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THE UNIVERSITY OF BRITISH COLUMBIA

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Pine Growth Trial - Drought Stress and Solbere Treatment
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Introduction

Exposure to biotic and abiotic stresses is a daily occurrence for most plants. This has necessitated the evolution of a range of protective adaptations. For example, structural modifications such as changes in leaf thickness, leaf size, leaf dry mass per unit area and presence of a light reflecting wax layer on the leaf surface, while short-term responses involve leaf movement (change in leaf angle or leaf rolling) and chloroplast movement. Often, as light intensity increases, chloroplasts gather along cell walls that are perpendicular to incoming light, with maximum chloroplast avoidance occurring at 500 µmol m^{-2} s^{-1}. Plants with defects in chloroplast relocation or continued exposure to high light intensity show light-dependent depression of leaf photosynthetic rates (photoinhibition) and the formation of reactive oxygen species (ROS). Reactive oxygen species are generated at both photosystem I, where electron transfer to oxygen forms hydrogen peroxide, and photosystem II (PSII) where singlet oxygen is produced by energy transfer to oxygen from the P680 reaction centre. It is the combined high light and ROS production that inactivate and prevent the repair of PSII, resulting in decreased maximum photosynthesis. Unchecked production of ROS and the resulting photooxidation eventually lead to irreversible chlorosis and necrosis of leaf tissue.

Environmental stressors rarely occur in isolation and high light stress coupled with drought can exacerbate reductions in photosynthesis. Particularly in C3 plants where there is no spatial separation of carbon assimilation and fixation reactions. Rubisco, the enzyme responsible for CO_2 fixation in plants, accepts both CO_2 and O_2 as substrates. Carboxylation of ribulose 1,5-bisphosphate will yield photosynthates, while oxygenation produces the waste product 2-phosphoglycolate for which the plant must expend energy to recycle. Rubisco must further be activated through removal of inhibitors from its catalytic site and the key enzyme involved, Rubisco activase, is activated by increased illumination. More light therefore increases carbon fixing, but simultaneously generates carbon dioxide from photorespiration as glycolate is recycled. As temperatures increase, solubility of CO_2 relative to O_2 decreases, causing greater Rubisco oxygenation reactions and energy losses. Concurrent drought stress, leading to stomatal closure further decreases internal CO_2 concentrations. While photorespiration may be important for maintaining electron flow and preventing photoinhibition under stress, its high energy requirements decrease photosynthetic efficiencies. Limiting occurrences which necessitate the use of non-photochemical quenching and photorespiration in plants has the potential to significantly increase plant productivity.

One way to limit the incidence of excess light is through a photoprotective covering on the plant leaf. Solbere is a proprietary product that is applied to plant leaves as a spray. It utilizes a mix of titanium dioxide and calcium carbonate that is intended to maximize plant growth, health, and yield. While the exact mechanism has yet to be established, there is a potential for Solbere treatment in to play a beneficial role in tree growth. One mechanism proposed is that a Solbere coating on plant leaves enables the plant to minimize chloroplast avoidance and maximize time spent in a light-harvesting state even during periods of strong sunlight. We aimed to conduct a pilot study on Lodgepole pine using two factors, Solbere spray application and water restriction.

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To examine the effects of Solbere as a photoprotective covering, Solbere and exposure to water-deficit in a two-factorial experiment was be conducted, where 500 lodgepole pine trees was examined: half were watered regularly (with and without application of foliar spray of Solbere), while the other half were subject to a water limiting (50% soil water holding capacity) environment (with and without application of the foliar spray of Solbere). The Solbere application was applied twice, at a 4% application rate, as per manufacture's instruction, at the beginning of the trial and after one-month of active growth. Greenhouse environmental conditions mimiced summer conditions with extended supplemental light during the day and consistent 23ºC greenhouse temperatures.

Overall plant performance was assessed by growth, but also impacts on photosynthesis. Leaf-level performance and monitoring of photo-oxidative stress was assessed as Fv/Fm via fluorometer measures of non-photochemical quenching. Measurements of overall photosynthetic performance, including carbon assimilation, transpirational rate, and conductance was assessed using a LiCOR 6400 equipped with a special gymnosperm-specific clamp (purchased), while long-term WUE was determined using carbon isotope discrimination (∆13C).

Experimental Design and Methods

A total of 412 Lodgepole pine (*Pinus contorta var. latifolia*) trees were planted into cone shaped pots (Figure 1). The trees were arranged on racks in 3 rows of 10 trees per rack. Each row of trees was spaced such that there was an empty row between rows (i.e. three of the five available rows were filled for each rack). In total, there were 16 racks of trees. 12 racks were filled to a full 30-tree capacity; the remaining 4 racks had 13 trees.

Figure 1. Planting of pine seedlings into cones and racks (June 24th).

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Two factors were simultaneously tested in this study - Solbere spray application (treated / untreated) and water restriction (water restricted / normal watering). Therefore, the trial had four treatments.

The groupings for the racks and treatments were as follows (Note: racks 4, 16, 8, 12 were the partially filled racks).

Water restricted-untreated:	Racks 1, 5, 7, 4
Water restricted-treated:	Racks 9, 11, 13, 16
Normal watering-untreated:	Racks 2, 3, 6, 8
Normal watering-treated:	Racks 10, 14, 15,12

The trees were planted late June and allowed to acclimate to the greenhouse conditions (Figure 2). After three weeks growth, half of the trees were sprayed with a 4% Solbere solution using a handheld spray bottle (Figure 3). Then, half the trees from each of the Solbere-treated and untreated groupings were raised to facilitate water stress (Figure 4), as the flood table watering system control the amount of water they received daily. While the trees receiving normal watering from the table watering system. Moreover, the watered trees also received bi-daily manual watering over the course of the experiment, to ensure no water stress.

Figure 2. Photo of trees after three-weeks acclimation growth in the greenhouse.

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Figure 3. Photos of pine trees with Solbere application (left are control trees, while right have a 4% Solbere application).

To determine the watering schedule for the water-restricted trees, five trees were oven dried to determine the dry weight of the cone + tree + soil. Thereafter, we were able to approximate a threshold weight loss in grams that corresponded with 50% water loss in soil of the water-restricted trees. On a daily basis, five trees from each water restricted rack were monitored for weight loss. We allowed the average of these trees to reach the 50% water loss threshold before watering all the water restricted trees. Using the threshold, the water restricted trees were watered roughly every three-four days, depending on daily temperatures in the greenhouse, which impacted water loss from the trees/soil. The normal watering trees received daily watering daily.

Figure 4. Photo showing racks placed in the flood tables for normal watering (front) and raised racks where the trees would be water stressed (back).

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Height and diameter measurements were taken on four occasions. Once the acclimation growth phase was complete. And, then the first measurements were taken on August 7th. Follow up measurements were taken on August 26th/27th, at which time a second application of Solbere was applied to maintain adequate coating for the last period of the growth trial (Figure 5). A final growth measurement was then taken on September 26th. Note: bud set was unavoidable even under artificial light cycles (17 hours of light) that simulated summer daylight hours. Thus, a final measurement was largely used as a confirmation of the fact that the trees were no longer growing in height, but the trees continued to increase in diameter.

Figure 5. Photos showing growth at the tip, where Solbere application is only apparent on half of the needles (right) compare to the control needs with no Solbere application (left).

Rates of photosynthesis and transpiration were measured using a LICOR 6400 portable photosynthesis system over two multi-day periods, including September 11-13 and September 24-25. Sampling was performed strictly between 8:00am-10:30am each morning. We gathered data for photosynthesis, conductance, and transpiration. A total of 40 trees were analysed,10 per treatment, randomly selected within each treatment block.

Gravimetric density was measured on 32 trees (8 trees per treatment) after removing the bark and branches from the stems. The diameter was recorded both before and after the bark removal to determine if increases in stem diameter were associated with increased woody material. The same samples used for density measurements were then ground into a woody flour using liqud nitrogen coupled to a genogrinder, and compressed into pellets for analysis of Delta 13 values via isotope mass spectroscopy.

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Results and Data Analysis

LICOR: Photosynthesis, Conductance, and Transpiration

Normal Watering

	Photosynthesis (μmol CO2 m^{-2} s^{-1})		Conductance (mol H2O m^{-2} s^{-1})		Transpiration (mmol H2O m^{-2} s^{-1})	
	Average	St Dev	Average	St Dev	Average	St Dev
Untreated	13.27	3.57	0.24	0.07	4.14	1.15
Treated	11.61	2.89	0.27	0.07	4.72	1.17

Water-restricted

	Photosynthesis (μmol CO2 m^{-2} s^{-1})		Conductance (mol H2O m^{-2} s^{-1})		Transpiration (mmol H2O m^{-2} s^{-1})	
	Average	St Dev	Average	St Dev	Average	St Dev
Untreated	10.86	1.92	0.18	0.08	3.36	1.22
Treated	8.29	3.02	0.17	0.11	3.31	1.78

LICOR data was analysed using SAS. A total of 40 samples were included in the analysis. A multivariate analysis of variance (MANOVA) was performed to compare differences in the average photosynthesis (Photo), conductance (Cond), and transpiration (Trmmol) both between treated and untreated samples, as well as between water restricted and normal watering.

Summary of findings from LICOR

- There was no interaction effect between the two factors for Photo, Cond, or Trmmol (Treated*Dry Type III SS Pr > F = 0.5774).
- For Photosynthesis (Photo), there was a difference between treated and untreated samples (Page 2: Type III SS, Pr>F = 0.0244). Treated samples had lower photosynthesis rates.
- For Conductance (Cond), there was a difference between water restricted and normal watering (Page 4: Type III SS, Pr>F = 0.0127). Water restricted samples had lower conductance rates.

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- For Transpiration (trmmol), no significant differences were apparent for either factor. However, transpiration rates were notably close for water restriction (Page 6: Type III SS, Pr>F = 0.0621).

Diameter Change

	Average (mm)	St. Dev.
Water Restricted-Untreated	1.34	0.54
Water Restricted-Treated	1.72	0.57
Normal watering-Untreated	1.53	0.54
Normal watering-Treated	1.67	0.43

T-tests showed more diameter change (growth) in treated trees than untreated trees under water restriction ($p < 0.001$). During normal watering, trees showed more growth than the water-restricted trees when both were untreated ($p=0.0365$). However, there was no difference in diameter change between normal watering and water-restricted trees when both groups were treated. Finally, there was no difference in diameter growth between treated and untreated trees when both had normal watering.

Height Change

	Average (cm)	St. Dev.
Water Restricted-Untreated	4.16	0.54
Water Restricted-Treated	3.67	0.57
Normal watering-Untreated	4.97	1.73
Normal watering-Treated	4.65	0.89

T-tests showed more height change (growth) in trees watered normally compared to water restricted trees, when the trees were untreated ($p<0.001$) and when the trees were treated ($p<0.001$). There was no difference between treated and untreated groups for either water restricted or normal watering conditions.

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Note - approximate volumes of the trees were calculated based on the final height and diameter measurements of the trees, assuming the trees represent a cone (no taper was considered). Comparisons between the treatments were based off these volumes.

Volume Change

	Average
Water Restricted-Untreated	1.91
Water Restricted-Treated	2.79
Normal watering-Untreated	2.91
Normal watering-Treated	3.33

Density

	Average (mm)	St. Dev.
Water Restricted-Untreated	456.79	27.20
Water Restricted-treated	464.39	33.42
Normal watering-Untreated	459.41	65.18
Normal watering-Treated	464.97	35.88

T-tests performed in excel showed no differences in density between any of the treatments.

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Percent Xylem of Stem Diameter

	Average (% xylem)	St. Dev.
Water Restricted-Untreated	70.84	4.30
Water Restricted-Treated	78.11	6.07
Normal watering-Untreated	75.59	5.54
Normal watering-Treated	78.55	3.69

T-tests performed in excel showed greater % xylem in the treated trees compared to untreated trees when under water restriction ($p = 0.0152$).

Delta 13

	d13C	St. Dev.
Water Restricted-Untreated	-28.20	0.56
Water Restricted-treated	-29.31	1.01
Normal watering-Untreated	-29.84	1.04
Normal watering-Treated	-30.26	1.11

T-tests performed in excel showed lower d13C in treated trees compared to untreated trees when under water restriction ($p=0.0268$). Also, trees with normal watering had lower d13C compared to water restricted trees when there was no treatment ($p=0.0051$).

Summary of Findings

Based on these findings it appears that Solbere treatment has a positive impact on growth as there appears to be an increase in biomass (volume), which is largely driven by an increase in diameter growth, as the Solbere treated trees were slightly shorted than untreated trees control trees regardless of normal watering cycles or when subject to water removal (water restricted). Moreover, the wood density was unchanged, regardless of treatment (water status or application of Solbere). Therefore, more volume at a similar density translates into an increase in total available biomass. A closer

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inspection of the girth growth, as measured by diameter, indicated that the observed increase in diameter was related to increased deposition of xylem tissue (wood), as the ratio of xylem to phloem (bark) increased with Solbere treatment, and was the same regardless of watering status.

The observed increase in biomass deposition was originally believed to be related to improved rates of photosynthesis, however, our LICOR data does not support these claims, as assimulation rates appear to be lower in the Solbere treated trees, regardless on water status. Our data also show that there was a difference between water restricted and normal watering, where the water restricted samples had lower conductance rates, as would be expected. And, Solbere treatment showed slightly higher conductance during normal watering conditions (not statistically significant). Similar trends were apparent in transpiration. On a first inspection, it appears that photosynthetic rates are therefore not directly related to increased biomass in this pine trial.

One possible explanation may be that the Solbere treatments increase the reflectance of solar radiation, which may result in lower assimilation rates, but also may have reduced the internal leaf temperature (needs to be validated with a thermal camera. Note, three were purchased, but did not measure leaf temperatures accurately). Potentially, the lower temperatures of the foliage permitted the stomates to remain open longer, which is supported by the conductance and transpiration rates, and therefore capture more carbon during the hotter periods. As a consequence, the capture carbon may have altered the carbon allocation, and therefore resulted in increased deposition of woody tissue, as well as starch stores (recommend evaluating starch accumulation). And, less carbon/energy into combatting photon stress and thermal adjustment. The delta 13 carbon isotope estimates (long-term water use efficiency) support the instantaneous estimates of water use derived from the LICOR instrument.