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## Ecophysiological Mechanisms Underlying Aspen to Conifer Succession

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Ecophysiological Mechanisms Underlying  
Aspen to Conifer Succession

W. John Calder

A thesis submitted to the faculty of  
Brigham Young University  
in partial fulfillment of the requirements for the degree of  
Master of Science

Sam St. Clair  
Loreen Allphin  
Bruce Webb  
Dennis Egget

Department of Plant and Wildlife Sciences

Brigham Young University

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## ABSTRACT

### Ecophysiological Mechanisms Underlying

### Aspen to Conifer Succession

W. John Calder

Department of Plant and Wildlife Sciences

Master of Science

This thesis includes three studies. The first study examined how reductions in light availability and changes in soil chemistry that occur as conifers establish in aspen stands, differentially affects the regeneration success of aspen and conifers. We found that aspen were more sensitive to changes in light and soil than subalpine fir. For aspen, reduced light and conifer influenced soils significantly reduced height, biomass, photosynthesis and the production of secondary defense compounds. Subalpine fir seedlings were significantly reduced in photosynthesis, biomass and R:S under lower light conditions but showed no differences in physiology or growth when grown on the contrasting soil types. Subalpine fir seedlings were significantly reduced in photosynthesis, biomass and root:shoot ratio under lower light conditions but showed no differences in physiology or growth when grown on the contrasting soil types. Results from this study suggest that reduction in light and changes in soil chemistry associated with conifer succession place constraints on aspen growth and defense capacity, which may contribute to losses in aspen cover under longer disturbance return intervals.

The second study looked at regeneration dynamics of aspen and conifers as forest stands transition from canopy gaps to aspen dominated canopies to conifer dominated canopies. We found that as overstory conifer density increases, aspen decrease in density, basal area, and seedling establishment. Conifers were shown to establish closer to aspen as the canopy increased in conifer density. As this proximity relationship extended into the canopy there is increased mortality in both aspen and subalpine fir, suggesting both facilitation and competition.

Our third study looked at the physiological effects of smoke exposure on growth and primary and secondary metabolic responses of deciduous and conifer tree species. Twenty minutes of smoke exposure resulted in a greater than 50% reduction in photosynthetic capacity in five of the six species we examined. Impairment of photosynthesis in response to smoke was a function of reductions in stomatal conductance and biochemical limitations. In general, deciduous species showed greater sensitivity than conifer species. Smoke had no significant affect on growth or secondary defense compound production in any of the tree species examined.

Keywords: aspen decline, aspen succession, smoke, fire suppression hypothesis

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Brigham Young University

SIGNATURE PAGE

of a thesis submitted by

W. John Calder

The thesis of W. John Calder is acceptable in its final form including (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory and ready for submission.

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Date

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Sam St. Clair

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Date

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Bruce Webb

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Date

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Dennis Eggett

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Date

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Loreen Allphin

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Date

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Rodney Brown

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## Introduction

This thesis primarily examines the ecophysiological mechanisms that influence conifer expansion into aspen forests. Aspen is successional to conifers and it is thought that changes in fire regime and climate are allowing increased aspen displacement by conifers. Aspen is a valuable species that has a major influence on community diversity and ecosystem services. As such it, it is important to understand why aspen are succession to conifers. Our first study looked at how changes in soil and light that are associated with conifer succession, influenced aspen and subalpine fir primary metabolism, growth, and secondary metabolism. Our second study took place in the field and examined regeneration patterns and trends of overstory mortality through the various stages of forest establishment and succession including: canopy gaps, aspen dominated canopies and finally conifer dominated canopies.

As fire is typically the disturbance in aspen and conifer stands that resets the successional transition to aspen communities we undertook a smoke physiological study. Much work has been done examining the effects of smoke on seed germination but very little work has looked at out physiological effects from smoke. We exposed six tree species to smoke to better understand plants response to fires that typically dominate systems.

# Physiological mechanisms underlying aspen succession to conifers: the role of light and soil chemistry

W. John Calder<sup>1</sup>, Kevin Horn<sup>1</sup>, Samuel B. St. Clair<sup>1</sup>

<sup>1</sup>Department of Plant and Wildlife Sciences, Brigham Young University, Provo, Utah

## Abstract

Evidence suggests that longer fire return intervals may be contributing to patterns of aspen decline in portions of its western range by promoting succession to conifers. As conifers establish in aspen stands there are reductions in light availability and changes in soil chemistry. We hypothesize that these reductions in light availability and changes in soil chemistry, differentially affects the function and regeneration success of aspen suckers and conifer seedlings leading to shifts in forest composition. A field study was conducted to examine the responses of aspen and subalpine fir regeneration under contrasting light and soil chemistry conditions based on variation in overstory aspen/conifer composition (gap, aspen dominant, mixed and conifer dominant). Results from the field were confirmed in a greenhouse study in which aspen and subalpine fir were planted in soil cores collected under either dominant aspen or dominant conifer stands and grown in high or low light conditions. Aspen was substantially more sensitive to low light conditions and differences in soil chemistry than subalpine fir, a pattern that was consistent in both studies. For aspen, reduced light and conifer influenced soils significantly reduced height, biomass, photosynthesis and the production of secondary defense compounds that protect against animal and insect herbivores. The effects of conifer soil on reducing growth were significantly greater under high light than low light conditions. Subalpine

fir seedlings were significantly reduced in photosynthesis and biomass and under lower light conditions but showed no differences in physiology or growth across soil types. Unlike aspen, subalpine fir showed the ability to dynamically adjust its root:shoot. This appears to give it better shade tolerance and allows it to maintain growth rates on more nutrient limited conifer soils. Results from this study suggest that as conifers establish and increase in height and basal area within aspen stands, reductions in light and changes in soil chemistry place greater physiological and growth constraints on aspen than subalpine fir, with the likely outcome being, losses in aspen cover under longer fire return intervals.

## Introduction

Quaking aspen (*Populus tremuloides* Michx) is the only major upland deciduous tree species in North America, where it exerts a significant influence on the structure and function of subalpine and boreal forest systems. Aspen ecosystems are characterized as having high biodiversity that support a variety of animal, forbs, grass and shrub species (Debyle, 1985; Hollenbeck and Ripple, 2007). Aspen forest communities have high productivity and structural diversity that creates habitat and forage which is critical for both wildlife and livestock (Stam *et al.*, 2008). There is emerging evidence suggesting that aspen dominated watersheds produce significantly greater water yields than those dominated by conifers (Gifford *et al.*, 1984; LaMalfa and Ryle, 2008), which has important implications for hydrology in the western US.

Recent patterns of aspen decline and dieback suggest that current management strategies and changing environmental conditions may impose constraints on aspen vigor across portions of its western range (Worrall *et al.*, 2008; St Clair *et al.*, 2009). Yet, there are critical knowledge gaps regarding the extent and causes of aspen decline. In western North America, aspen commonly co-occur with conifer species (Smith and Smith, 2005; Strand *et al.*, 2009). Recent studies on fire history in subalpine and boreal forests suggest that both climate conditions (Buechling and Baker, 2004; Beaty and Taylor, 2007) and fire suppression by humans (Gallant *et al.*, 2003; Van Wagner *et al.*, 2006) has lengthened fire return intervals during the last century. There is evidence that the lengthening of fire return intervals through fire suppression has led to increases in conifer dominance that has reduced aspen cover through competitive interactions (Gallant *et al.*, 2003 ; Bergen and Dronova, 2007). Other studies have shown that aspen cover has remained relatively stable during the 20<sup>th</sup> century (Kaye *et al.*, 2003; Kulakowski *et al.*,

2006) with some decline in areas where the dominant pre-disturbance vegetation was conifer (Kulakowski *et al.*, 2004; Kashian *et al.*, 2007). However, it is unclear why there is succession in some areas and not others.

Following fire, aspen regenerates asexually through root suckering (Fraser *et al.*, 2004; Paragi and Haggstrom, 2007). Emerging evidence suggests that establishing aspen stands then facilitate the establishment of conifers seedlings if a conifer seed source is present (Gradowski *et al.*, 2008). As conifers establish and increase in height and basal area within aspen stands, light penetration through the canopy is decreased (Stadt and Lieffers, 2000), and shifts in soil chemistry occur (Bartos and Amacher, 1998). These effects become more pronounced as conifer establishment increases in the absence of disturbance. How these changes differentially affect the regeneration success of aspen and conifers in the understory are factors that are likely to influence the successional trajectory of the future stand.

Aspen is considered shade intolerant relative to conifers (Kobe and Coates, 1997; Wright *et al.*, 1998). Reduction in light availability can also substantially reduce aspen phenolic glycosides and condensed tannins (Hemming and Lindroth, 1999; Osier and Lindroth, 2006). Both of which are major defense compounds produced in aspen leaves (Hwang and Lindroth 1997)

Soil conditions are thought to be an important factor determining the successional status of aspen. It has been hypothesized that certain soil types (hypersaline shales and high clay soils with low infiltration rates) may slow conifer succession by inhibiting conifer seedling establishment and development (Betancourt, 1990). Conifer seedling establishment and survival can be enhanced under aspen dominated stands compared to conifer stands (Shepperd and Jones, 1985; Gradowski *et al.*, 2008). Differences have been observed in soil chemistry under conifer

and aspen stands (Bartos and Amacher, 1998). A recent study shows that the bioavailability of macronutrients (N, P, K and Mg) decrease significantly as aspen stands become seral to conifer (unpublished data). Aspen growth shows sensitivity to reductions in nutrients (Hemming and Lindroth, 1999; DesRochers *et al.*, 2003). Decreases in nutrients (N-P-K) have been shown to stimulate condensed tannin production but have little effect on phenolic glycosides (Donaldson *et al.*, 2006; Osier and Lindroth, 2006). The allocation to secondary metabolites under lower nutrient conditions is associated with decreased growth capacity (Donaldson *et al.*, 2006; Osier and Lindroth, 2006).

The objective of this study was to determine how changes in light and soil chemistry associated with different successional stages in aspen-conifer stands affects the physiology, growth, and defense of aspen suckers and subalpine fir seedlings in the understory. We tested the following hypotheses: 1) aspen shows greater physiological and growth sensitivity to low light conditions than subalpine fir; 2) shifts in soil chemistry that occur during conifer succession differentially affect aspen and subalpine fir physiology and growth rates; and 3) aspen are better defended (high concentrations of leaf defense compounds) under high light conditions and on the more nutrient rich soils that are present in the earlier stages of succession.

## Materials and Methods

### *Greenhouse study*

A split plot experiment was used to test how changes in soil chemistry and light environment affect aspen and subalpine fir regeneration. Light level was the whole plot treatment. High light consisting of 70% full sun light by using 30% shade cloth that hung through the greenhouse (to mimic a pure aspen stand) and low light consisting of 20% full light

from placing the soil cores in shade boxes with 50% shade cloth (to mimic a pure conifer stand). Soils (conifer soil or aspen soil) were the sub-plot treatment and tree species (*Populus tremuloides* or *Abies lasiocarpa*) were the sub-sub-plot treatment. The experiment was replicated four times.

Soil cores in which the aspen and subalpine fir were planted were collected from Telephone Hollow on the Uinta National Forest (40°18'29.67 N, 111°14'35.64 W, elevation 2491 m). Soil cores were collected underneath a pure subalpine fir stand and a pure aspen stand that were adjacent to each other. The soil cores were extracted by driving PVC pipe (10 cm in diameter and 20 cm in length) into the soil and carefully removing them by using a shovel to keep the soil profiles intact. Caps with drainage holes were placed on the bottoms of the cores.

Aspen ramets were grown from root cuttings collected in May of 2007 from a single aspen clone in the vicinity of Telephone Hollow. Aspen root sections ~10 cm in length and approximately 0.5 cm in diameter were placed in vermiculite for 10 days at which point emerging suckers developed. Suckers were excised from the root section using a razor blade and were then dipped in a solution of 0.4% indolebutyric acid (to encourage root initiation) in ethanol for five seconds before being transferred to peat moss plugs. These transplants were then placed in a growth chamber under low light (100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), 80% relative humidity at 20° C. After 10 days when root formation was visible, the roots were carefully washed and the young plants were carefully transferred into the soil cores. At the same time aspen suckers were being transferred to the soil cores, first year subalpine fir seedling were collected at Telephone hollow and planted into the soil cores. The establishing aspen and subalpine fir trees in soil cores were maintained in the growth chamber for another week while root establishment occurred.

On June 20, 2007 the aspen suckers and subalpine fir seedlings in the soil cores were transferred into the greenhouse and the study was initiated. The aspen and subalpine firs were grown for two seasons in a climate controlled greenhouse at Brigham Young University in Provo Utah (40°14'41.32"N, 111°38'56.94"W). The trees were watered using an automated watering system that delivered 300 ml of water three times a week. At the end of the first growing season when the aspen had lost their leaves, the aspen and subalpine fir were moved to a walk in cooler of 2.7° C to maintain dormancy through the winter. Light levels in the cooler were maintained at ~20  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 8.5 hours a day (8:30am – 4pm). They were returned to the greenhouse on May 8, 2008.

In the greenhouse, mean temperature and relative humidity in the 30% shaded blocks (maximum light levels were 1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) during the day was  $25 \pm 0.08$  °C and  $42 \pm 0.23\%$ . In the 80% shaded blocks (max PPFD 350  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) mean temperature and relative humidity during the day was  $24 \pm 0.07$  °C and  $45 \pm 0.2\%$ . During the night mean temperature and relative humidity was uniform between the two light treatments ( $19 \pm 0.08$  °C and  $51 \pm 0.3\%$ ).

### *Field study*

Seven sites spread relatively uniformly across the Fish Lake National Forest in central Utah were selected for the field study (at 38°74'30.38"N, 111°65'40.53"W; 38°48'21.16"N, 112°07'59.96"W; 38°58'85.64"N, 111°67'03.82"W; 38°76'80.71"N, 111°68'54.24"W; 38°69'67.14"N, 111°53'12.40"W; 38°53'95.73"N, 111°68'60.35"W; 38°14'38.66"N, 112°20'51.67"W). Elevations ranged from 2,700m to 3,000m. Sites were selected based on the presence of four adjacent transitions in stand composition: predominantly conifer (>80% conifer

stems), predominantly aspen (>80% aspen stems), equal mix of aspen and conifer (~50% aspen and conifer stems), and a gap that had no overstory influence. Stand composition and density within each transition zone was determined using the point quarter method along a 50 meter transect (Cottam and Curtis, 1956) with a correction from Pollard (1971). An aspen sucker nearest the 15, 30 and 45 m points along the transect that was less than 100 cm in height was selected for measurements. Each aspen sucker was measured for photosynthesis (as described below), height and stem diameter, and leaf samples were collected for lab analysis.

Leaf area index (LAI) was measured using the AccuPAR LP-80 ceptomoter (Decagon Devices, Pullman, Washington) every seven meters along the transect. Two measurements were made at each point and then averaged. Measurements taken above the understory vegetation were used to estimate overstory LAI.

### *Leaf analysis*

Needles and leaves that were collected from the greenhouse and field were stored on dry ice during transport and stored in the lab at -80° C. Aspen leaves were freeze dried to preserve phenolic glycosides. Subalpine fir needles were oven dried at 60° C for 72hrs. Leaf and needle material was homogenized in a Wiley Mill using a #10 screen.

Condensed tannins were quantified for both aspen and subalpine fir. Condensed tannins were extracted from approximately 50 mg of leaf material was placed in 2 ml screw-cap micro-centrifuge tubes suspended in 1ml of 70% acetone-10 mM ascorbic acid solution. The samples were then vortexed on high at 4° C for 20 minutes. The liquid supernatant was then removed and placed in a separate micro-centrifuge container ,and the extraction was then repeated. The concentration of tannins was then quantified spectrophotometrically (SpectraMax Plus 384,

MDS, Toronto, Canada) using the modified butanol-HCL method described in Porter *et al* (Porter *et al.*, 1986) with purified tannin standard isolated from aspen leaves (Hagerman and Butler, 1980).

The phenolic glycosides, salicortin and tremulacin, were extracted from approximately 50 mg of aspen leaf tissue (subalpine fir does not contain significant levels of phenolic glycosides), which was placed in 2 ml screw cap micro-centrifuge tubes and suspended in methanol. The samples were then vortexed on high for 5 minutes. The liquid supernatant was then removed and placed in a separate micro-centrifuge container and the extraction was repeated. Final concentrations of 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, 5µm) 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).

For phosphorus analysis, leaf samples were ashed in a muffle furnace at 495° C for 12 hours, dissolved in 2ml of 100mM HCl, and analyzed spectrophotometrically (SpectraMax Plus 384, MDS, Toronto, Canada) according to the methods of Murphy and Riley (Murphy and Riley, 1962). Nitrogen was measured by placing 50 mg of dry leaf material in a tin capsule and analyzed in a nitrogen analyzer (TruSpec, CN Determinator, LECO Cooperation, St. Joseph, Michigan, USA) using the combustion method (Campbell, 1991).

### *Gas Exchange*

Light response curves were conducted on youngest fully expanded leaf or branch of needles (that filled the entire chamber area) using a gas exchange systems with a blue-red light

source (Li-Cor 6400 and 6400-40, Li-Cor Biosciences, Lincoln, NE, USA) at ambient temperature and humidity. Leaf chamber CO<sub>2</sub> concentrations were controlled at 385ppm using a CO<sub>2</sub> mixer. The light response curve was measured at each of the following light levels: 2000, 1500, 1000, 500, 200, 100, 50, and 0  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . Measurements were initiated by sealing the leaf in the chamber where one leaf or branch per plant was measured for gas exchange. After CO<sub>2</sub> and water vapor concentrations in the leaf chamber reached a steady state (60-90 seconds), rates of photosynthesis were logged and light adjusted to the next PPFD. Light response curves in the greenhouse were taken from 9:45 to 14:30 on July 3 and 4, 2008 and field measurements were taken from July 9-17, 2008.

### *Growth*

The greenhouse study was completed on July 29, 2008. Aboveground plant biomass was clipped at the soil surface, measured for height using measuring tape and then placed in a paper bag. Roots were collected, surfaced rinsed and placed in paper bags. Both shoot and root samples were placed in a drying oven at 60° C for 72h which resulted in complete drying of the tissues. The samples were measured for mass using an analytical balance.

### *Soil Analysis*

Three soil cores (10 x 23 cm) from each treatment were analyzed for pH using a pH meter in a saturated soil paste. Phosphorus extracted with sodium bicarbonate solution and analyzed with the method from Olson, et al (1954). Potassium was also extracted with sodium bicarbonate solution and analyzed according to the method from Schoenau and Karamonos (Schoenau and Karamonos, 1993). Calcium and magnesium were analyzed from a saturated

extract that was red using an atomic absorption spectrometer (SpectraMax Plus 384, MDS, Toronto, Canada). Iron was extracted with DTPH and determined using inductively coupled plasma spectroscopy (Iris Intrepid II XSP, Thermo Electron Cooperation, Waltham, MA, USA) (Dahlquist and Knoll 1978). For the determination of soil nitrogen, 50 mg of dry soil was placed in a tin capsule and analyzed in a nitrogen analyzer (TruSpec, CN Determinator, LECO Cooperation, St. Joseph, Michigan, USA).

### *Statistical Analysis*

Measurements of growth, photosynthesis (at the 2000  $\mu\text{mol m}^{-2}\text{s}^{-1}$  light point), foliar chemistry and soil chemistry were tested for differences using analysis of variance (ANOVA). Mean comparisons among treatment groups were determined using a Tukey adjusted t-test. Homogeneity of variance and normality were examined using Shapiro-Wilk W statistics and equal variance tests. Data that did not meet the assumptions for the parametric tests were transformed using Box-Cox transformations. From the field we transformed condensed tannins ( $\lambda = -0.2$ ), nitrogen ( $\lambda = -0.8$ ) and phosphorus ( $\lambda = -0.2$ ). From the greenhouse we transformed aspen biomass ( $\lambda = \log$ ), condensed tannins ( $\lambda = -0.4$ ), phosphorus ( $\lambda = -1.2$ ) and leaf nitrogen ( $\lambda = 1$ ). Specific leaf area data from the greenhouse was unable to meet the parametric assumption so a Wilcoxon rank test was run. The value reported in table 2 is a chi-square value. Statistical analysis was performed using JMP version 7 statistical software (SAS Institute, Cary, NC, USA).

## Results

### *Soils*

Aspen soil had significantly greater N, Ca and Mg while the conifer soil had significantly greater P and Fe (table 1). There were no significant differences in soil pH, potassium or bulk density between the two soil types.

#### *Light response curves*

Significant differences in treatment effects on photosynthesis for both species occurred in the light saturating portion of the light response curves (figures 1 and 2). Aspen and subalpine fir grown under higher light had significantly greater rates of photosynthesis than those grown in lower light conditions. Aspen had significantly higher rates of photosynthesis when grown on aspen soils (figure 1). In contrast, rates of photosynthesis of subalpine fir seedlings were not significantly affected by soil type. In the field study, photosynthesis rates were significantly greater in aspen suckers growing in gaps than suckers growing under conifer, mixed or aspen canopy types (figure 2).

#### *Growth (greenhouse experiment)*

In the high light treatment, aspen had significantly greater height and biomass when growing on aspen soil. In contrast, there was no significant soil effect on aspen growth responses under the low light treatment (figure 3). Neither light nor soil treatments significantly influenced the root:shoot ratio in aspen (figure 4).

For subalpine fir in the greenhouse, high light conditions resulted in significantly greater biomass but no difference in height (figure 3). The only measure of growth significantly influenced by soil conditions was an increase in the root:shoot in subalpine fir seedlings growing on conifer soil (figure 4).

### *Leaf nutrients and morphology*

Aspen in the greenhouse study had higher leaf nitrogen concentration when grown under low light conditions with no significant effect of soil (table 2). Foliar P concentrations of aspen were affected by soil treatments in low light, where low light and conifer soil resulted in significantly greater P concentrations (table 2). In the field, aspen N concentrations were greater under mixed and conifer stands than aspen stands and gaps (Table 3), while P concentrations significantly lower in the gap compared to mixed and conifer stands (table 3).

For subalpine fir seedlings light and soil factors in the greenhouse study had no significant effects on foliar N levels (table 2). Foliar P levels were significantly greater in subalpine fir seedlings grown on conifer soils.

Aspen's specific leaf area was found to be significantly higher (thinner leaves) under shade treatment in the greenhouse (table 2). In the field, leaves became thinner as conifer density increased (table 3).

LAI did not have any significant difference between the canopies.

### *Foliar Defense Chemistry*

In the greenhouse study, both phenolic glycosides and condensed tannins concentrations in aspen were significantly greater under high light conditions (table 2). Soil type had no significant influence on tannins levels in aspen leaves. However, conifer soils did appear to reduce phenolic glycoside concentrations in aspen under high light conditions but not low light conditions as indicated by the significant interaction term (table 2). Tannins levels in subalpine fir seedlings showed a significant difference among treatments but significant differences were

not detected by the Tukey adjusted t-test (table 2). In the field, phenolic glycosides and condensed tannins were significantly higher in aspen suckers growing in gaps than either mixed or conifer stands (table 3).

## Discussion

The differences in light conditions and soil chemistry in our study differentially influenced the physiology and growth of subalpine fir and aspen. This finding is consistent with literature that has documented differences in the response of conifers and deciduous tree species to light availability and soil chemistry (Wright *et al.*, 1998; Hemming and Lindroth, 1999; Messier *et al.*, 1999; Wittmann *et al.*, 2001). In general, these data supported our hypotheses that aspen would 1) show greater physiological and growth sensitivity to low light conditions than subalpine fir; 2) shifts in soil chemistry that occur during conifer succession differentially affect aspen and subalpine fir physiology and growth rates; and 3) aspen are better defended (high concentrations of leaf defense compounds) under high light conditions and more nutrient rich soils. These hypotheses were supported as aspen showed greater sensitivity to reductions in light levels and shifts in soil chemistry that occur as forest canopies are increasingly dominated by conifers.

### *Light effects on growth*

Conifer establishment in aspen stands decreases light penetration to the forest floor (Stadt and Lieffers, 2000). When we simulated that change in the greenhouse, aspen had reduced rates of photosynthesis (figure 1) and nearly an 80% decrease in total biomass (figure 3). Previous studies had shown that aspen show a decline of growth with decreasing light levels (e.g.

(Hemming and Lindroth, 1999; Osier and Lindroth, 2006) and that aspen grown under low light conditions will have lower photosynthetic saturation points (Wright *et al.*, 1998; Wittmann *et al.*, 2001). Structurally, observed differences in photosynthesis may be partially driven by changes in leaf thickness with changing light conditions. Aspen grown in high light had lower SLA (thicker leaves) than those grown in low light. Thicker leaves represent greater amounts of photosynthetic machinery per unit leaf area and thus higher photosynthetic capacity at saturating light levels (Taiz and Zeiger, 2006). Subalpine fir also showed reductions in photosynthesis and biomass with decreasing light availability but they were not as severe as seen in aspen (Figures 1).

Aspen height growth was drastically reduced under lower light conditions and subalpine fir height growth was not (Figure 3). The lack of difference in subalpine fir is consistent with trends in shade tolerant species, as they generally alter lateral growth over height under decreasing light (Parent and Messier, 1995).

#### *Light effects on primary and secondary metabolism*

The reduction of aspen foliar N and P observed in aspen leaves under high light conditions is consistent with nutrient dilution in tissues experiencing increased growth rates (Roumet and Roy, 1999). Reductions of tannins under lower light (table 2) have been observed in studies of aspen defense chemistry (Osier and Lindroth, 2006). A novel result was that light reduction also significantly reduced phenolic glycoside levels. It has generally been thought that genotype most strongly influences the production of phenolic glycosides, and that light and other environmental factors have a much smaller effect (Osier and Lindroth, 2006).

### *Soil and light effects*

For aspen, light appears to have the strongest effect in constraining primary and secondary metabolism. In the greenhouse, when light is not limiting, soil effects are manifested in reduced photosynthesis, height, and biomass (figure 1 and 3). The significant interaction of light and soil was defined by significant soil effects under high light but not low light conditions. In the field these conditions of high light and conifer influenced soils will take place under canopy gaps within conifer stands. Our measurement of LAI shows that we found no significant differences between canopies in light availability (figure 5). This appears to be the result of gaps in the canopy, as seen by the large error bars (figure 5). In the absence of fire, it is predicted that canopy gaps such as these will form and expand (Hill *et al.*, 2005). In these gaps aspen may be regenerating at full sunlight but they will continue to be impaired by the effects of the conifer influenced soils that we observed in the greenhouse.

When subalpine fir are grown on conifer soil they increase the root:shoot ratio (figure 4) and this is correlated with its maintenance of biomass across soil types (figure 3). Aspen however, show no change across soil types and this is correlated with a decrease in biomass (figure 3). As decreased root mass per unit soil area is correlated with decreased nutrient uptake (Aerts and Chapin, 2000), we hypothesize that this adjustment in root:shoot ratio allows subalpine fir to acquire sufficient limiting nutrients on the conifer soil to maintain growth (figure 1, 3 and 4).

### *Ecological Implications*

Aspen's decreased height growth on conifer soils would result in greater time required for aspen to extend into the overstory to compete for full sunlight. This is critical to aspen's

regeneration success as it is shade intolerant relative to conifers (Kobe and Coates, 1997; Wright *et al.*, 1998). The ability of ramets to extend higher into the canopy is perhaps the most significant need for aspen success in the presence of conifers. Studies examining aspen dieback have found that lack of recruitment and poor regeneration commonly occur in areas of intense browsing pressure (Kaye *et al.*, 2005; Strand *et al.*, 2009). As browsing in aspen increases so does succession to conifers (Kashian *et al.*, 2007; Strand *et al.*, 2009). For aspen to be unaffected by browsing they would need to extend above the browse line but this will occur more slowly under shifts in light and soils conditions that occur as conifers expand into aspen forests.

Reductions in light also reduced key defense compounds in aspen which further compounds the herbivory problem. Condensed tannins are commonly thought of as an anti-herbivory compound but their effect in aspen systems is unknown as they have not shown any effect on aspen adapted herbivores (Bryant *et al.*, 1987; Ayres *et al.*, 1997; Hwang and Lindroth, 1998; Donaldson and Lindroth, 2007). Phenolic glycosides, however, have shown significant biological activity against aspen adapted herbivores (Hwang and Lindroth, 1998; Osier and Lindroth, 2004; Donaldson and Lindroth, 2007) and elk preferential consume aspen that has lower concentrations of phenolic glycosides (Wooley *et al.*, 2008). Recent studies indicate that the reductions in phenolic glycosides from 22-24% to 16-17% that we observed in response to light reduction in this study will increase aspen susceptibility to insect and mammal defoliation considerably (Donaldson and Lindroth, 2007; Wooley *et al.*, 2008).

These two factors of reduced height and reduced defense give further insight into the mechanisms driving succession that have been observed with ungulate herbivory (Kashian *et al.*, 2007; Strand *et al.*, 2009). Aspen with lower defense chemistry and lower growth rates are more

likely to be consumed by herbivores, thus preventing aspen recruitment into the overstory and maintenance of the stand.

## Tables and figures

**Table 1.** Analysis of soils used in greenhouse study

	pH	N (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Bulk Density
Aspen	5.6 ± 0.12	0.33 ± 0.02	11 ± 0.8	372 ± 21	244 ± 28	40 ± 5.4	74 ± 7.9	1.04 ± 0.01
Conifer	5.7 ± 0.07	0.13 ± 0.01	60 ± 2.2	254 ± 51	121 ± 19	19 ± 3.5	121 ± 13	1.11 ± 0.05
<i>P</i> -value	0.4	<0.01	0.04	0.09	0.02	0.03	0.04	0.33

*P* < 0.05 indicates a statistically significant difference in soil chemistry.

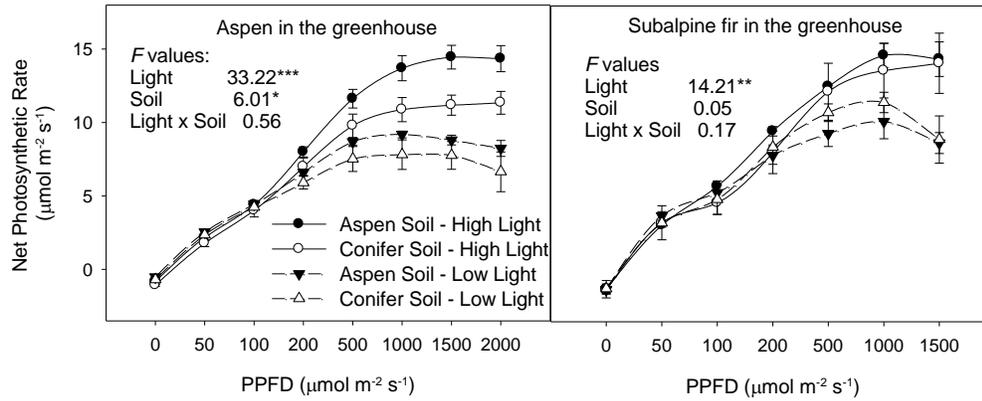


Figure 1. Photosynthetic light response curves of aspen suckers and subalpine fir seedlings from the greenhouse study with means and standard errors presented. In the greenhouse “high light” was 70% of full sunlight and “low light” was 20% of full sunlight which is representative of light conditions underneath a pure aspen and pure conifer stand. An ANOVA of the means from the last light point on Aspen returned a  $p$ -value of  $<0.001$  and subalpine fir returned a  $p$ -value of 0.03. Significance denoted by \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ .

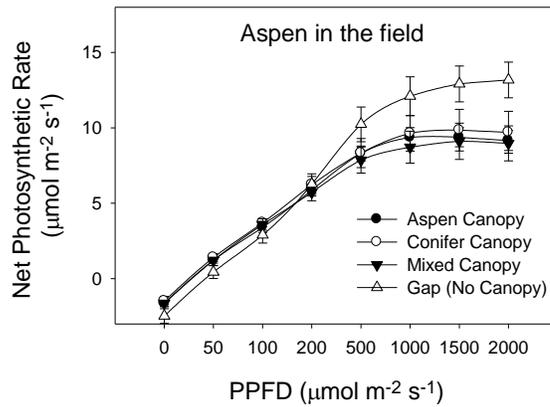


Figure 2. Photosynthetic light response curves of aspen suckers from the field study with means and standard errors presented. In the field we performed light response curves on aspen suckers underneath aspen dominant stands (>80% aspen), mixed aspen and conifer stands (50/50 of each), conifer dominant stands (>80% conifer) and gaps with no overstory influence. An ANOVA of the means from the last light point returned  $p=0.04$ .

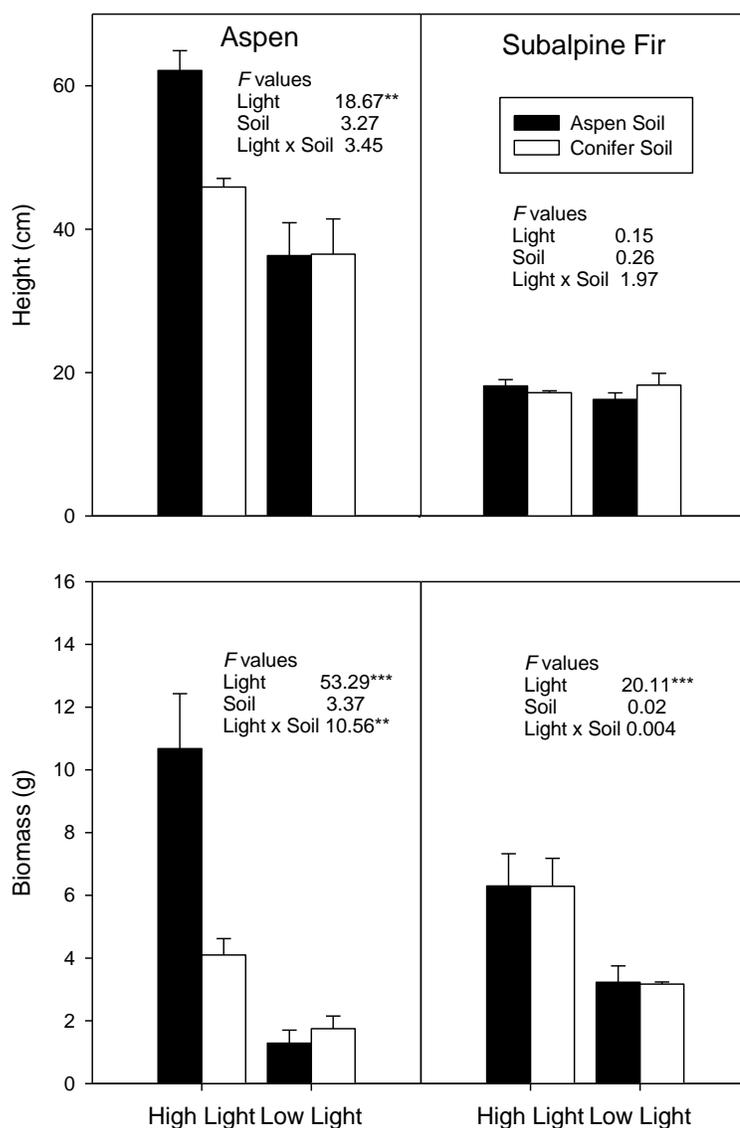


Figure 3. Means and standard errors for growth responses of aspen and subalpine fir grown in the greenhouse under contrasting soil light and soil conditions. Subalpine fir showed reduce biomass and height under lower light conditions but was not sensitive to soil type. Aspen also had reduced growth responses under lower light conditions and had reduced growth on conifer soils but only under high light conditions. Significance denoted by \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ .

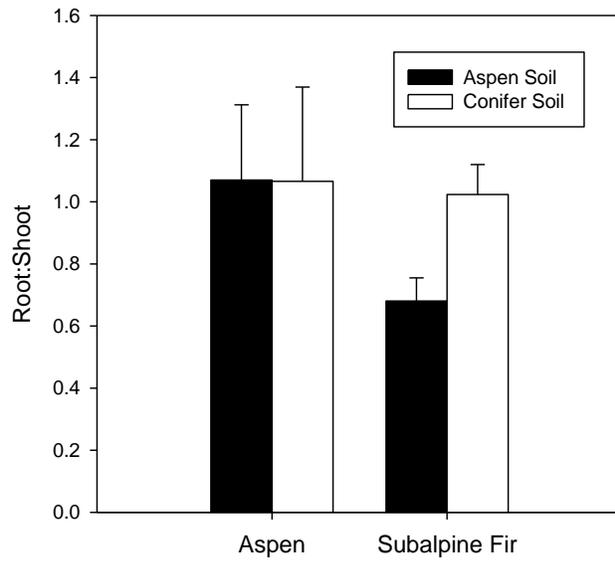


Figure 4. The means and standard error of root to shoot ratios of aspen and subalpine fir grown on contrasting soils in 80% full sunlight. There was no significant response in aspen but subalpine fir showed a significantly different allocation of resources.

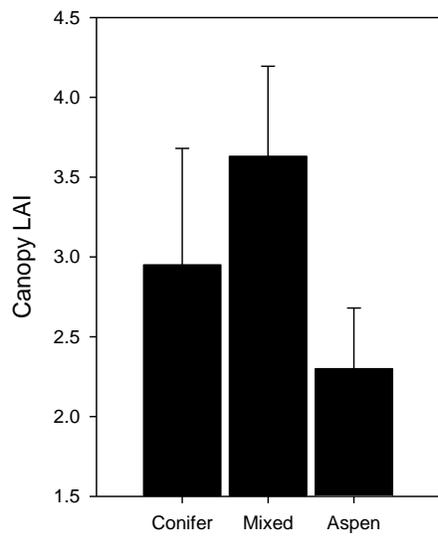


Figure 5 . Mean and standard error of leaf area index values (a measure of canopy light interception) in pure aspen and conifer stands (>80%) or mixed aspen-conifer stands (50% of each). Note the substantial variation in means as denoted by the large error bars. This was largely a function of large numbers of canopy gaps.

Table 2. The effects of light and soil on foliar chemistry and structure. Means presented with standard error.

Differences within species are denoted by superscripts. Significant differences presented as \* P <0.05 \*\* P <0.01

\*\*\* P <0.001

**Table 2.** Greenhouse foliar analyses

Treatment	Nitrogen (%)	Phosphorus (mg/g)	SLA cm <sup>2</sup> g <sup>-1</sup>	Phenolics (% dry mass)	Tannins (% dry mass)
<b>Aspen</b>					
High Light					
Aspen soil	0.94 ± 0.13 <sup>b</sup>	1.16 ± 0.03 <sup>b</sup>	137.16 ± 5.92	24.05 ± 0.73 <sup>a</sup>	2.42 ± 0.81 <sup>a</sup>
Conifer soil	0.92 ± 0.11 <sup>b</sup>	1.41 ± 0.17 <sup>ab</sup>	149.10 ± 3.37	21.78 ± 1.65 <sup>ab</sup>	1.91 ± 0.70 <sup>a</sup>
Low Light					
Aspen soil	1.99 ± 0.11 <sup>a</sup>	1.64 ± 0.14 <sup>ab</sup>	303.81 ± 8.07	16.27 ± 1.39 <sup>b</sup>	0.34 ± 0.05 <sup>b</sup>
Conifer soil	1.96 ± 0.04 <sup>a</sup>	2.02 ± 0.46 <sup>a</sup>	372.89 ± 32.12	17.86 ± 1.85 <sup>ab</sup>	0.29 ± 0.04 <sup>b</sup>
<i>F</i> -values					
Light	105.66 <sup>***</sup>	19.82 <sup>**</sup>	NA	50.21 <sup>***</sup>	41.44 <sup>***</sup>
Soil	0.1051	6.31 <sup>*</sup>	NA	0.04	1.03
Light x Soil	0.0031	0.38	NA	4.59 <sup>*</sup>	0.09
<b>Subalpine fir</b>					
High Light					
Aspen soil	1.22 ± 0.07	1.48 ± 0.11 <sup>b</sup>	NA	NA	8.13 ± 0.28 <sup>a</sup>
Conifer soil	1.25 ± 0.07	1.85 ± 0.11 <sup>ab</sup>	NA	NA	7.57 ± 0.23 <sup>a</sup>
Low Light					
Aspen soil	1.07 ± 0.07	1.43 ± 0.12 <sup>b</sup>	NA	NA	9.52 ± 0.54 <sup>a</sup>
Conifer soil	1.24 ± 0.11	2.12 ± 0.22 <sup>a</sup>	NA	NA	9.20 ± 1.02 <sup>a</sup>
<i>F</i> -values					
Light	1.03	0.62	NA	NA	6.16 <sup>*</sup>
Soil	1.31	12.96 <sup>**</sup>	NA	NA	0.52
Light x Soil	0.78	1.18	NA	NA	0.03

**Table 3.** Field foliar analyses for aspen

Treatment	Nitrogen (%)	Phosphorus (mg/g)	SLA cm <sup>2</sup> g <sup>-1</sup>	Phenolics (% dry mass)	Tannins (% dry mass)
Aspen overstory	1.62 ± 0.14 <sup>bc</sup>	3.16 ± 0.18 <sup>a</sup>	159.47 ± 6.09 <sup>b</sup>	21.52 ± 4.50 <sup>ab</sup>	1.41 ± 0.44 <sup>a</sup>
Mixed aspen/conifer	2.31 ± 0.22 <sup>a</sup>	3.78 ± 0.34 <sup>a</sup>	195.51 ± 10.68 <sup>a</sup>	18.55 ± 1.51 <sup>ab</sup>	0.46 ± 0.22 <sup>b</sup>
Conifer overstory	2.3 ± 0.42 <sup>ab</sup>	3.79 ± 0.57 <sup>a</sup>	204.08 ± 7.60 <sup>a</sup>	16.00 ± 1.91 <sup>b</sup>	0.41 ± 0.25 <sup>b</sup>
No overstory	1.53 ± 0.14 <sup>c</sup>	2.37 ± 0.31 <sup>b</sup>	125.46 ± 4.67 <sup>c</sup>	24.05 ± 2.52 <sup>a</sup>	3.42 ± 1.44 <sup>a</sup>
<i>F-value (ANOVA)</i>	3.21*	4.74**	20.71***	4.64*	8.61***

Means presented with standard error. Significant differences presented as \* P <0.05 \*\* P <0.01 \*\*\* P <0.001

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Aspen and subalpine fir regeneration success and mortality along successional gradients in aspen-conifer forests

W. John Calder<sup>1</sup>, Eric Smith<sup>1</sup>, Loreen Allphin<sup>1</sup> & Samuel B. St. Clair<sup>1</sup>

<sup>1</sup>Department of Plant and Wildlife Sciences, Brigham Young University, Provo, Utah

## Abstract

Quaking aspen has been in decline and succession to conifers in much of the West while maintaining dominance in regions of Colorado. This decline has been attributed to the increase of fire suppression that historically provided the disturbance to initiate aspen suckering. We studied an area in south-central Utah where there has been some evidence of aspen decline with the objective of characterizing patterns of establishment and trends of overstory mortality through the various stages of forest establishment and succession including: canopy gaps, aspen dominated canopies and finally conifer dominated canopies. As overstory conifer density increases, aspen decrease in density, basal area, and regeneration success. A facilitation effect was observed in which conifer seedlings were shown to have higher establishment rates next to mature aspen trees particularly as the canopy increased in conifer density. Mature aspen that had a younger maturing conifer tree within 1 meter of its base had significantly higher mortality rates. This suggests that as conifer seedlings that establish next to aspen trees mature they compete with the aspen tree for necessary resources.

Keywords: Aspen; *Populus tremuloides*; Aspen Decline

## Introduction

In its western range, aspen (*Populus tremuloides*) typically regenerate clonally through root suckering following disturbances (often fire) that leads to mortality of the overstory canopy (Fraser *et al.*, 2004; Paragi and Haggstrom, 2007). In western North America, aspen commonly co-occur with conifer species (Smith and Smith, 2005; Strand *et al.*, 2009). Recent studies on fire history in subalpine and boreal forests of western North America, suggest that both climate conditions (Buechling and Baker, 2004; Beaty and Taylor, 2007) and fire suppression by humans (Gallant *et al.*, 2003; Van Wagner *et al.*, 2006) has lengthened fire return intervals during the 20th century. Lengthening of fire return intervals likely increases conifer dominance that has reduced aspen cover through competitive interactions (Gallant *et al.*, 2003; Bergen and Dronova, 2007)

Although aspen decline and succession has been reported, it is not ubiquitous throughout the West. In Colorado and Montana, numerous studies have found that aspen cover is within historical limits (Kaye *et al.*, 2003; Kulakowski *et al.*, 2006). Manier and Laven (Manier and Laven, 2002) found on portions of the western slopes of the Rocky Mountains that conifers have been displacing aspen over the last century, but this has not led to a change in total aspen cover as losses to conifers are offset by gains in shrublands (Manier and Laven, 2002).

The conifers associated with aspen are considered shade tolerant relative to aspen (Kobe and Coates, 1997; Wright *et al.*, 1998). In its western range aspen have been thought of as a nurse crop with conifers establishing more successfully under aspen stands (Shepperd and Jones, 1985; Gradowski *et al.*, 2008). Manier and Laven (Manier and Laven, 2002) suggested that aspen may facilitate conifer expansion by being the primary overstory establisher in rangelands.

Our initial observations in Utah's aspen-subalpine fir communities suggest that subalpine fir (*Abies lasiocarpa*) establishment occurs with greater frequency under forest stands with a significant aspen component. The central objective of this study was to characterize how overstory composition at different successional stages influences aspen and subalpine fir regeneration success and survivorship of overstory trees as the stand matures. We tested the following hypotheses: 1) overstory aspen facilitates aspen and subalpine fir regeneration; 2) conifer establishment negatively affects aspen abundance and vigor as measured by reductions in aspen regeneration, overstory density and mortality.

## Materials and Methods

### *Field sites*

Seven sites that provided good coverage across the Fish Lake National Forest in central Utah were selected for the field study (at 38°74'30.38"N, 111°65'40.53"W; 38°48'21.16"N, 112°07'59.96"W; 38°58'85.64"N, 111°67'03.82"W; 38°76'80.71"N, 111°68'54.24"W; 38°69'67.14"N, 111°53'12.40"W; 38°53'95.73"N, 111°68'60.35"W; 38°14'38.66"N, 112°20'51.67"W). Elevations ranged from 2,700m to 3,000m. Site selection was based on the presence of a continuous overstory stand with four distinct stand compositions: predominantly conifer (>80% conifer stems), equal mix of aspen and conifer (~50% aspen and conifer stems), predominantly aspen (>80% aspen stems), and a canopy gap without any direct overstory influence. Estimates of stand composition to meet the above criteria were estimated visually and then confirmed as described below.

### *Field measurements*

We used the point quarter (Cottam and Curtis, 1956) and distance to nearest neighbor methods to assess stand densities along a fifty meter transect within each of the zones with measurement points every seven meters along the transect. From the tree measured in each quarter we measured the distance to the nearest overstory neighbor, understory aspen, and understory conifer. Tree species, bole diameter at breast height (DBH) and survivorship were recorded. To estimate densities from the point quarter and nearest neighbor, we used the estimate modified from Pollard (Pollard, 1971):

$$N_p = (4(4n-1)) / (\pi \sum r^2_{ij})$$

Where  $N_p$  is the estimated density,  $n$  is the number of points along a transect, and  $\sum r^2_{ij}$  is the sum of the squared distances from each measurement. The two overstory distance measurements were then combined to determine density according to Diggle (Diggle, 1975):

$$N_p = \sqrt{N_{pa} * N_{pb}}$$

Where  $N_{px}$  is the density estimate as calculated above from point to nearest plant and plant to nearest neighbor.

Regeneration density of aspen and subalpine fir was measured using a one meter squared quadrat placed along both sides of the transect every seven meters.

### *Statistical analysis*

Individual transects were treated as sampling clusters with densities averaged between each transect. Either a Kruskal-Wallis Z-test with Bonferonni correction or ANOVA was run to compare the means of seedling densities, basal area, and mature stem densities. The Kruskal-Wallis Z-test was used for multiple comparisons on data that did not meet the parametric assumptions of an ANOVA test. If the data met parametric assumptions then pairwise comparisons with a Tukey adjusted t-test was run to see the differences among means from the ANOVA test. Data that was not parametric but responded to transformations was transformed with a box-cox transformation. Those transformed were, aspen regeneration density ( $\lambda = -0.04$ ), distance to understory aspen ( $\lambda = 0.4$ ) and distance to understory conifer ( $\lambda = 0.2$ ) (table 1). Homogeneity of variance and normality was tested using Shapiro-Wilk W statistics and equal variance tests. A chi-square test was used to compare the differences in aspen and subalpine fir mortality within 1>m of the other overstory tree. Statistical analysis was run on JMP version 8 statistical software (SAS Institute, Cary, NC, USA) and NCSS version 7.1.4 (NCSS, LLC., Kaysville, Ut, USA).

## Results

Aspen regeneration was not statistically different across the four zones (figure 1). As the canopy transitioned from aspen to conifer, overstory aspen density and basal area decrease significantly (table 1). Subalpine fir regeneration increased significantly ( $p$ -value <0.01) from the gap to the mixed and conifer stands (figure 1). Though it appears that there is a difference between the gaps and aspen canopy, the statistics were not parametric and a Kruskal-Wallis z-test with Bonferonni correction was unable to detect any differences.

Subalpine fir basal area and density significantly increased from aspen to mixed stand, with no significant difference between the mixed and conifer stands (table 1). Conifer densities increased significantly in the transition from aspen to conifer stand, while basal area did not. In comparing basal area and stem densities from the mixed stands for all species there was no significant difference (table 1).

In comparing the distance from an overstory tree to nearest understory conifer or aspen we found that conifer seedlings establish closer to aspen than conifers (figure 2). The effects of species and stand and their interaction had a significant effect on the distance of an understory conifer to a mature aspen (figure 2). Comparing the distance from a mature tree to the nearest understory aspen we found a significant effect from stands and species (figure 2). Aspen regeneration occurred on average further from a conifer than an overstory aspen and as the stand increased in conifer cover the distance to both overstory species increased (figure 2).

Associated with the decline of aspen was significantly increased mortality with aspen < 1 m away from a subalpine fir. In aspen dominated stands 4% of the aspens sampled were dead, while 17% of aspen that had a subalpine fir < 1 m away were dead (table 1). In mixed stands there was no significant increase of mortality with 13% aspen mortality while 20% of aspen within >1m of a subalpine fir were dead. In the conifer stands aspen mortality was the greatest with 38% aspen mortality and 66% dead within <1m of a subalpine fir (table 1).

We also examined the mortality of subalpine firs that were associated within < 1m of an aspen. In the aspen stand no conifer mortality was observed. In the mixed stand 9% were dead with 18% dead when they were within <1m of an aspen, though this was not significant (table 1). In the conifer stand there was significantly greater mortality with 15% mortality in general and 34% mortality when growing within <1m of an aspen (table 1).

## Discussion

As conifer increasingly dominated the stand we observed decreases in aspen, basal area and density (figure 1, table 1). In that transition, subalpine fir basal area and density increased (figure 1, table 1) and conifers seedlings were shown to establish in closer proximity to mature aspen trees (figure 1, table 1). The pattern of conifers establishing close to or under aspen has been suggested before with aspen as a nurse crop (Shepperd and Jones, 1985). Our study showed that as overstory conifer densities increased, the distance from a conifer seedling to a mature aspen narrowed (figure 2). This relationship has not been shown before and the mechanism or possible benefit of this interaction is unknown. Others have found that white spruce (*Picea glauca*) establish more readily under aspen canopies than conifer canopies (Gradowski *et al.*, 2008). White pines (*Pinus strobes*) have been found to have greater than expected growth when in the proximity of aspen (Peterson and Squiers, 1995).

There are different hypotheses for why conifers would establish more readily in aspen stands, such as increased soil moisture, buffering of climate extremes (Shepperd and Jones, 1985). Callaway and Walker (Callaway and Walker, 1997) reviewed the literature on competition and facilitation and noted that many species are shown to be nurse plants for competitors. Initially the establishment under a mature tree does not appear to harm the nurse plant, but as the beneficent plant matures, competition leads to increased mortality of the nurse plant (Callaway and Walker, 1997).

Our study found increased aspen mortality with increasing conifer composition and when found in close proximity to sizeable conifer trees (table 1). Most studies examining aspen mortality have done so looking at browsing pressure. Those that have looked at aspen mortality

as a result of increased conifer density have not found any significant increase (Kaye *et al.*, 2005). We found increased mortality of aspen within < 1m of subalpine fir (table 1). This relationship appears to have a particularly negative effect on aspen in the later stages of succession. As the canopy became dominated by conifers, subalpine firs < 1 m from an aspen also experienced increased mortality (table 1). These results suggests that the nursing relationship is only beneficial to subalpine firs and has the greatest positive effects in the early stages of succession.

In the case of aspen acting as a nurse crop for the establishment of subalpine fir, it may not be a simple case of competition and succession but both may be benefiting by this relationship. In western North America, aspen commonly co-occur with conifer species (Strand *et al.* 2009). The successional processes that determine whether aspen maintains dominance in the forest or eventually is replaced by conifers are poorly understood. Short fire return intervals (40-80 years) remove conifers and triggers aspen regeneration resulting in young aspen stands that remain vigorous and healthy. The paradox is that aspen generally regenerate through root suckering after a disturbance (Fraser *et al.*, 2004; Paragi and Haggstrom, 2007) but aspen are resistant to burning that would initiate suckering (Brown and Simmerman, 1986). Aspen is therefore dependent on the presence of more flammable conifers to produce a mixed stand that is susceptible to burning (Cumming 2001). The establishment of conifers throughout the canopy allows fire to carry both horizontally and vertically through the stand leading to removal of the crown that releases the clone from apical dominance that leads to widespread suckering (Fraser *et al.*, 2004, Brooks, 2009) .

Changing climate scenarios are also likely to have an impact on aspen regeneration through fire. In aspen's western range, the climate is projected to change to increased droughts

and overall warmer and dryer conditions (ICPP, 2007). A dryer climate will shorten fire intervals and result in more intense and extensive fires. These greater intensity fires stimulate aspen regeneration more than low intensity fires (Fraser *et al.*, 2004; Keyser *et al.*, 2005) which may then lead to gains in aspen cover. Though it remains to be seen how aspen will persist in dryer conditions.

## Tables and figures

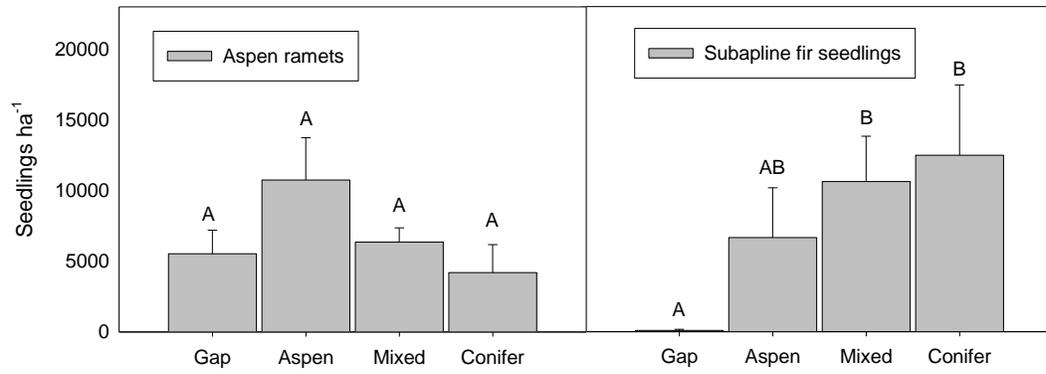


Figure 1. Seedling densities from different stands of overstory composition. Significant differences are denoted by different letters.

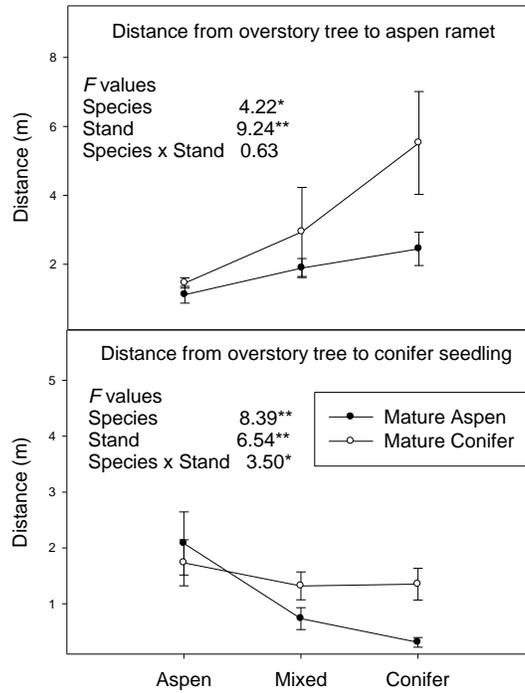


Figure 2. Comparisons of the distance from an overstory tree to the nearest understory tree by stand type. P-value <0.05\*, <0.01\*\*.

Table 1. Basal area, density, and % dead of mature trees across different stands. Significant differences within species are denoted by different superscripts. % dead shows those dead within 1m of heterospecific, i.e. aspen within >1m of subalpine fir and subalpine fir within >1m of an aspen. Significance denoted by: \*  $p$ -value <0.05.

Table 1. Basal area, stem and seedling densities of aspen and conifer species by stand

Stand	Species	Basal Area (m <sup>2</sup> ha <sup>-1</sup> )	Stems (ha <sup>-1</sup> )	% Dead within 1 m of opposing species
Aspen	Aspen	48.9 ± 7.8 <sup>a</sup>	2023 ± 395 <sup>a</sup>	17% (c <sup>2</sup> 4.81*)
Mixed	Aspen	36.6 ± 5.6 <sup>ab</sup>	1432 ± 173 <sup>a</sup>	20% (c <sup>2</sup> 1.48)
Conifer	Aspen	16.7 ± 8.3 <sup>b</sup>	492 ± 199 <sup>b</sup>	66% (c <sup>2</sup> 5.78*)
	$p$ -value	<0.05*	<0.01**	
Aspen	Subalpine fir	3.4 ± 1.8 <sup>b</sup>	170 ± 58 <sup>b</sup>	0%
Mixed	Subalpine fir	23.9 ± 3.3 <sup>a</sup>	942 ± 109 <sup>a</sup>	10% (c <sup>2</sup> 1.65)
Conifer	Subalpine fir	24.7 ± 6.2 <sup>a</sup>	1042 ± 239 <sup>a</sup>	34% (c <sup>2</sup> 7.25*)
	$p$ -value	<0.001***	<0.01**	
Aspen	Other conifers	5.3 ± 2.7 <sup>a</sup>	35 ± 19 <sup>b</sup>	NA
Mixed	Other conifers	15.8 ± 4.0 <sup>a</sup>	432 ± 146 <sup>ab</sup>	NA
Conifer	Other conifers	17.9 ± 4.3 <sup>a</sup>	444 ± 110 <sup>a</sup>	NA
	$p$ -value	0.17	<0.01**	

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## Physiological effects of smoke exposure on deciduous and conifer tree species

William J. Calder<sup>1</sup>, Greg Liffert<sup>1</sup>, Max Moritz<sup>2</sup>, Samuel B. St.Clair<sup>1</sup>

<sup>1</sup>Department of Plant and Wildlife Sciences, Brigham Young University, Provo, UT

<sup>2</sup>Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA

### Abstract

Smoke from forest fires can persist in the environment for months and while there is a substantial amount of literature examining the effects of smoke exposure on seed germination, the effects of smoke on leaf function are nearly uninvestigated. There is evidence that smoke limits pathogens and we hypothesize that plants may alter their defense chemistry strategy in response to fire and that smoke may be an important cue. The objective of this study was to compare growth and primary and secondary metabolic responses of deciduous and conifer tree species to short smoke exposure. Twenty minutes of smoke exposure resulted in a greater than 50% reduction in photosynthetic capacity in five of the six species we examined. Impairment of photosynthesis in response to smoke was a function of reductions in stomatal conductance and biochemical limitations. In general, deciduous species showed greater sensitivity than conifer species. Smoke had no significant affect on growth or secondary defense compound production in any of the tree species examined.

Keywords: Smoke; fire suppression hypothesis; secondary defense

## Introduction

Fire has shaped terrestrial plant communities for the last 350 million years (Agee, 1993). With each ignition of wildfire large amounts of smoke are produced that can persist in the environment for months (Radojevic, 2003). Nearly all of the studies that examine the effects of smoke on plant growth have been tied to seedling germination, e.g. (Daws *et al.*, 2007). While much work has been done on the effects of smoke exposure inducing seed germination, little is known about how smoke influences primary and secondary metabolism. A better understanding of the physiological responses of plants to smoke is pertinent as longer growing seasons and increased drought frequency and duration projected from climate change are expected to result in an increase in wildfires (ICPP, 2007).

Davies and Unam (Davies and Unam, 1999) studied the effects of forest fires in Indonesia on photosynthesis and found that despite increases in CO<sub>2</sub> from the fires, photosynthetic rates were lowered. Gilbert and Ripley (Gilbert and Ripley, 2002) showed that smoke exposure reduced stomatal conductance, CO<sub>2</sub> assimilation rate, and intercellular CO<sub>2</sub>. Smoke could decrease photosynthesis through high temperatures (Daie and Campbell, 1981), increasing vapor pressure deficits (Guehl and Aussenac, 1987), or decreased light availability (Davies and Unam, 1999). In addition, there are compounds in smoke that are physiologically active, e.g. NO<sub>2</sub> (Keeley and Fotheringham, 1997), CO<sub>2</sub>, SO<sub>2</sub>, and O<sub>3</sub> (Robinson *et al.*, 1998). O<sub>3</sub> has been linked to the destruction of chlorophyll (Peiser and Yang, 1977) and has also been shown to inhibit the K<sup>+</sup> ion channel that regulates guard cell function, resulting in an inhibition of stomatal opening (Torsethaugen *et al.*, 1999). SO<sub>2</sub> causes reductions in stomatal conductance, strongly inhibits photosynthetic oxygen evolution and electron transport, and inactivates Calvin-

cycle enzymes (Silvius *et al.*, 1975; Shimazaki and Sugahara, 1979; Kimmerer and Kozlowski, 1981; Shimazaki *et al.*, 1984; Wellburb, 1985).

Smoke itself has been proposed to inhibit fungal pathogens (Schwartz *et al.*, 1995). Schwartz and Hermann (Schwartz *et al.*, 1995) hypothesized that fungicidal properties of smoke could limit fungal infection. Moritz and Odion (Moritz and Odion, 2005) found a strong relationship between the absence of infection of *Phytophthora ramorum* and time since last burn and suggested that fire may inhibit pathogen activity by increasing soil Ca as Ca is crucial for plant resistance to disease (Marschner, 1995). Another possible mechanism for fire reducing pathogens that Moritz and Odion (Moritz and Odion, 2005) suggested is that soil environments after fire support various microbial communities that have antagonistic properties to fungal pathogens (Reaves *et al.*, 1990). Other research has found evidence that drier microclimates following fire can limit fungal pathogens (Holzmueller *et al.*, 2008). The possible ecological influences of smoke, while suspected for decades (Parmeter, 1977) have received little attention recently. Smoke is a highly complex chemical cocktail that may provide information to plants in ecosystems that have recently experienced fire.

Butenolide 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one has been identified as a compound in smoke that induces germination (Flematti *et al.*, 2004). It is unknown how the seed perceives the butenolide but there is evidence that it enhances water uptake capacity necessary for germination (Jain *et al.*, 2008). It is thought that the smoke is interpreted by the plant as a signal that conditions are favorable for germination, e.g. (Roche *et al.*, 1997). A similar signaling pathway could exist for leaf-pathogen relationships that operate through changes in defense chemistry in response to smoke.

Condensed tannins are thought to protect cell walls against microbial and fungal penetration (Cooper and Owen-Smith, 1985) and phenolic glycosides have been found to deter insect herbivory in trembling aspen (Donaldson and Lindroth, 2007). Many researchers have investigated the costs of these compounds in terms of fitness, reviewed in (Purrington, 2000; Strauss *et al.*, 2002). Here we examine whether observed changes in leaf-pathogen interactions following fire is mediated by changes in defense chemistry following smoke exposure.

Here we investigate the responses of three deciduous (*Populus tremuloides*, *Acer glabrum*, *Quercus gambelii*), and three conifer (*Pinus ponderosa*, *Pseudotsuga menziesii*, and *Picea pungens*) tree species to short term smoke exposures. We hypothesize that exposure to smoke will 1) reduce rates of photosynthesis and stomatal conductance; 2) lead to a decrease in plant growth; and 3) alter foliar defense chemistry.

## Materials and Methods

Six tree species (*Populus tremuloides*, *Acer glabrum*, *Quercus gambelii*, *Pinus ponderosa*, *Pseudotsuga menziesii*, and *Picea pungens*) in their second year of growth were used as treatment units. *Populus tremuloides* were grown from wild root cuttings, and the remaining five species were obtained from two tree nurseries (Sun Mountain growers, in Kaysville, Utah, and Plants of the Wild, Tekoa, Washington) as potted and bare root seedlings.

Prior to transplant, seedlings roots were washed and plant mass was measured. Within each species, trees of similar mass and height were used in the experiment. On March 26 - 27, 2008, each tree was transplanted into a peat based medium of Canadian Sphagnum peat moss with gypsum, coarse perlite and lime (Sunshine Mix #1, Sun Gro Horticulture, Bellevue, WA) in pots 23.5cm x 11.5cm<sup>2</sup>. Four grams of Osmocote Smart Release Plant Food with 14-14-14 was

added to each pot to provide the necessary nutrients for growth. The trees were left to grow in the greenhouse for the remainder of the summer and were watered to saturation twice a week.

### Smoke Treatment

From May 26-30, 2008, one plant of each species was replicated five times in smoke exposures staggered in time (one replicate each day, over a five day period). Smoke exposure occurred for 20 minutes. A second cycle of smoke exposure on the same plants occurred from June 9-13, 2008.

The smoke chamber was fabricated from a sealed plastic cooler (95cm x 38cm x 45cm). Equal parts of dried leaf material obtained from each of the six tree species in the study were used to generate the smoke. The leaves were combusted in a glass funnel fitted into a flask that was connected to the top of a glass flask. The flask was cooled in an ice bath to eliminate temperature increases inside the chamber. For 25 seconds, 500 mg of leaf material mixture was burned to ash with a lighter and the smoke was pulled through plastic tubing into the chamber using a vacuum connected to tubing at the bottom of the cooler. A fan inside of the cooler dispersed the smoke and a florescent light inside the cooler provided low light levels. Temperatures inside of the smoke chamber never exceeded 35° C, as measured by a Hobo U10-003 data logger (Onset Computer Corporation, Pocasset, MA). A second chamber identical to the first was used for control treatments. All procedures were exactly the same for the control chamber with the exception that leaf material was not placed in the glass funnel.

After smoke exposure, the treated plants were removed to measure rates of photosynthesis and stomatal conductance with a gas exchange system (LI-COR 6400, Li-Cor Biosciences, Lincoln, NE). Photosynthetic measurements were taken at a photosynthetic photon

flux density (PPFD) of  $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$  with the 6400-04 LED blue-red light source at ambient temperature and humidity. Two measurements were taken at  $\text{CO}_2$  concentrations of 385 ppm and 1000 ppm respectively with  $\text{CO}_2$  concentrations being achieved using the  $\text{CO}_2$  mixer.

Measurements were initiated by sealing the leaf in the chamber where one leaf per plant on the youngest fully expanded leaf or branch of needles was measured for gas exchange. After  $\text{CO}_2$  and water vapor concentrations in the leaf chamber reached a steady state (60-90 seconds), rates of photosynthesis and stomatal conductance were logged. Measurements were taken immediately after smoke exposure, 30 minutes after exposure, and then every 70 minutes until 310 minutes after exposure.

## Growth

On July 29, 2008 the trees were harvested for growth and mass measurements. Stems were measured and cut off at soil level and both roots and shoots were dried at  $60^\circ \text{C}$  for 72 hours to obtain dry mass using an analytical balance (GeneMate GP-600, ISC Bioexpress, Kaysville, UT, USA).

## Secondary Chemistry

When the trees were harvested for growth, leaves and needles were removed from the live trees and packed on dry ice before being moved to storage at  $-80^\circ \text{C}$  for later analysis of phenolic glycosides and condensed tannins. Leaves were freeze dried and needles were oven dried at  $60^\circ \text{C}$  for 48h. Leaf and needle material were then crushed in a Wiley Mill using a #10 screen. Tannins were quantified for all species using a modified butanol-HCL method described in Porter *et al* (Porter *et al.*, 1986), where approximately 50 mg of leaf material was placed in 2

ml screw-cap micro-centrifuge tubes suspended in 1ml of 70% acetone-10 mM ascorbic acid solution. The samples were then vortexed on high at 4° C for 20 minutes. The liquid supernatant was then removed and the extraction was repeated. The concentration of tannins was then quantified spectrophotometrically using purified tannins as a standard (SpectraMax Plus 384, MDS, Toronto, Canada).

The phenolic glycosides, salicortin and tremulacin, were extracted from approximately 50 mg of aspen leaf tissue (the other species do not contain significant levels of phenolic glycosides) where the tissues was placed in 2 ml screw cap micro-centrifuge tubes and suspended in methanol. The samples were then vortexed on high for 5 minutes. The liquid supernatant was then removed and the extraction was repeated. Final concentrations of 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).

### Statistical Analysis

We ran a Student's t-test to test for the difference in defense chemistry and growth. Repeated measures analysis of variance (ANOVA) was used to test the effects of smoke exposure on rates of photosynthesis from 30 minutes after exposure to 310 minutes after exposure using time as the 'within' factor (Gumpertz and Brownie, 1993). Homogeneity of variance and normality were tested with Shapiro-Wilk W statistics and equal variance tests. Data that did not meet the assumptions for the parametric tests were tested using a Wilcox rank

sum test, while the data in the graphs were untransformed. Statistical analysis was performed using JMP version 7 statistical software (SAS Institute, Cary, NC, USA).

## Results

All of the species showed significant differences in stomatal conductance and rates of photosynthesis 30 minutes after exposure except for Douglas-fir. Aspen and ponderosa pine showed the greatest reductions in photosynthesis (figure 1).

The repeated measures analysis on rates of photosynthesis at 1000 ppm CO<sub>2</sub> showed a significant time effect (p-value <0.001) in which rates of photosynthesis recovered from smoke exposure as time progressed. At 1000 ppm there was also a significant interaction between time and species type (deciduous and conifer) (p-value 0.0073) with deciduous species showing slower recovery (figure 2). At 385 ppm CO<sub>2</sub> time was the only significant effect in the repeated measures model (p-value 0.0209). Species type was marginally significant (p-value 0.0709).

The conifers had a unique response to smoke exposure where a number of samples exposed to smoke had higher rates of photosynthesis than the control. This was not found with all of the samples, meaning the other samples did not exceed the control in rates of photosynthesis. This is partly responsible for the large error bars seen in figure 2.

Smoke did not significantly affect growth, condensed tannins (figure 3), or phenolic glycosides (data not shown).

## Discussion

The data are consistent with our first hypothesis that smoke exposure reduces rates of photosynthesis. Comparing photosynthetic responses at ambient and saturating CO<sub>2</sub>

concentrations suggests that smoke affects photosynthetic function by reducing stomatal conductance and through impairment of biochemical function (Figure 2). Our results show for the first time that photosynthetic sensitivity to smoke occurs across a diverse sampling of tree species and that there is wide ranging variation in sensitivity among those species. Because of the complexity of smoke it is hard to pinpoint how the underlying mechanisms of smoke affect photosynthesis.

Physically, smoke has high vapor pressure deficits that can lead to reductions in stomatal conductance and photosynthesis (Guehl and Aussenac, 1987). Chemically, over 100 compounds have been identified in smoke including NO<sub>2</sub> and SO<sub>2</sub> (Radojevic, 2003). How these chemicals interact to affect photosynthesis is mostly unknown but it is possible that they will have additive as well as individual effects. By itself, NO<sub>2</sub> has shown little effect on stomatal conductance or photosynthesis (Saxe, 1986; McAinsh *et al.*, 2002). SO<sub>2</sub> reduces stomatal conductance, inhibits photosynthetic oxygen evolution and electron transport, and inactivates Calvin-cycle enzymes (Silvius *et al.*, 1975; Shimazaki and Sugahara, 1979; Kimmerer and Kozlowski, 1981; Shimazaki *et al.*, 1984; Wellburb, 1985). When combined, NO<sub>2</sub> and SO<sub>2</sub> mixtures have shown to have an additive inhibition of photosynthesis (Bull and Mansfield, 1974). Long term exposures to NO<sub>2</sub> and SO<sub>2</sub> show subsequent reductions in superoxide dismutase and glutathione reductase (Wingsle and Hallgren, 1993) both of which function in plants as antioxidant response (Alscher, 1989; Wingsle and Hallgren, 1993). The disabling of antioxidant enzyme function in conjunction with high levels of ozone, a powerful pro-oxidant, during extended smoke exposure may promote oxidative stress.

Different compounds in smoke, such as NO and NO<sub>2</sub>, affect species in varying degrees (Saxe, 1986). We found that the conifer species initially recovered from the smoke exposure

faster than the deciduous species (figure 2) and after thirty minutes of smoke exposure, Douglas-fir had completely recovered (initial decreased rates of photosynthesis not shown) (figure 1). Why the conifers initially recovered faster than the deciduous trees is unknown. Plant species can develop tolerance to pollutants that are known to affect photosynthesis, e.g. (Swanepoel *et al.*, 2007). The greater tolerance in conifers could be a result of the different fire strategies, in which gamble oak, rocky mountain maple, and aspen employ a survival strategy of overstory mortality followed by asexual regeneration at some later time (Brown, 1958; Kurzel *et al.*, 2007; Lentile *et al.*, 2007). In contrast, conifer species including ponderosa pine and Douglas-fir employ a strategy of fire resistance with their thick bark that allows the overstory to survive (Kimmins, 2004). We hypothesize that species that employ a strategy of fire resistance would have a greater need to develop mechanisms of tolerance to avoid the negative effects of smoke for extended periods of time.

Our data do not support our second hypothesis that smoke exposure can reduce tree growth rates. It is likely that two 20-minute smoke exposures are insufficient to elicit a growth response. In natural systems plants can be exposed to smoke on scales of weeks to months (Davies and Unam, 1999). Smoke exposures of such length are impractical in controlled studies but tree ring data could potentially be used to examine correlations between growth rates and smoke extent over the summer season if other confounding factors can be accounted for.

There was also no evidence to support our second hypothesis of an alteration of secondary defense metabolism. There are a few possible reasons for why we did not find any significant differences. First, plants may simply not have evolved using smoke as an important signaling cue in fire-prone systems. Second, while we looked at two important defense compounds based on quantity and function we certainly did not conduct a comprehensive survey

of secondary metabolic responses. Finally, it is also possible that the signature of our smoke exposures (timing, intensity) were not adequate for eliciting a defense response.

Because there are many compounds in smoke and we know so little about how they can affect plants, we have much to learn about the influence of smoke on plant function. The fact that different plant species can show varying responses to smoke, in addition to the potential for different plant species to produce its own complex suite of compounds, suggests there may be some intriguing roles for smoke in plant and ecosystem function.

## Figures

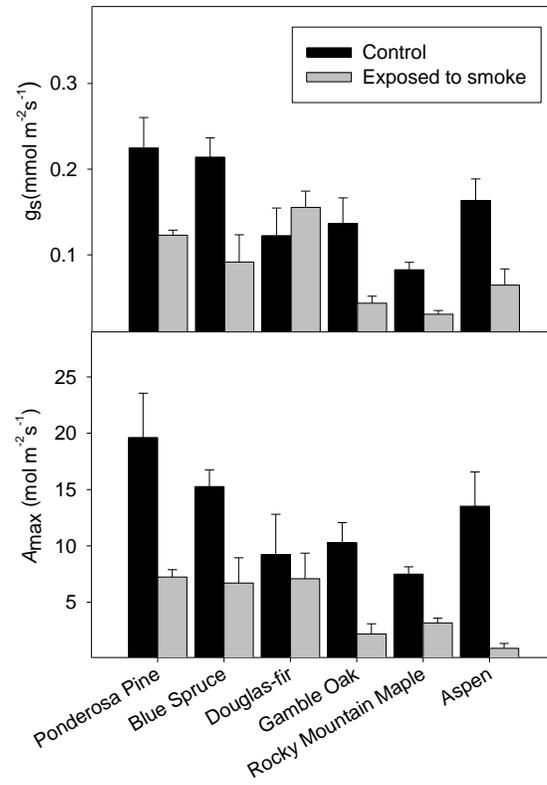


Figure 1.  $A_{\text{max}}$  (maximum rates of photosynthesis) and  $g_s$  (stomatal conductance) after 30 minutes of smoke exposure. The only species without significant differences (at  $<0.05$  level) was Douglas-fir.

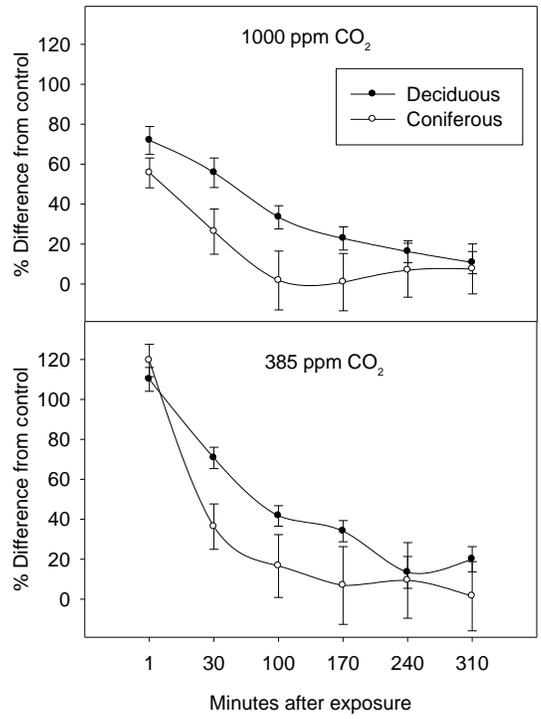


Figure 2. Rates of photosynthesis expressed as percent difference from control at both 385 and 1000 ppm CO<sub>2</sub>.

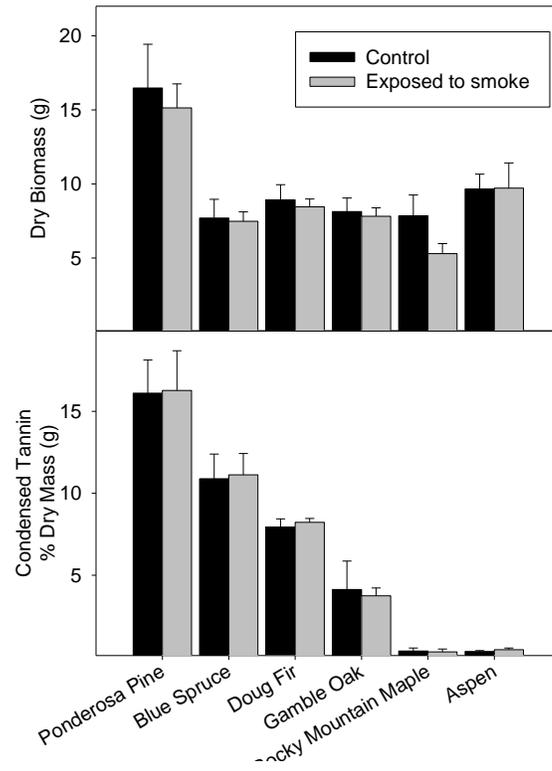


Figure 3. Comparisons of biomass and condensed tannins in smoke exposed samples and controls. There were no significant differences between any of the species

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