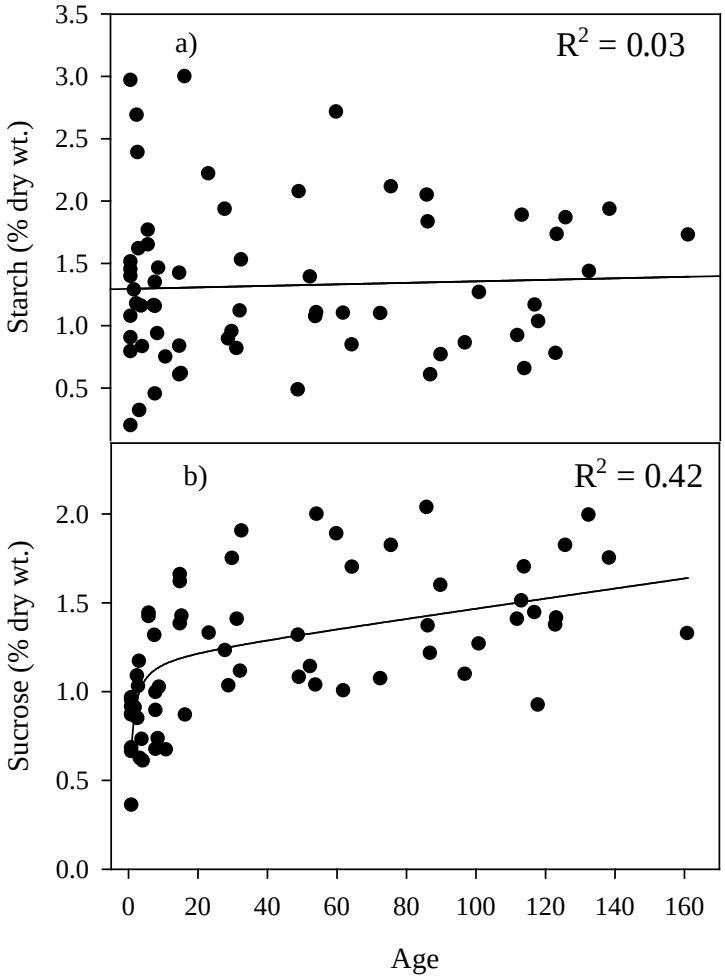

Figure 6**.** Age-related variation in foliar sucrose and starch for the eight aspen clones. Linear and hyperbola regression provided the best fit for foliar (a) starch (P = 0.744) and (b) sucrose (P = 0.0002) data.

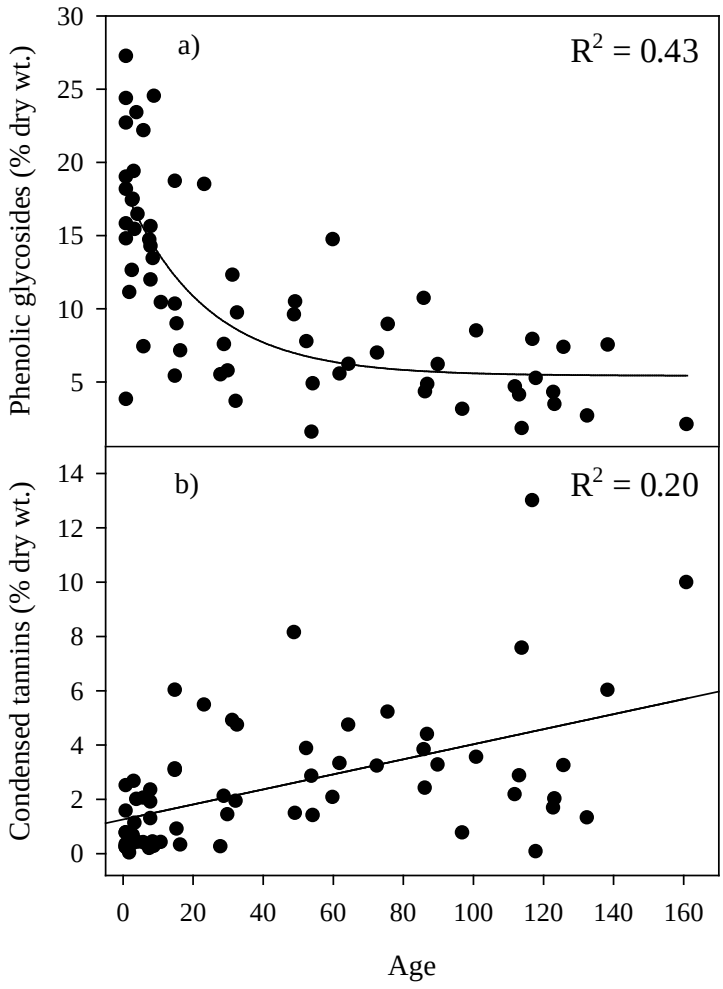

Figure 7**.** Age-related variation in phenolic glycosides and condensed tannins for the eight aspen clones. Exponential decay and linear regression provided the best fit for leaf (a) phenolic glycosides (P = 0.0002) and (b) condensed tannins (P = 0.015) data.

Chapter 2

The influence of pre-fire forest succession status and environmental conditions on post-fire aspen regeneration success

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Summary:

Fire is an essential component of many forest ecosystems and fire exclusion policies and other anthropogenic factors have altered disturbance regimes and increased the length of fire cycles in many aspen dominated areas leading to increased aspen succession to conifers. The objective of this study was to better understand how post-fire aspen regeneration success is influenced by increasing conifer abundance under longer fire return intervals and changing environmental conditions. 66 sites were selected from the Sanford fire complex located in the Dixie National Forest in southern Utah. We measured aspen regeneration density and height as response variables and canopy composition and density, soil characteristics, slope and aspect as independent variables. Results from this study demonstrated that the succession status of the former overstory has a strong influence on post-fire aspen regeneration success. Pre-disturbance conifer abundance and aspen densities were good predictors of aspen sucker regeneration success. Results demonstrate that as conifer presence increases in the overstory there is a decrease in post-fire regeneration success. Aspect significantly influenced aspen regeneration with aspen suckering density being significantly lower on north facing slopes than other aspects. Soil P and carbon were positively correlated with aspen regeneration density and regeneration varied significantly by soil texture classes. Recommendations for forest managers where sustainability of aspen is desired are: 1) prescribed burns when conifer stem density is greater than 80%; and 2) large prescribed burns especially is areas with high livestock and wildlife densities. .

Keywords: Fire, Aspen, Ecology, Succession, Prescribed Burns, Post-fire, Regeneration

Introduction

Succession processes and appropriately timed disturbance cycles are critical to the maintenance and health of many forest ecosystems (Attiwill 1994). Historically, disturbances and their timing were exclusively driven by natural events (herbivory, wind, flood, drought, frost, wildfire) that were in synchrony with succession processes (Bengtsson et al. 2000). Increasingly, anthropogenic influences (logging, fire suppression, roads) are drastically changing the types and timing of disturbance. Fire is an important disturbance process that is a necessary part of many forest ecosystems (Agee 1993). Fire clears out pathogens, contributes to organic matter decomposition, and cycles nutrients (Meentemeyer et al. 2008). Fire adapted ecosystems are dependent upon fire cycles to sustain the health of the ecosystem and to retain biodiversity (Parker et al. 2006). Land use practices and fire exclusion policies have significantly altered disturbance regimes and the length of fire return intervals often with negative effects (Margolis and Balmat 2009).

Early disturbance species may be particularly affected by lengthened fire return intervals due to fire exclusion (Reyes et al. 2010). Quaking aspen (*Populus tremuloides*) is an early disturbance tree species that benefits from relatively short fire return intervals. Fire stimulates root suckering in aspen which results in a vigorous regeneration response that allows aspen dominance during early succession stages (Frey *et al.* 2003).

Studies have shown that lengthening fire return intervals reduces aspen cover while increasing conifer dominance (Smith and Smith 2005, Gallant et al. 2003). Succession is a natural process in seral aspen stands but as fire return intervals lengthen under fire suppression, canopy composition can shift away from pure and mixed aspen/conifer stands toward climax conifer forests that may result in aspen loss (Bergen and Dronova 2007, Rogers 2002). Fire

exclusion leads to increased stand ages and densities, resulting in competitive interactions that can result in recruitment failure of species (Minnich et al. 2000). Forest Inventory and Analysis (FIA) data show significant declines of aspen in the Interior West relative to historic highs (Rogers 2002). Evidence suggests that climate patterns (Buechling and Baker 2004; Beaty and Taylor 2008; Rehfeldt 2009) and longer fire return intervals (Gallant *et al.* 2003; Smith and Smith, 2005) may be contributing to patterns of aspen dieback in its western range through increased competitive interactions with conifers. As aspen are lost to climax conifer forests there are there are reductions in biodiversity (Debyle 1985; Hollenbeck & Ripple 2007), and watershed yields (LaMalfa and Ryel 2008).

The objective of this study was to better understand how post-fire aspen regeneration success is influenced by conifer expansion under longer fire intervals. We hypothesized that 1) Post-fire aspen regeneration decreases with increasing pre-fire conifer stand composition and density; and 2) Environmental factors including slope, aspect, and soil characteristics also influence aspen regeneration success

Materials and methods

Study locations

In the spring of 2002, two separate prescribed burns (1417 total hectares) were planned in the Dixie National Forest in south-central Utah. These prescribed burns had the goal to reduce fuel load, prevent further pinyon/juniper invasion into sagebrush/grasslands, and stimulate aspen suckering (Sanford Prescribed Fire Review). The fires were initially ignited in April (Sanford)

and May (Adams Head) but by June, due to weather conditions they combined into what is now known as the 31,566 hectare Sanford Wildland Fire (37.9°N 112.2°W).

66 sites within the Sanford Fire Complex were selected for measurements and sample collection, which occurred on June 15-16, Aug 4-6, and Aug 26, 2009 (Figure 1). Site elevations ranged from 2440m to 3230m. Sites were chosen in locations where the fire had caused complete stand mortality. However, the dead trees of the former stand were still in place seven years after the fire, which allowed determination of tree diameter and stand density and composition (aspen and conifer species could be distinguished) as measures of pre-fire stand succession status. Site aspect, slope, soil chemistry and texture were also determined. Aspen sucker density and height were determined as measures of asexual regeneration vigor.

We assessed pre-fire overstory stand density using the point quarter method along 50m transects (Cottam and Curtis 1956) with a correction from Pollard (Pollard 1971) at each of the 66 site locations. Diameter at breast height (DBH) was determined on each tree included in the point quarter analysis. Post-fire sucker regeneration density, sucker height measurements was collected every 10m along transects using $1m^2$ quadrats. Soil samples were collected using a soil probe inserted 15 cm deep at every 10m point along each transect. Slope along each transect was calculated using a Clinometer (Haglof HEC, Langsele, Sweden) and aspect was determined with a compass.

Soil analysis

Soil Total nitrogen and carbon were determined on 20 mg of dry soil samples analyzed on a nitrogen and carbon analyzer (TruSpec, CN Determinator, LECO Cooperation, St. Joseph,

Michigan, USA) using the combustion method (Campbell, 1991). Phosphorus was extracted with Sodium bicarbonate solution and analyzed with the methods of Olson et al. (1954). Soil texture was determined using the hydrometer method (Day 1965).

Statistical analysis

In the statistical models tree diameter, stand composition, and densities of the former burned overstory along with aspect, slope, soil chemistry and texture were classified as explanatory variables. Aspen asexual regeneration (suckering) density and height were defined as response variables. Each individual transect was treated as an independent data point with data averaged across each transect. Differences in aspen regeneration density, height, aspect, and soil texture were tested using analysis of variance (ANOVA). To find the best model for predicting aspen regeneration response a stepwise regression model was used with aspen regeneration (stems ha^{-1}) and height as the main effects. All data were tested for normality and homogeneity of variance by visually inspecting the data and using Shapiro–Wilk W statistics to determine the goodness of fit of the data in normal-quantile plots. A Box–Cox power transformation was applied to aspen regeneration density, soil N, soil carbon, total stand density, aspen density, and conifer density to satisfy the assumptions of normality. All other data were found to be normally distributed. The statistical analysis was performed using JMP version 8 statistical software (SAS Institute, Cary, NC, USA).

Results

Stepwise multiple regression models did not identify additional factors that strongly increased the explanatory power of the simple regression models and so our results and discussion will focus on single factor regression models. Aspen regeneration density was negatively correlated with pre-fire conifer composition (R^2 = 0.52, $p<0.0001$,) (Figure 2) and positively correlated with pre-fire aspen density (number of aspen relative to the ground area) (R^2 = 0.52, $p<0.0001$) (Figure 2). Soil carbon and P, and aspen and conifer DBH were also positively correlated with aspen regeneration density (Table 1). Regeneration stem height showed a negative correlation with pH (R^2=0.12, p=0.0049) and a positive correlation with pre-fire aspen DBH (R^2=0.37, $p<0.0001$, Figure 4) (Table 1). All other explanatory variables were not significantly correlated with aspen stem height (Table 1).

In the ANOVA analysis north facing aspects were found to have significantly lower levels of aspen sucker regeneration than other aspects ($p<0.0001$, Figure 5). Differences in aspen regeneration density on different soil texture classes was significant at $P \leq 0.1$ with loam soils supporting higher levels of aspen regeneration than clay loam or sandy loam soils (F=2.43, p=0.0962, Figure 6).

Discussion

Successional influences on regeneration capacity

In this study, pre-fire overstory stand characteristics (conifer and aspen composition and densities) were found to strongly impact aspen regeneration success. Data supported our first hypothesis and demonstrated that as the percent of conifers increased relative to aspen there was

a linear decrease in post-fire aspen regeneration density (Figure 2). In contrast, high aspen densities increased aspen sucker regeneration levels post-fire (Figure 2). Bartos et al. (1991) found the timing of the fire and pre-fire sucker characteristics could be used as predictors of regeneration success. Quantification and use of pre-fire stand succession status to predict of aspen regeneration success post-disturbance is a novel contribution to the literature.

The lengthening of fire intervals has been theorized to increase conifer abundance thus decreasing aspen cover through competitive interactions (Gallant *et al.* 2003; Bradley et al. 1992). As conifers establish in aspen stands there are reductions in the levels of light that reach the forest understory (Stadt and Lieffers 2000). Aspen are shade intolerant (Kobe and Coates 1997) and suffer under reduced light when compared to conifer performance in the same conditions (Wright et al. 1998). Shifts in soil chemistry that occur as conifers expand in aspen stands (Mallik et al. 2008) reduce aspen regeneration vigor (Van Breemen and Finzi 1998, Calder et al. unpublished). Reductions in aspen photosynthesis, biomass and defense have been observed under reduced light levels and on conifer modified soils, while having little effect on conifer seedlings (Calder et al. unpublished). Increases in the length of fire return intervals also results in ageing aspen stands which have been found to have reduced vigor than younger aged stands (Smith et al. 2010). We hypothesize that as conifer abundance increases, the reductions in regeneration success that we have observed are due to the physiological sensitivity of aspen to changing light and soil conditions through competitive interactions.

Herbivory

High browsing pressure is a second way in which aspen is lost from the landscape (Kaye et al. 2005; Strand et al. 2009). Short aspen suckers that regenerate after fire are susceptible to

herbivory by mammal herbivores. Lower light levels and changes in soil chemistry caused by conifer dominance slows aspen height growth (Calder et al. unpublished) which increases the time required for aspen suckers to grow beyond the browse line, increasing the probability of herbivory (Kay 1990; Bartos et al. 1991). In contrast, the height growth of conifers is less sensitive to soil and low light constraints (Calder et al. unpublished, Parent and Messier 1995).

Our data demonstrate that aspen stands with larger average DBH's, produced suckers that grow more quickly (Figure 3). Higher sucker densities have been correlated with increasing preharvest basal area (Graham et al. 1963) and site index (Stoeckler and Macon 1956), presumably in large part because of higher densities of roots in the mature stand (Frey et al. 2003). The roots of more vigorous aspen clones have been found to produce greater suckering (Tew 1970). We hypothesize that stands which have a larger pre-fire average stem DBH may contain bigger, more vigorous root systems which leads to faster sucker height growth. Producing higher stem densities is another way that aspen are able to tolerate herbivory. Pre-fire successional status has a large impact on aspen regeneration densities (Figure 1). High sucker densities may overwhelm herbivore browsing and increase the chance of stand replacement. There are many examples in the literature of aspen regeneration failure because of heavy herbivory of regenerating aspen stems in areas with high wildlife densities (Kay 1990; Bartos et al. 1991). In this study, aspen regeneration was highly successful in the presence of high wildlife densities that exist in the Dixie National Forest (~8000 individual elk and ~73,000 deer post season in 2002) (Dixie 2004). Regeneration levels were strong across our study sites with an average regeneration of ~37000 stems ha^{-1} seven years after the disturbance took place. Regeneration may have been so successful in the presence of high wildlife densities because the

large size of the disturbance overwhelmed the ability of the herbivores to consume all the suckers.

Environmental Factors

Our second hypothesis that environmental parameters (slope, aspect, and soil conditions) have a strong influence on post disturbance aspen regeneration success was partially supported as aspect and soil characteristics significantly influenced aspen regeneration success but slope did not. Other studies have shown that physiographic variables including elevation, slope, and aspect had small influences on aspen regeneration vigor following fire and were generally not strong indicators of an aspen stands health (Rogers et al. 2010; Bartos et al. 1991). While slope appears to have little effect on aspen regeneration vigor our data does suggest that aspect is important as aspen regeneration density was much lower on north facing slopes (Table 1; Figure 3). Initially we suspected that differences in regeneration that were observed on north facing aspects were due to increased conifer competition in those locations but looking at conifer densities across aspects we found no significant differences (Table 1). Differences in overstory aspen densities across aspects were found to exist, with lower densities existing on the north slopes (Figure 3). Since overstory aspen density was positively correlated with regeneration density a reduced aspen presence on north slopes may explain the lower regeneration response.

Our analysis indicates that soil characteristics have important influences on aspen regeneration vigor. Both soil P and carbon were positively correlated with aspen regeneration density (Table 1) and regeneration varied significantly by soil texture classes. Different soil types have different water holding capacities. Increased soil moisture and water availability in the

Loam soils may lead to the ability of the root systems to produce more dense regeneration. Our observations on differences in loam soils showed higher sucker densities that grew slower than other soil types. This may be due to the trade off that exists in resource allocation, increases in suckers production leads to less resources available for sucker growth. Phosphorus is an essential nutrient for plants and is the limiting growth factor in many forest systems. It is likely that this increase in foliar nutrient availability to the suckers stimulated higher photosynthetic rates and growth. This suggests a potential indirect role of nutrient availability on the numbers of suckers established after a disturbance (Fraser et al. 2002). Few studies have linked growth in young sucker stands to nutrient availability but our results support the theory that increases in levels of phosphorus and other micronutrients leads to the observed increasing growth rates.

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Tables and Figures

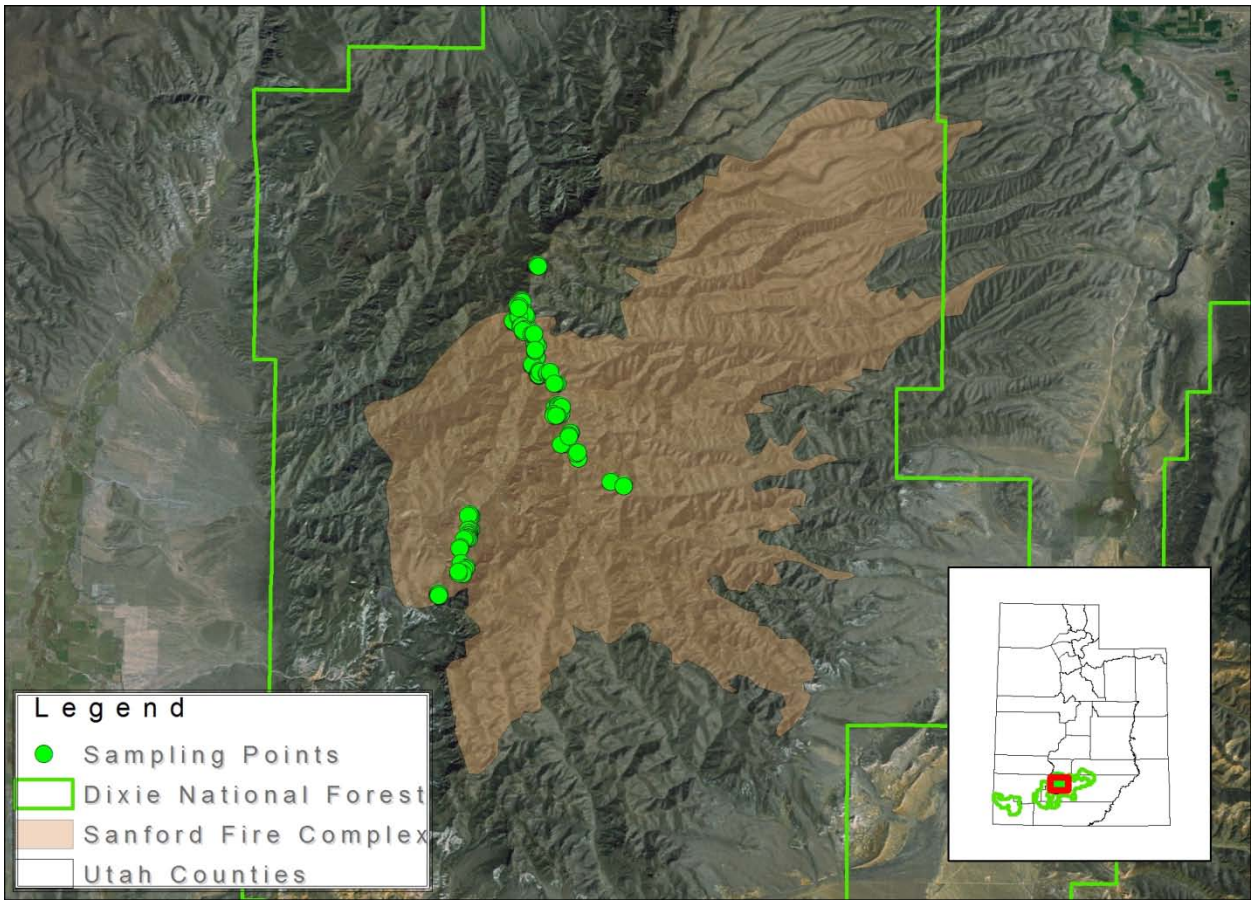

Figure 1**:** GIS data retrieved from the National Forest Service. Sampling points represent transect terminus. Although some points appear to be outside the Sanford Fire, on site analysis showed the fire boundaries of the GIS data to be approximate and the points were within the Sanford burn area boundary.

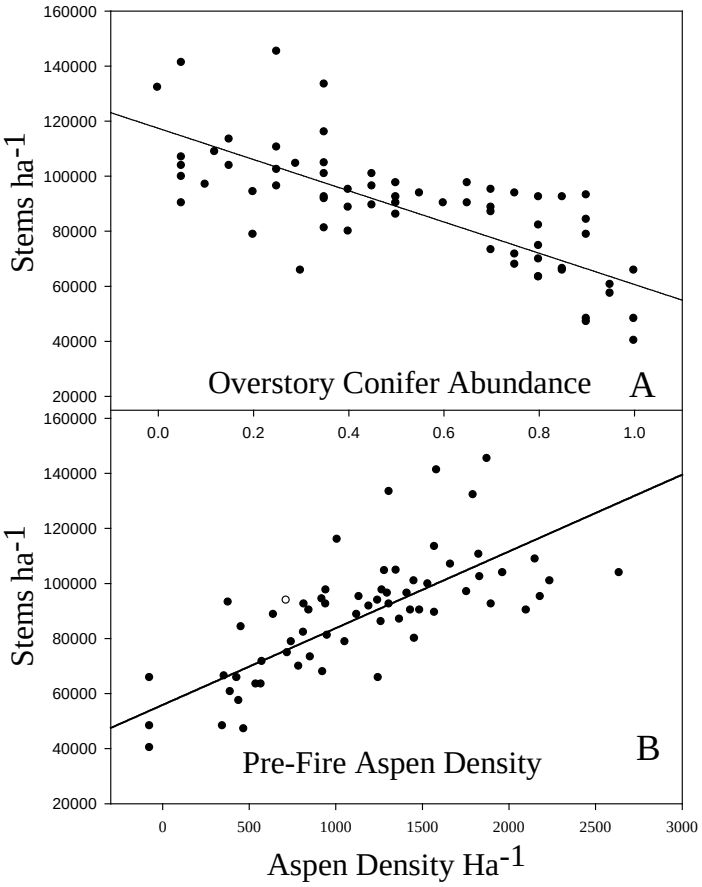

Figure 2**:** A: Conifer composition (relative to all other trees in the stand) compared to transformed aspen regeneration densities. B: Transformed pre-fire aspen density relative to ground area compared to transformed aspen regeneration densities. Both pre-fire aspen abundance and aspen regeneration densities were transformed using box-cox transformations. Regression analysis were both significant at ***P ≤ 0.001.

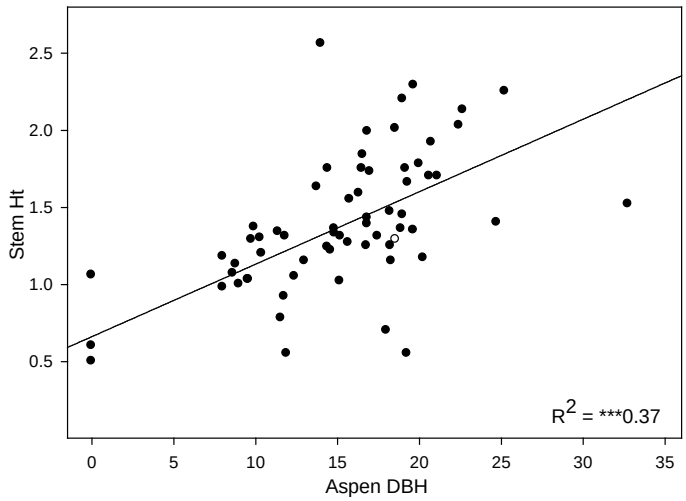

Figure 3**:** Average pre-fire aspen overstory DBH (diameter breast height) compared against stem regeneration height. (F=37.3765, ***p<0.0001) Significance designated as *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001

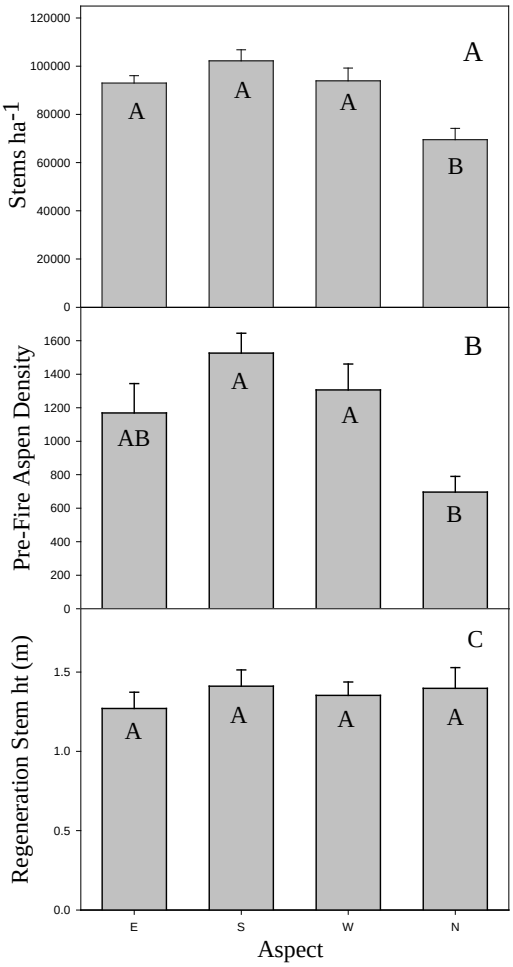

Figure 4: A: Post-fire transformed aspen sucker densities at various aspects. B: Transformed pre-fire aspen density (relative to ground area) for each aspect. C: Aspen sucker average height at various aspects. Different letters represent statistically different means in the ANOVA.

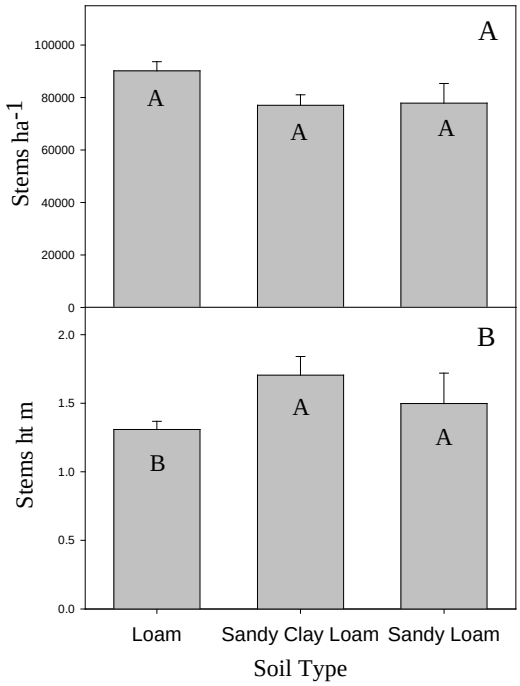

Figure 5: A: Transformed post-fire sucker densities from each soil type found within the complex. B: Average sucker height from each soil class. Different letters represent statistically different means in the ANOVA.

	Conifer Composition	Slope	carbon	phosphorus	Total N	pH	Conifer Density	Aspen Density	Aspen DBH	Conifer DBH
Sucker Regeneration Density	-0.52***	7E-04	+0.08**	+0.17***	0.06*	0.04	+0.09**	+0.52***	+0.15***	+0.05**
Stem Height	0.03	0.11	0.02	0.005	0.04	-0.12**	0.03	0.02	+0.37***	0.002

Table 1: Correlation coefficients indicating the relationship between aspen regeneration transformed data and aspen regeneration stem height. Significance designated as *P ≤ 0.010; **P ≤ 0.05; ***P ≤ 0.01