



# Impacts of Planting Drought Hardened Loblolly Pine Seedlings Under Various Drought Conditions

Tom Stokes



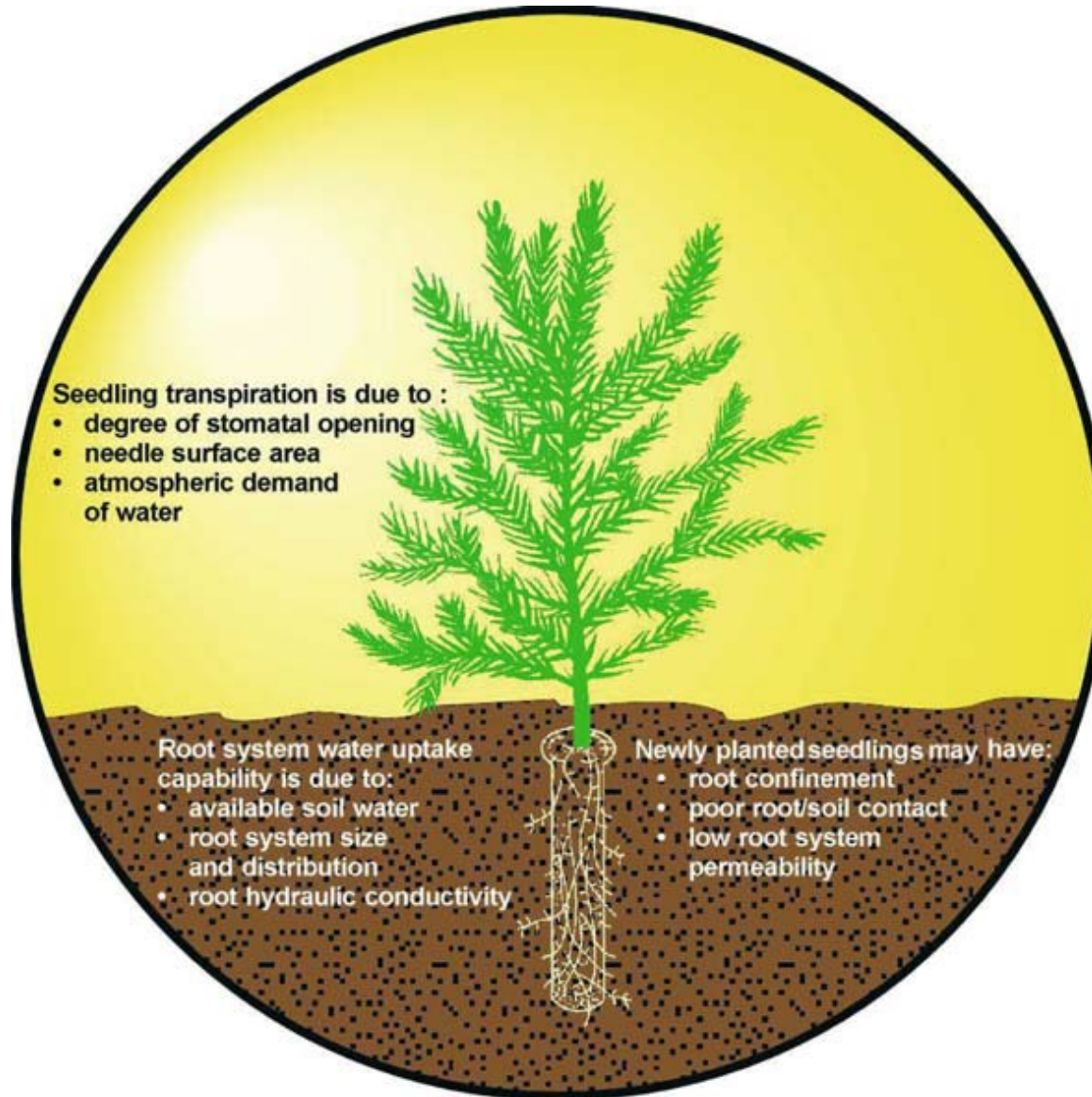
# Introduction

- Seedlings grown in nursery under optimal growing conditions
  - Irrigation
  - Fertilization
  - Competition control
  - Etc.
- Outplanting in harsh environmental conditions
  - Physiology
  - Morphology
  - Anatomy



# Introduction

- Surviving Drought
  - Water loss through transpiration
  - Root water uptake capability
  - Overcome newly planted conditions



Grossnickle (2005)

# Introduction

- Drought conditioning seedlings
  - To intentionally limit irrigation during the growth phase in a nursery
    - This practice began in the 1<sup>st</sup> half of the 20<sup>th</sup> century in an arid region in the pacific northwest.
    - Became more prevalent after a graph was published in 1974 indicating greater survival of drought stressed seedlings.
    - Some studies have shown greater survival with drought conditioned seedlings.
    - However, many recent studies have shown that survival does not increase with reductions in irrigation in many bareroot Pinus species.

# Introduction

- Benefits of Drought Conditioning
  - Increased root to shoot ratio
  - Decrease in succulent foliage
  - Increase in soluble sugars
- Risks of Drought Conditioning
  - Depletion of stored carbohydrates
  - Increase in cavitated xylem conduits
  - Predispose to future stress events (legacy effects).

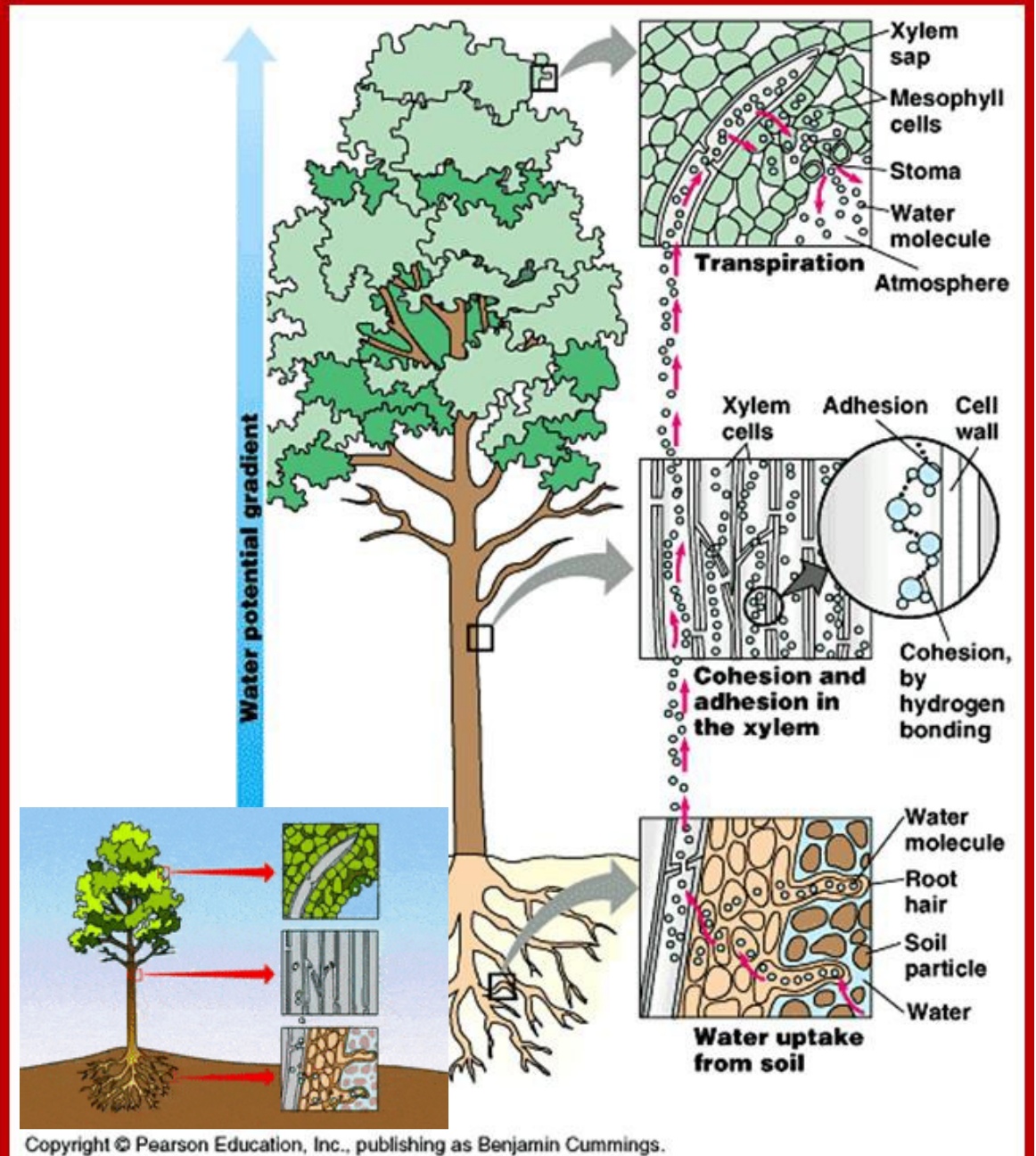
# Introduction

- To understand the effects of drought conditioning we must:
  - Understand how water moves through a plant
  - How cavitation and embolisms occur
  - How embolisms spread
  - How embolized xylem MAY be repaired



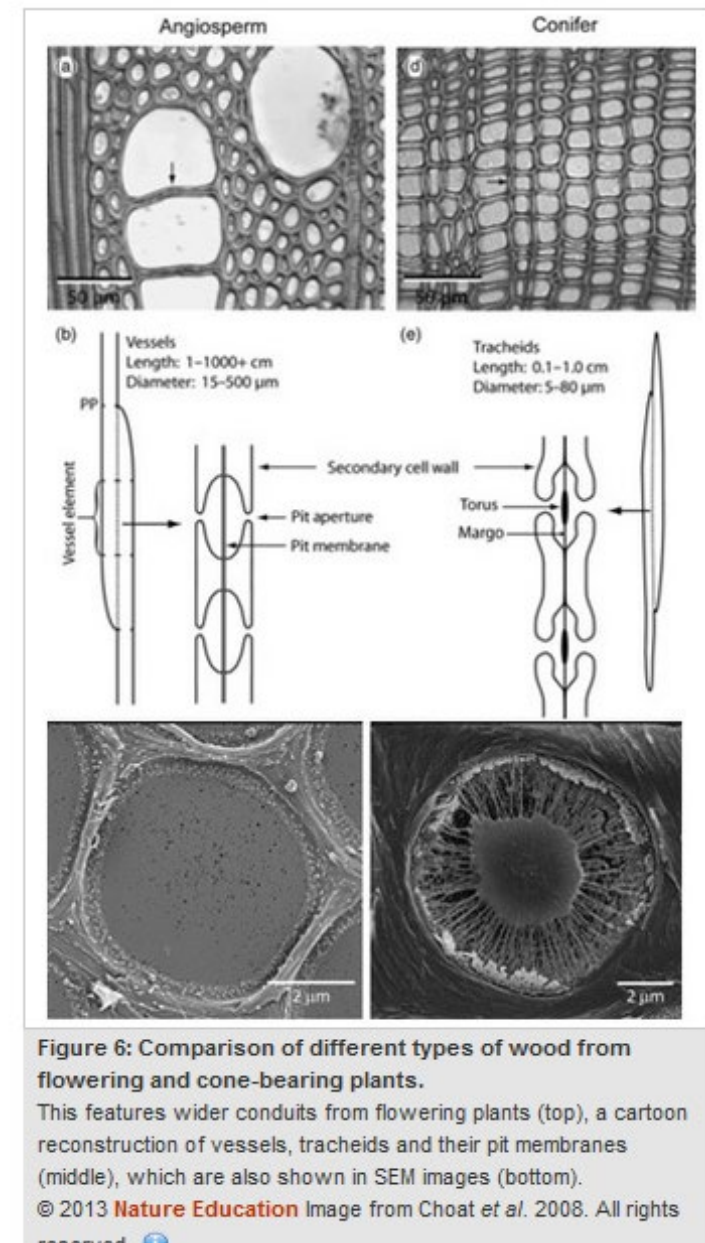
# Introduction

- How water moves through a tree.
  - Transpiration
    - Water moves out of stomata
    - Water moves down concentration gradient which creates negative pressure
    - Water is replaced by water from xylem
  - Cohesion and adhesion in the xylem.
    - Xylem water column is maintained by the cohesion of water and adhesion to the cell walls
  - Water uptake from soil
    - Water is pulled from root cortex into xylem cells
    - Water is pulled from the soil into the roots



# Introduction

- Xylem cavitation and Embolism
  - Breakage of the xylem water column due to water stress or injury
    - Entry of air into the xylem conduits
    - Embolisms move primarily through the pit membrane
- Species and individuals differ in their vulnerability to cavitation – trade-offs between vulnerability and water flow
- Size, structure and number of pits important traits

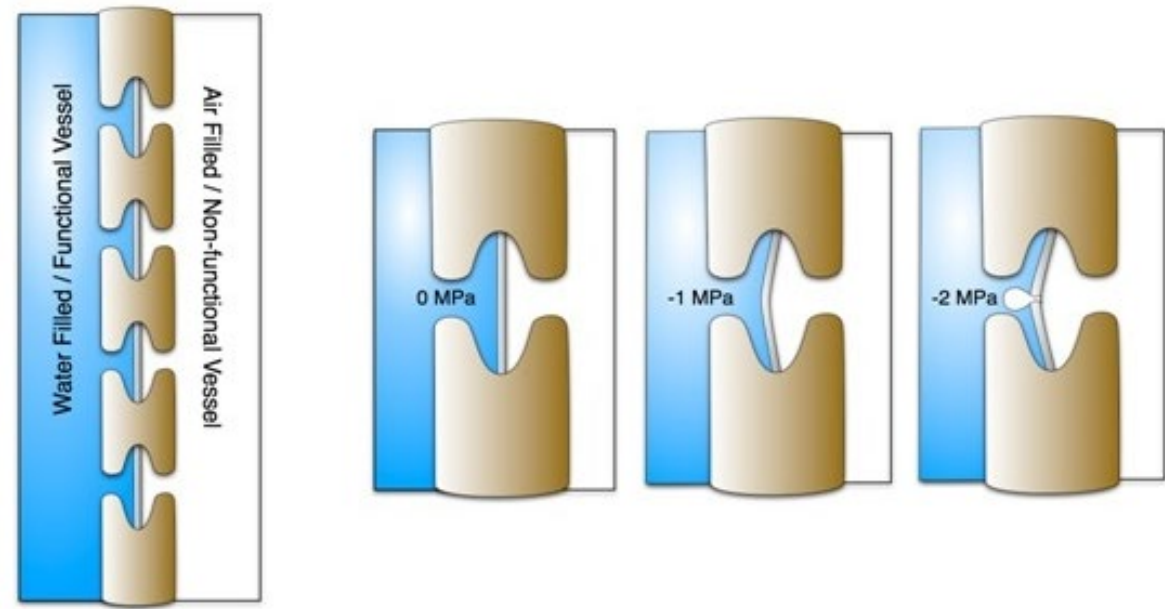


McElrone et al. (2013)




# Introduction

- How embolisms spread
  - Air seeding
    - Air bubble moves from air filled xylem into water filled xylem across the pit membrane when a threshold pressure is reached



**Figure 9: Air seeding mechanism.**

Demonstrates how increasing tension in a functional water filled vessel eventually reaches a threshold where an air seed is pulled across a pit membrane from an embolized conduit. Air is seeded into the functional conduit only after the threshold pressure is reached.

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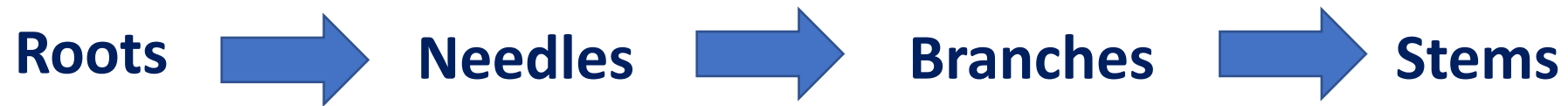
McElrone et al. (2013)

# Introduction

- Embolism repair
  - Some species have shown the ability to rapidly repair embolized xylem conduits
    - For refilling of xylem conduits either:
      - Freeze-thaw cycles –which will dissolve the gas back into water, or
      - Positive root pressure – from movement of solutes to the roots.
  - Several recent studies, especially with conifers, have shown a lack of a mechanism to refill embolized xylem conduits.
    - New xylem will have to be made – significant carbon cost to the plant.

# Introduction

- Vulnerability to embolism within a plant







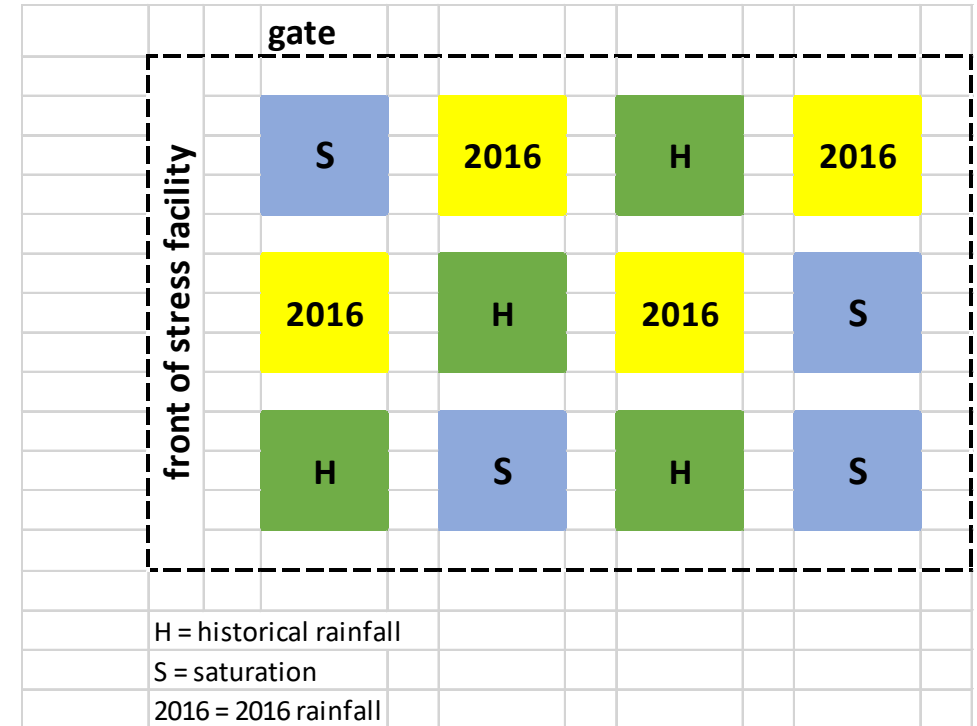
# Objectives

- Determine the physiological effects of reduced water availability on drought hardened one-year-old bareroot loblolly pine seedlings and subsequent recovery after rewatering.



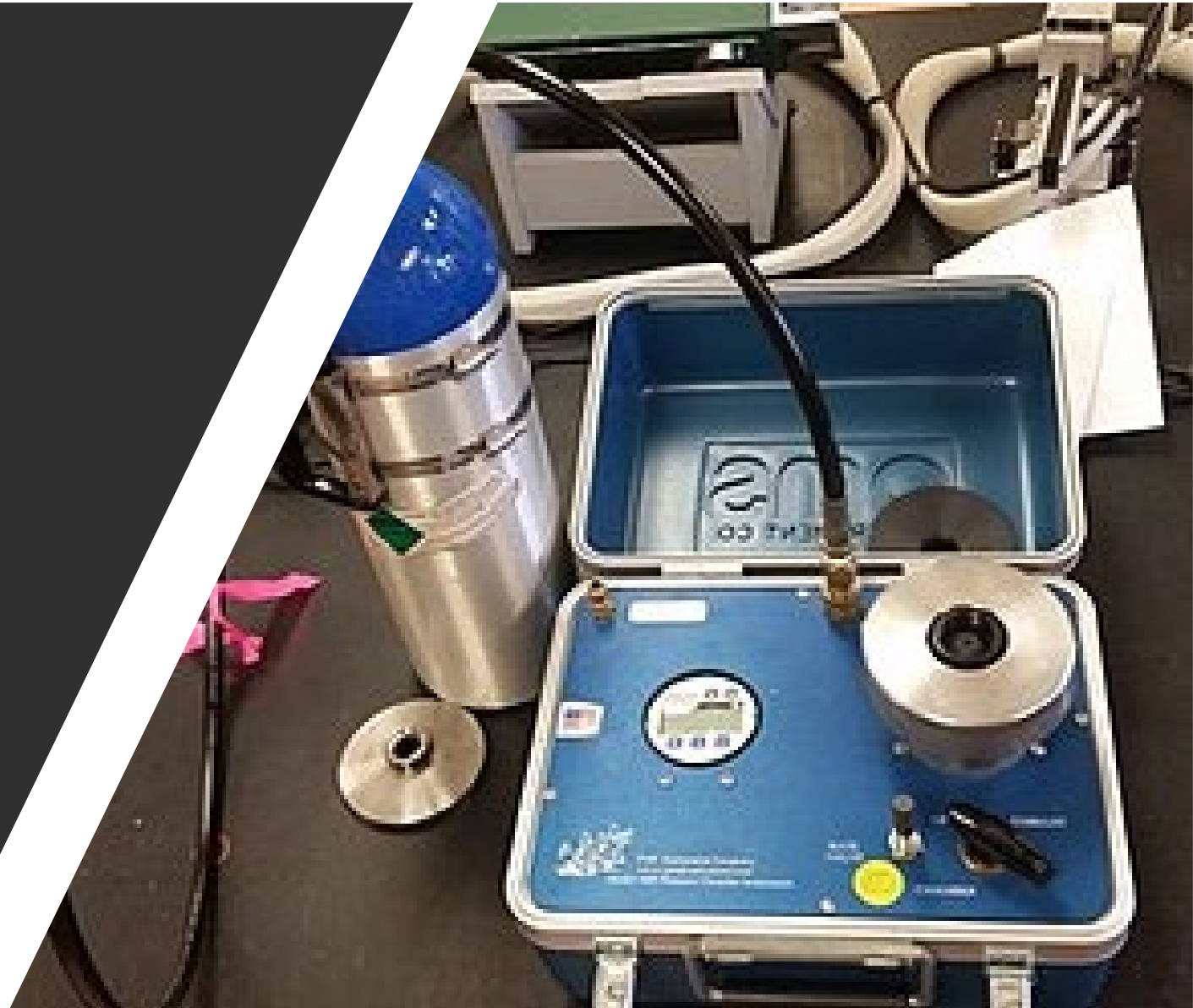
# Methods

- Randomized complete block
  - 4 replications of 3 treatments
- Treatments
  - **Saturation** – watered to saturation
  - **Historical** – 16.38 ml/seedling/week
  - **Drought** (2016) – 0-19.66 ml/seedling/week
- Recovery
  - After treatment phase of study all seedlings were well-watered for 6 months



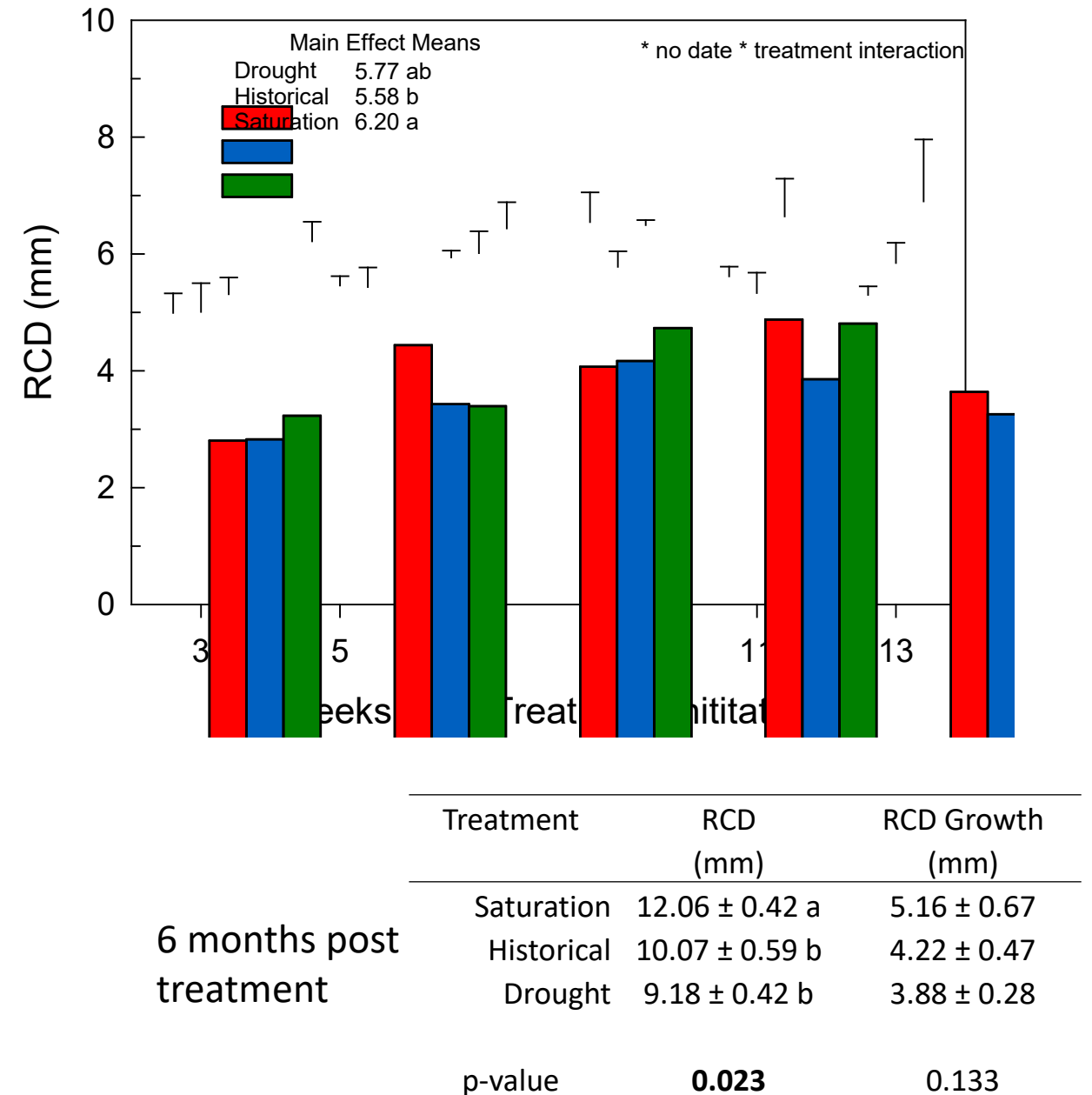
# Methods

- Measurements
  - RCD
  - Root and shoot biomass
  - Stem water potential
  - Stem embolism



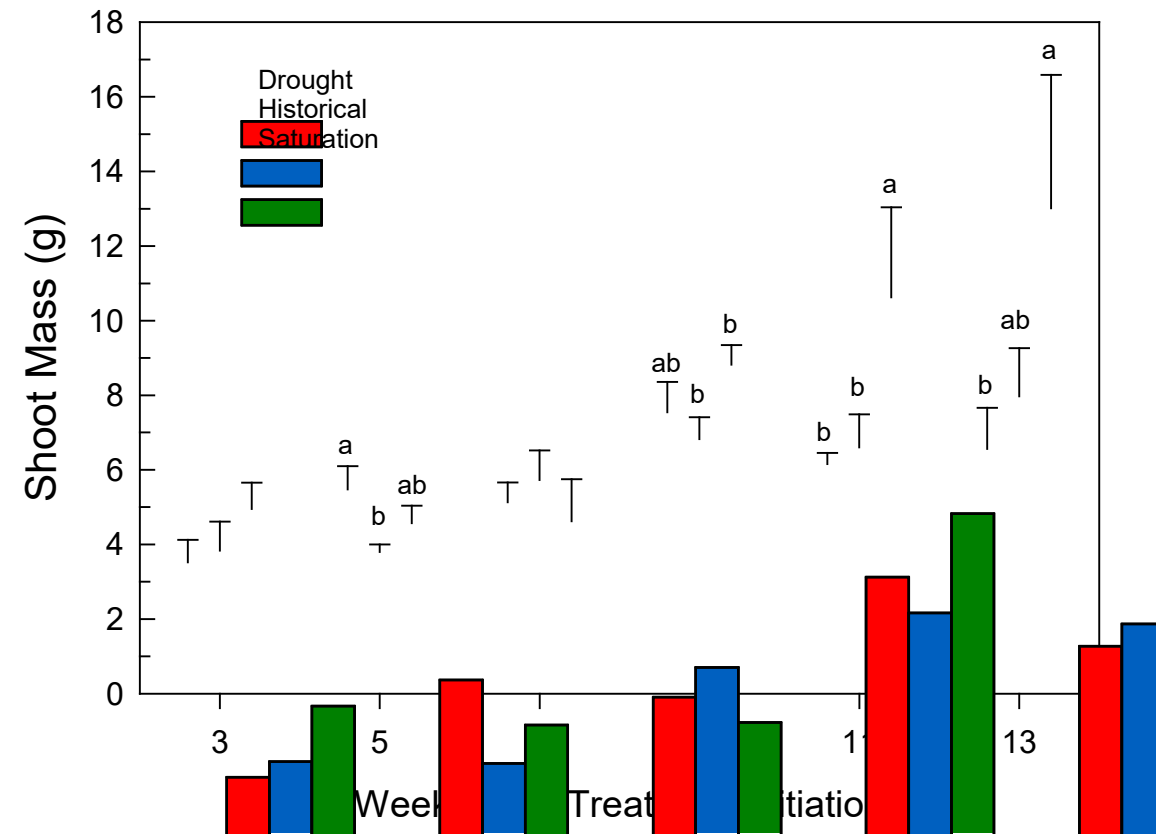
# Results

- Treatment phase:
  - RCD was decreased by 10% in the historical treatment compared to the saturation treatment
- Six months post treatment:
  - RCD in both drought and historical treatments were decreased on average 21.5%



# Results

- Treatment phase:
  - As treatment progressed, decreases were observed in shoot mass in the drought and historical treatment compared with saturation.
- Six months post treatment:
  - There was a strong trend for decreased shoot mass in the drought treatment compared to saturation
  - No treatment differences were observed for shoot mass growth



6 months post treatment

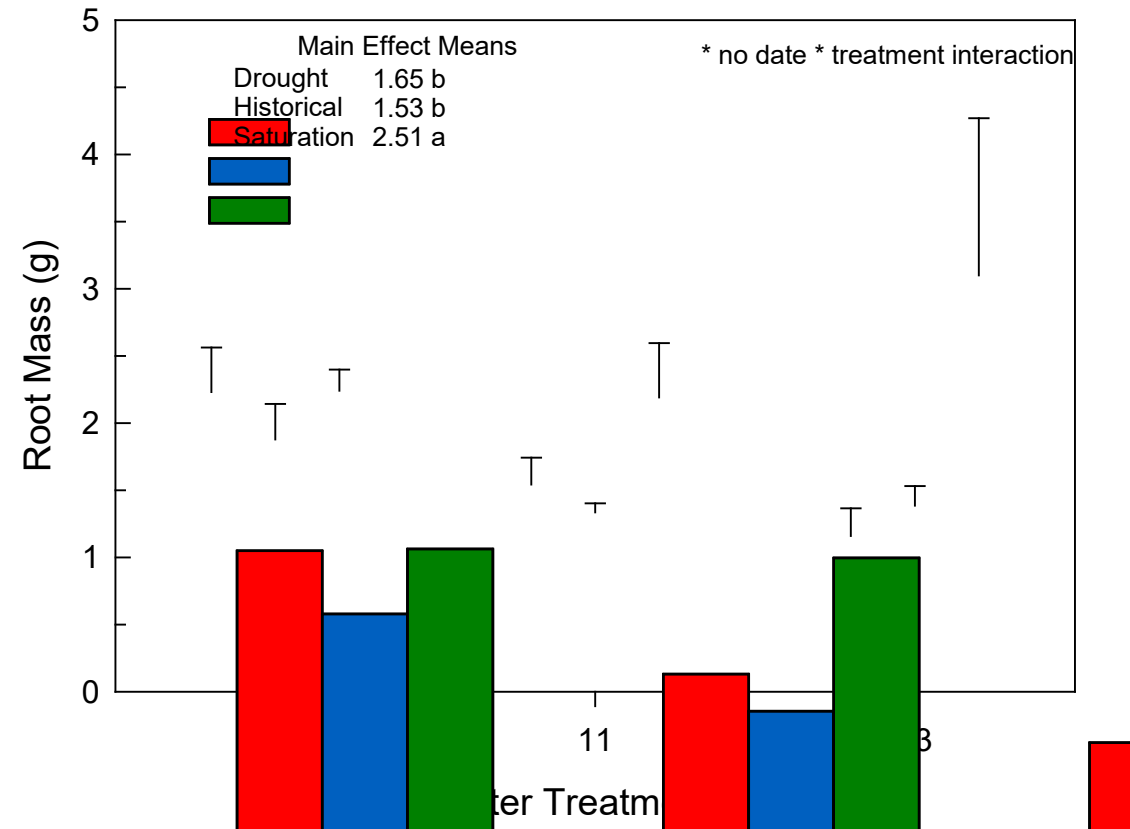
Treatment	Shoot Mass (g)	Shoot Mass Growth (g)
Saturation	51.09 ± 3.69	38.08 ± 3.63
Historical	42.76 ± 5.57	34.79 ± 6.38
Drought	33.92 ± 2.27	27.36 ± 1.58
p-value	0.088	0.249



# Results

- Treatment phase:
  - Drought and historical treatments decreased root mass on average by 36.6%
- Six months post treatment:
  - No treatment differences were observed for root mass or root mass growth

note: no differences in root:shoot ratios

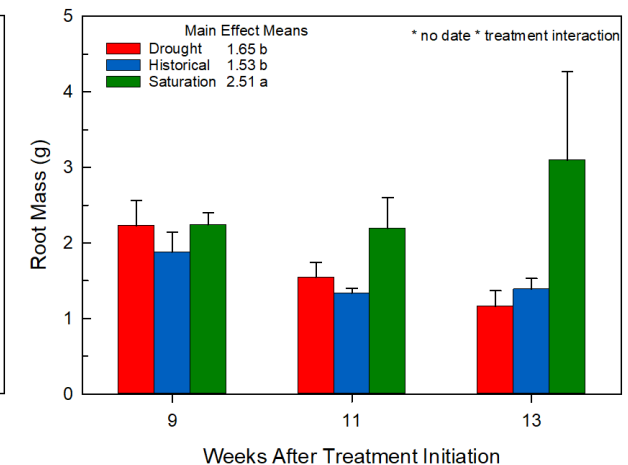
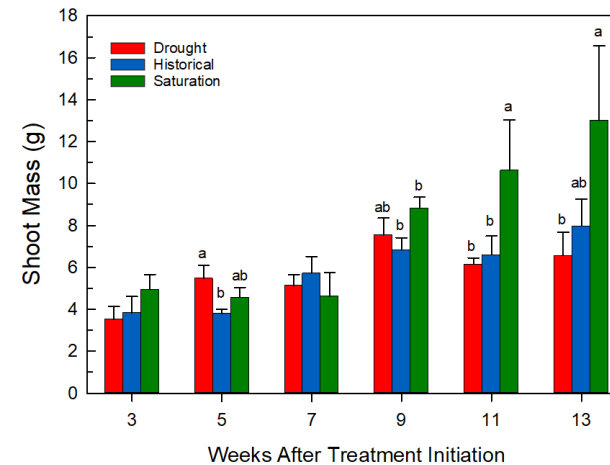
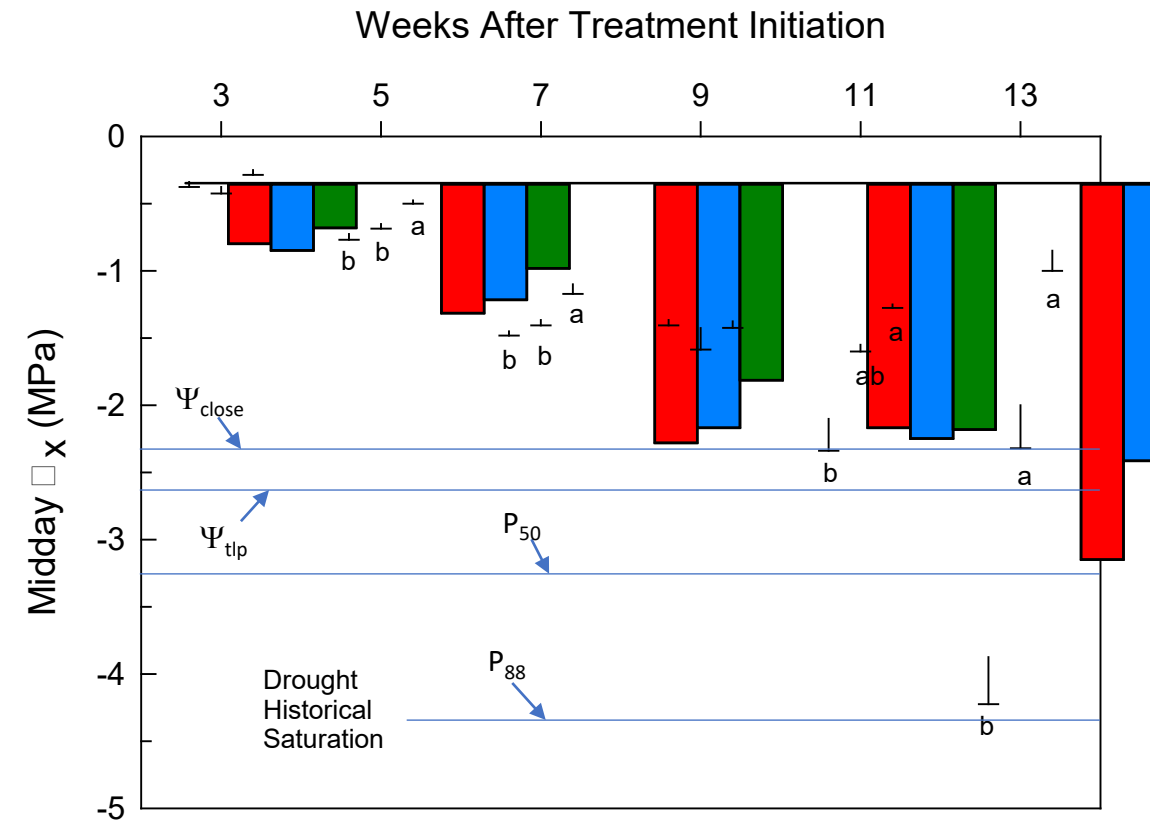


6 months post treatment

Treatment	Root Mass (g)	Root Mass Growth (g)
Saturation	7.20 ± 0.59	4.10 ± 0.63
Historical	5.61 ± 0.64	4.22 ± 0.69
Drought	5.02 ± 0.74	3.86 ± 0.85
p-value	0.178	0.902

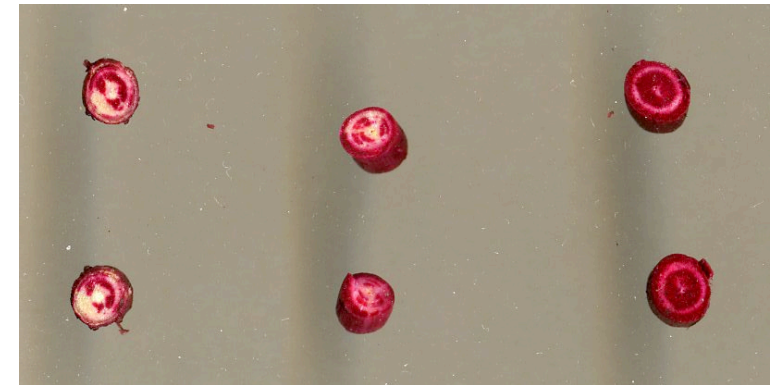
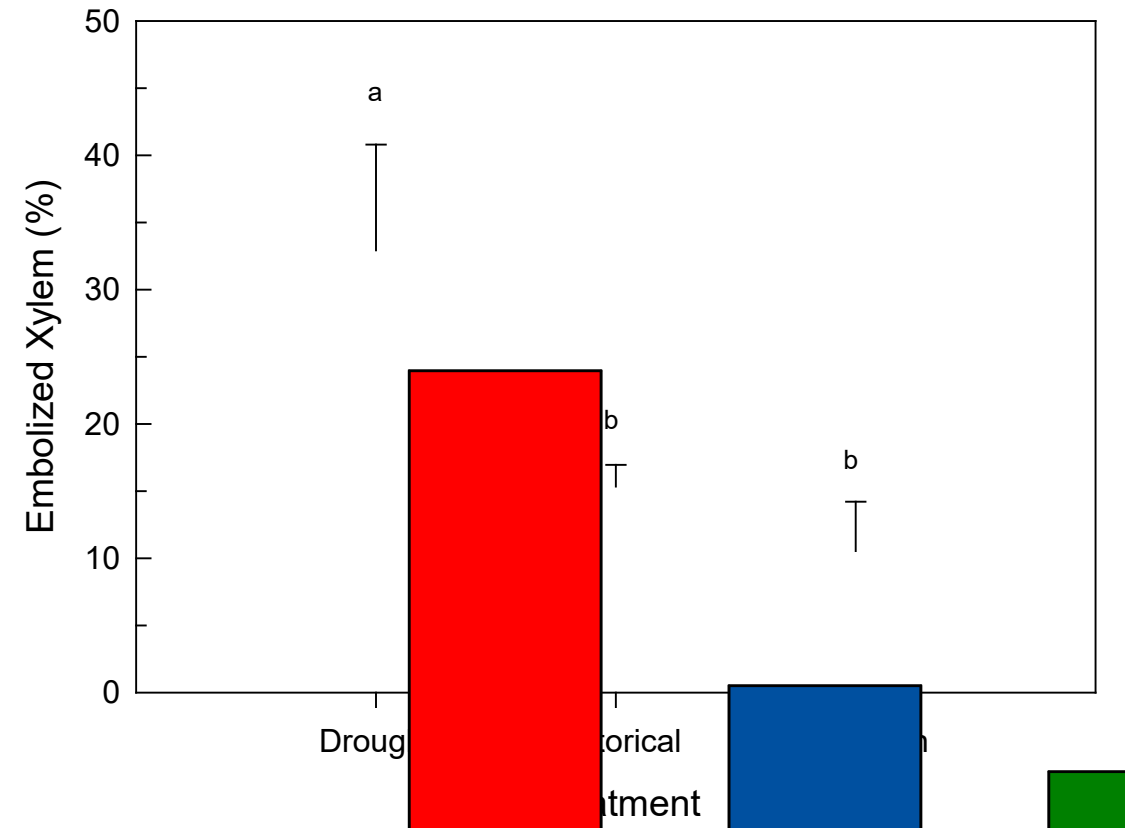
# Results

- Treatment phase:
  - By week 13,  $\Psi_x$  in the drought treatment was decreased by 355% compared to the saturation treatment.
  - Shown is an estimate of  $\Psi_{tlp}$  (-2.6 MPa). When  $\Psi_x$  declines below  $\Psi_{tlp}$ , damage to both seedling physiology and cell structure can occur.
  - Estimates of  $P_{50}$  and  $P_{88}$  are shown for mature loblolly pine at -3.3 and -4.4 MPa, respectively.
  - In 2-year-old loblolly pine,  $\Psi_{close}$  has been estimated at -2.3 MPa. (90% stomatal closure).
  - Stomatal closure possibly resulted in reductions of root and shoot biomass.



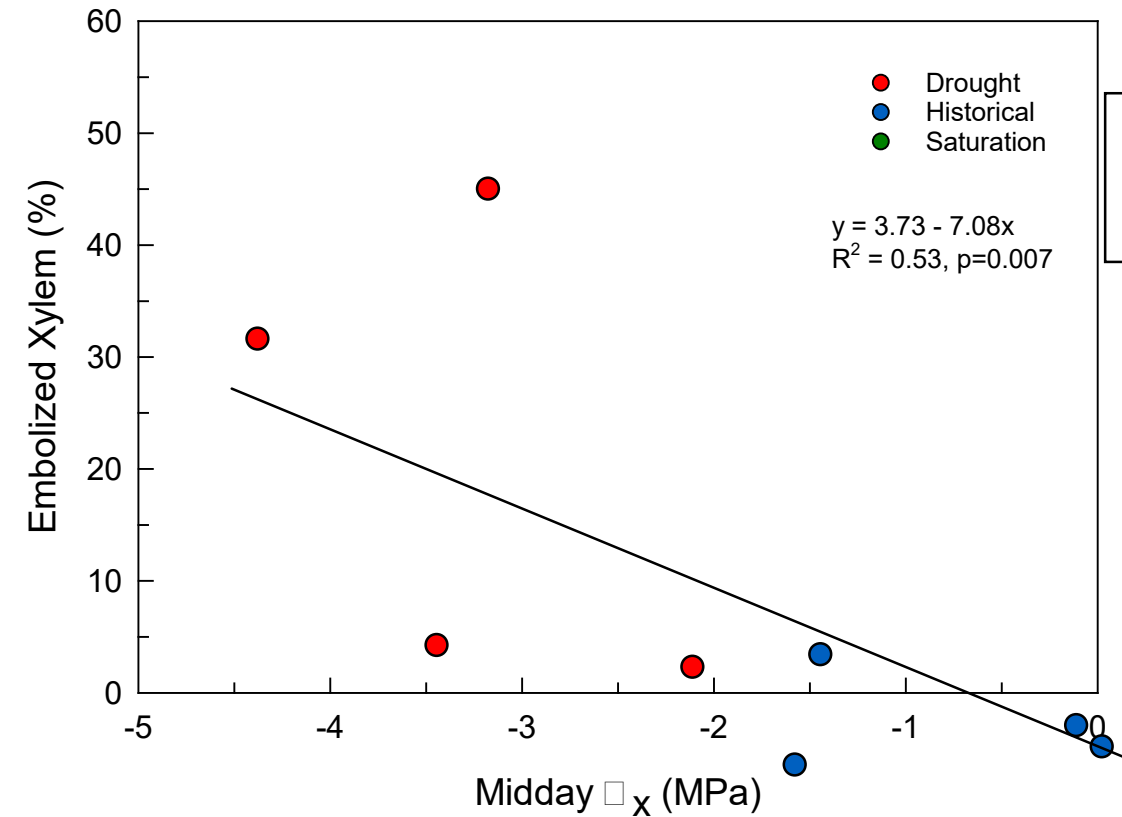
# Results

- Six months post treatment:
  - Percent embolized conducting tissue in the drought treatment was 33% higher than the historical and saturation treatment.
  - Possible evidence of lack of refilling mechanism in loblolly pine.



# Results

- Six months post treatment:
  - Percent embolized xylem 6 months after drought alleviation was linearly related to midday  $\Psi_x$  at the end of the treatment phase.





# Management Implications

- Under drought conditions, drought hardened seedlings had reduced size, growth and  $\Psi_x$  reached critical levels of hydraulic failure.
- Loblolly pine in this study demonstrated a lack of an efficient mechanism to refill embolized xylem conduits which can delay growth and predispose them to future stress events



## RESEARCH REPORT 21-04

### IMPACTS OF PLANTING DROUGHT HARDENED LOBLOLLY PINE SEEDLINGS UNDER VARIOUS DROUGHT CONDITIONS

by  
Tom Stokes, Ryan Nadel, Nina Payne, and Scott Enebak

#### INTRODUCTION

Increases in temperature combined with greater frequency of drought events has led to more unfavorable planting conditions for forest seedlings (Souden et al. 2019, Rehschuh et al. 2020) in the southern United States. At outplanting, seedlings may experience abiotic and biotic stresses that lead to limited growth or seedling mortality (Kozolowski and Davies 1975). Several factors can play a role in a seedling's ability to overcome such stresses, such as root system size and architecture, root-soil contact, and root hydraulic conductivity (Grossnickle 2005). This is required, as a recently planted seedling's root system must adequately supply water throughout the stem and out the transpiring foliage (Grossnickle 2005).

Forest nurseries sometimes use the practice of drought hardening seedlings to reduce the adverse effects of moisture stress when planted in dry conditions (Zhang et al. 2018). Drought hardening is achieved by exposing seedlings to drought conditions in the later stages of seedling culture by reducing irrigation thereby, theoretically, increasing the seedling's ability to adapt to subsequent droughts (Thomas 2009). The practice of drought hardening seedlings began in the first half of the 20<sup>th</sup> century with the establishment of a nursery in a relatively arid region in the Pacific Northwest with the hopes of producing seedlings that would have greater resistance to drought (Engstrom 1949, South and Nadel 2020). This practice was further validated when Lavender and Cleary (1974) published a graph indicating greater survival of drought stressed seedlings compared to non-stressed seedlings although no data was presented to support their findings (South and Nadel 2020).

While some studies have shown morphological and physiological adjustments in seedlings from drought hardening that could be beneficial (Sloan et al. 2020, Seiler and Johnson, 1985), very few studies have shown increased growth and survival of seedlings planted under dry conditions after drought hardening has occurred (Salvador et al. 2004, Morgan et al. 1984, Pita and Pardos 2001). In fact, studies have shown that increased survival does not occur with reductions in summer or fall irrigation in many bareroot *Pinus* species (South and Nadel 2020, Minko 1976, Williams et al. 1988, Dierauf and Chandler 1991).

Even with the possibility of beneficial physiological adjustments to seedlings, there are still a number of questions on the short- and long-term effects of drought hardening on forest tree seedlings. For example, drought stressing



# Future Work

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- Repeat this study with and without drought conditioned seedlings.
- Determine whether drought conditioning has greater harm than benefit.
- Determine if there is an optimum level of drought conditioning





# Future Work

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- 3 families of one-year old containerized loblolly pine.
- 3 treatments
  - **Control** – no drought conditioning
  - **DC-WP** – Three sequential cycles of drought conditioning targets defined by predawn water potential measurements
  - **DC-CW** – Three sequential cycles of drought conditioning targets defined by container weights.
- 4 replications.





# Future Work

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- Phases
  - Drought Conditioning Phase
  - Outplanting
  - Early Drought
    - No water?
  - Recovery
  - Late Drought?





# Future Work

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- Measurements
  - Stem Water Potentials
    - Conditioning
    - Drought
    - Recovery
  - Container Weights
    - Conditioning
  - Xylem Embolisms
    - Completion of conditioning
    - Completion of drought
    - Completion of recovery
  - Chlorophyll Fluorescence?



# Future Work



- Additional work
  - Chlorophyll Fluorescence
    - Determine if CF can be used to detect levels of water stress, or
    - If a threshold value of water stress can be determined where catastrophic hydraulic failure occurs.

# Questions?

