Southern Forest
Nursery Management Cooperative

Planting Drought Hardened Loblolly Pine Seedlings Under Various Drought Conditions

Tom Stokes

Southern Forest Nursery Management Cooperative 2021 Contact Meeting

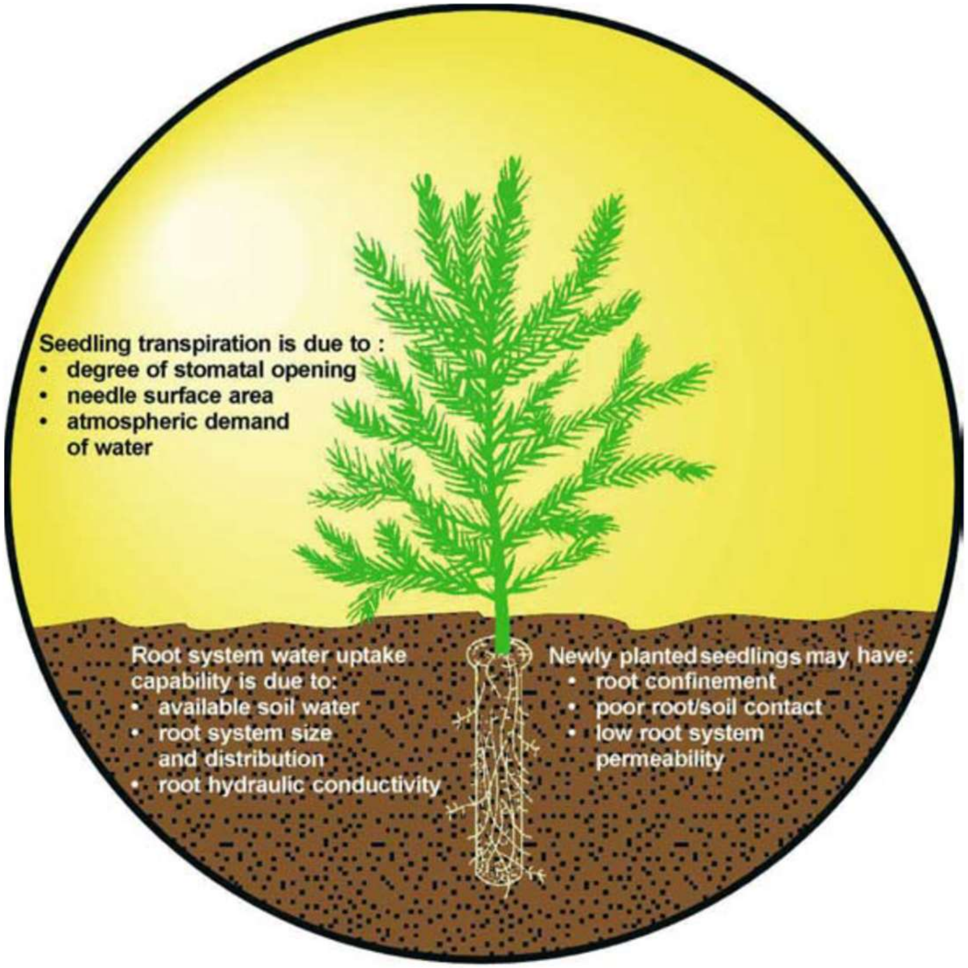
Introduction

- 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 1st half of the 20th 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

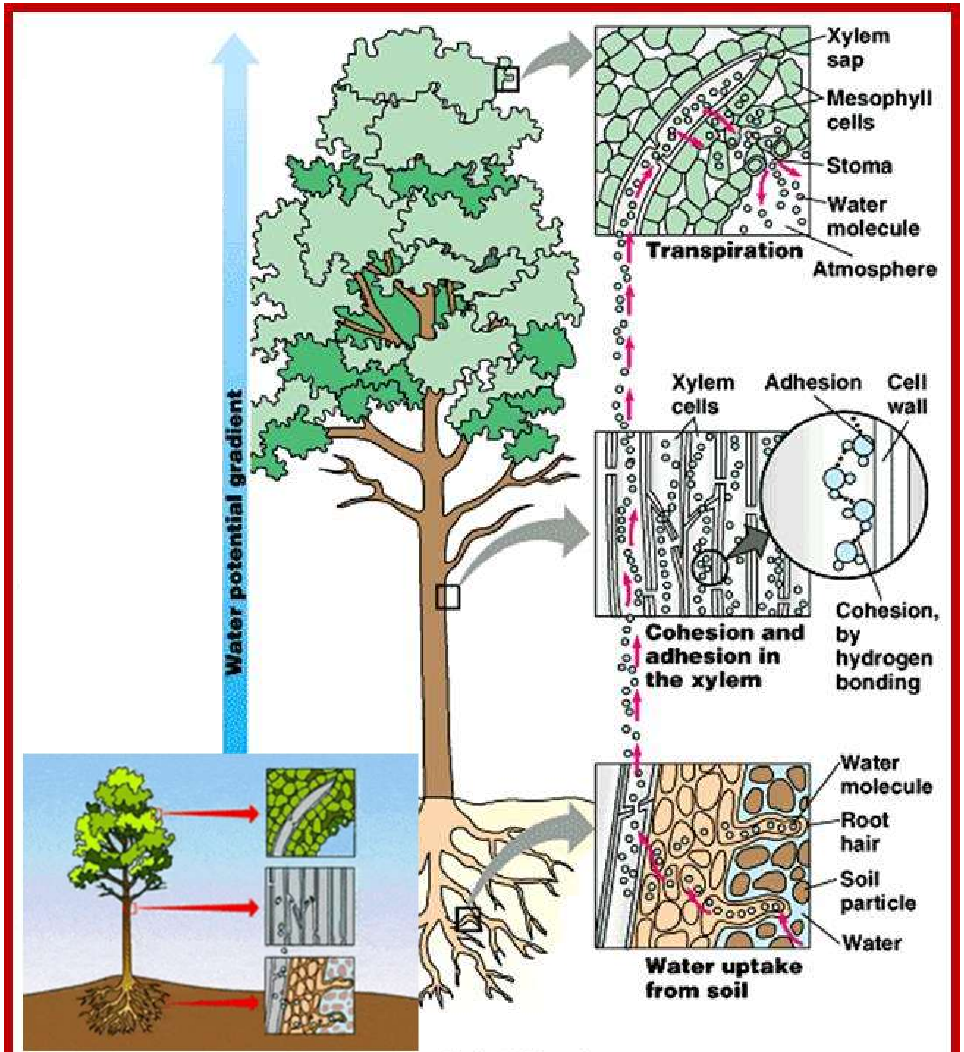
- Risks of Drought Conditioning
  - Depletion of stored carbohydrates
  - Increase in cavitated xylem conduits
  - Predispose to future stress events (legacy effects).

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

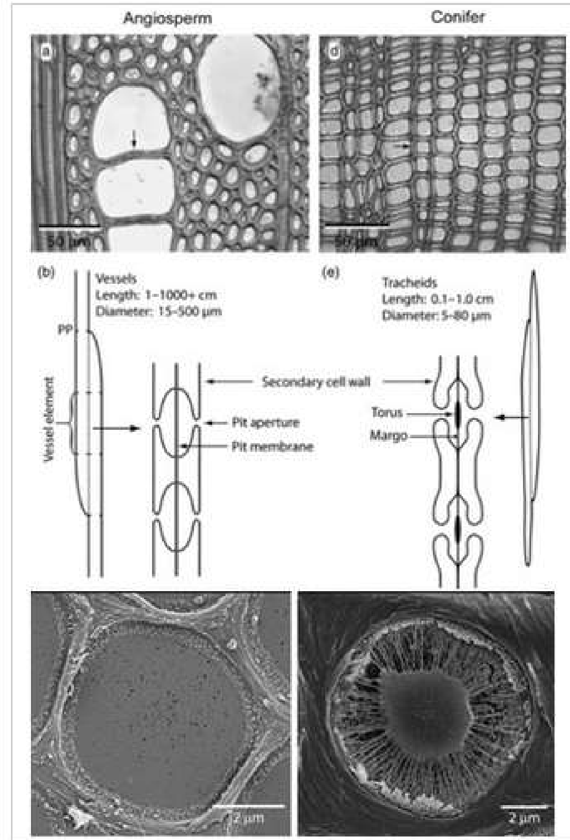


Figure 6: Comparison of different types of wood from flowering and cone-bearing plants.
This features wider conduits from flowering plants (top), a cartoon reconstruction of vessels, tracheids and their pit membranes (middle), which are also shown in SEM images (bottom).

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

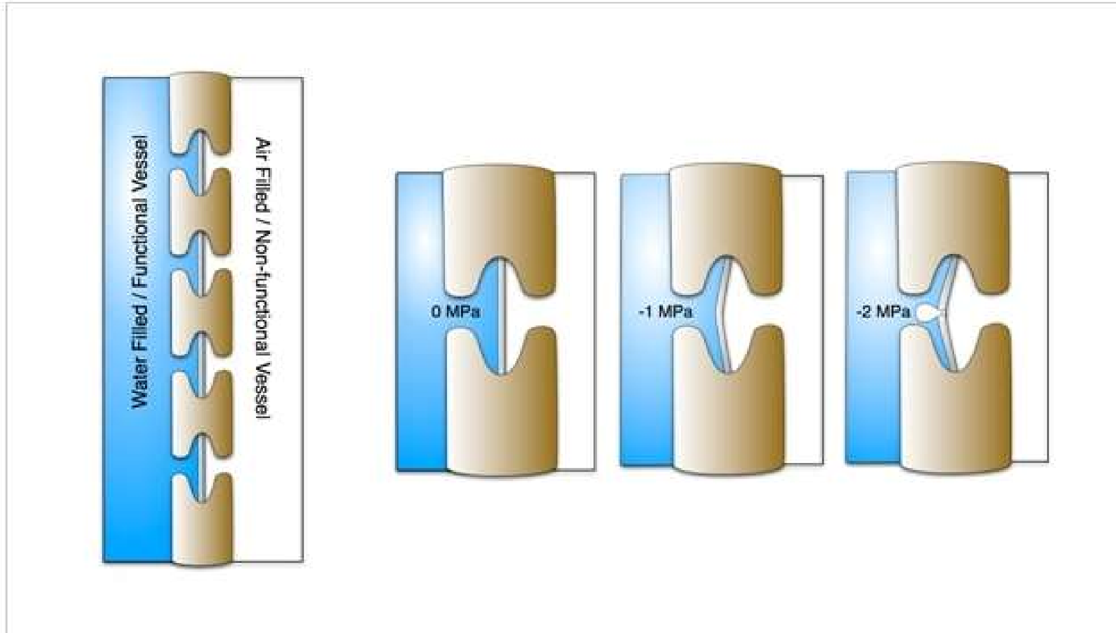


Figure 9: Air seeding mechanism.
Demonstrates how increasing tension in a functional water filled vessel eventually reaches a threshold where an air seed is pulled across a pit membrane from an embolized conduit. Air is seeded into the functional conduit only after the threshold pressure is reached.

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.

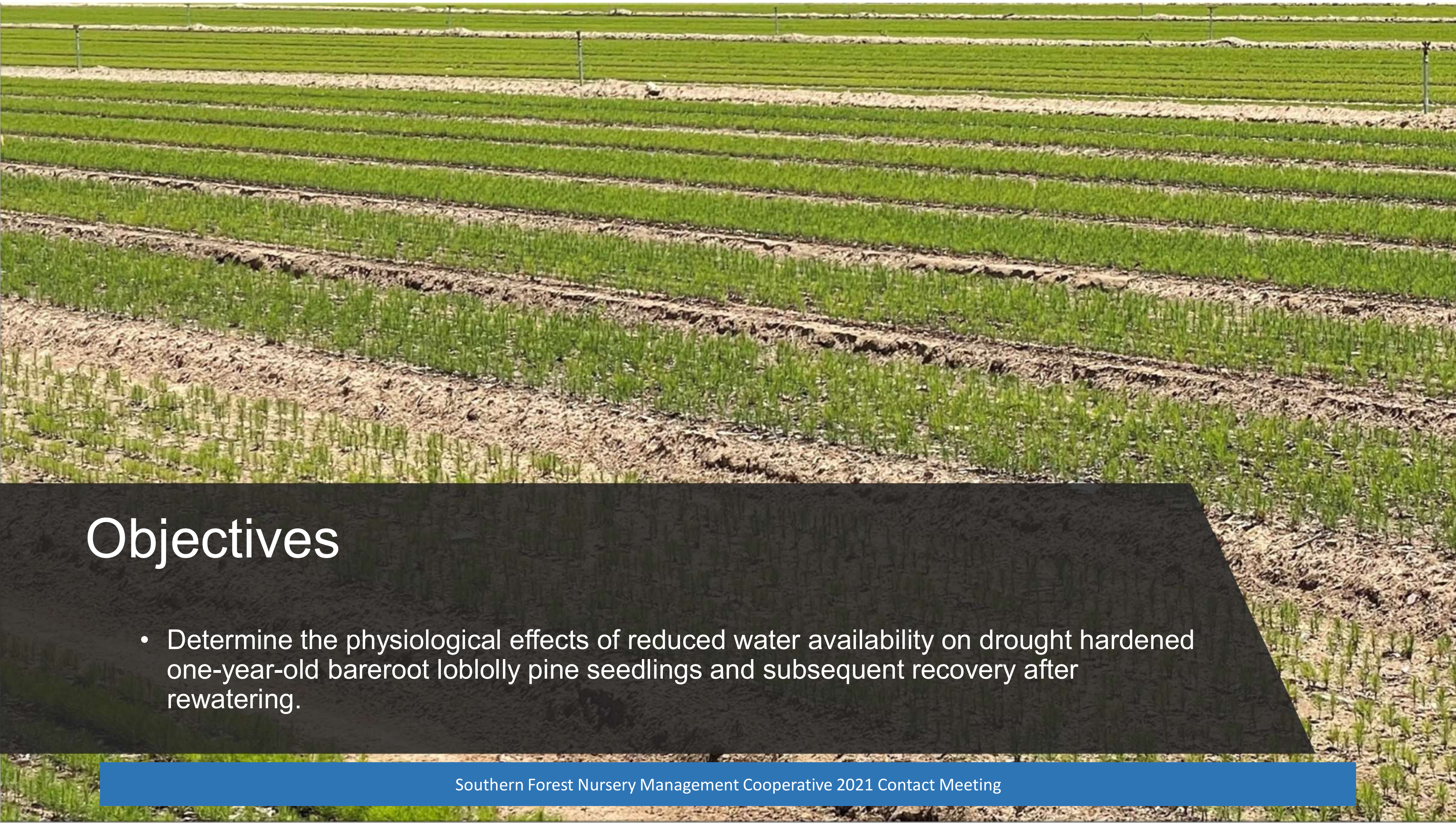
  - Several recent studies, especially with conifers, have shown a lack of a mechanism to refill embolized xylem conduits.
    - New xylem will have to be made – significant carbon cost to the plant.

Introduction

- Vulnerability to embolism within a plant

Roots → **Needles** → **Branches** → **Stems**

Wake Up

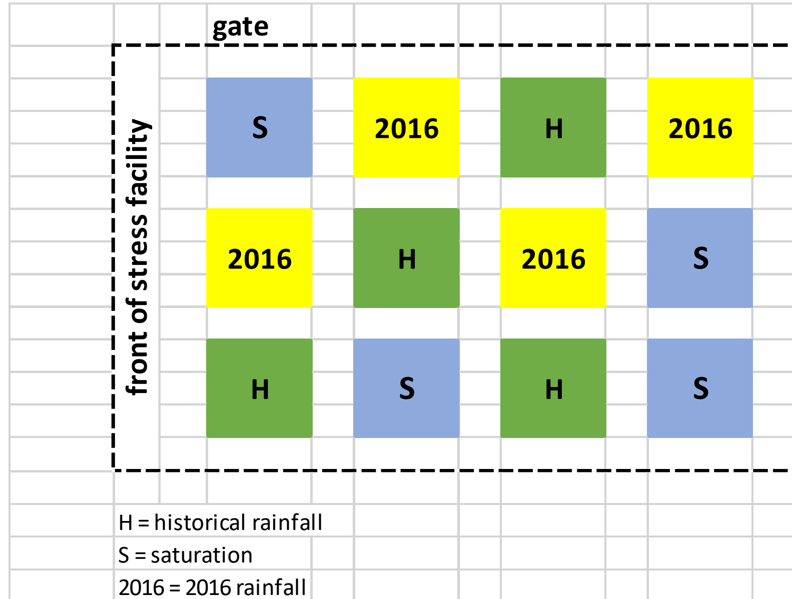


Objectives

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

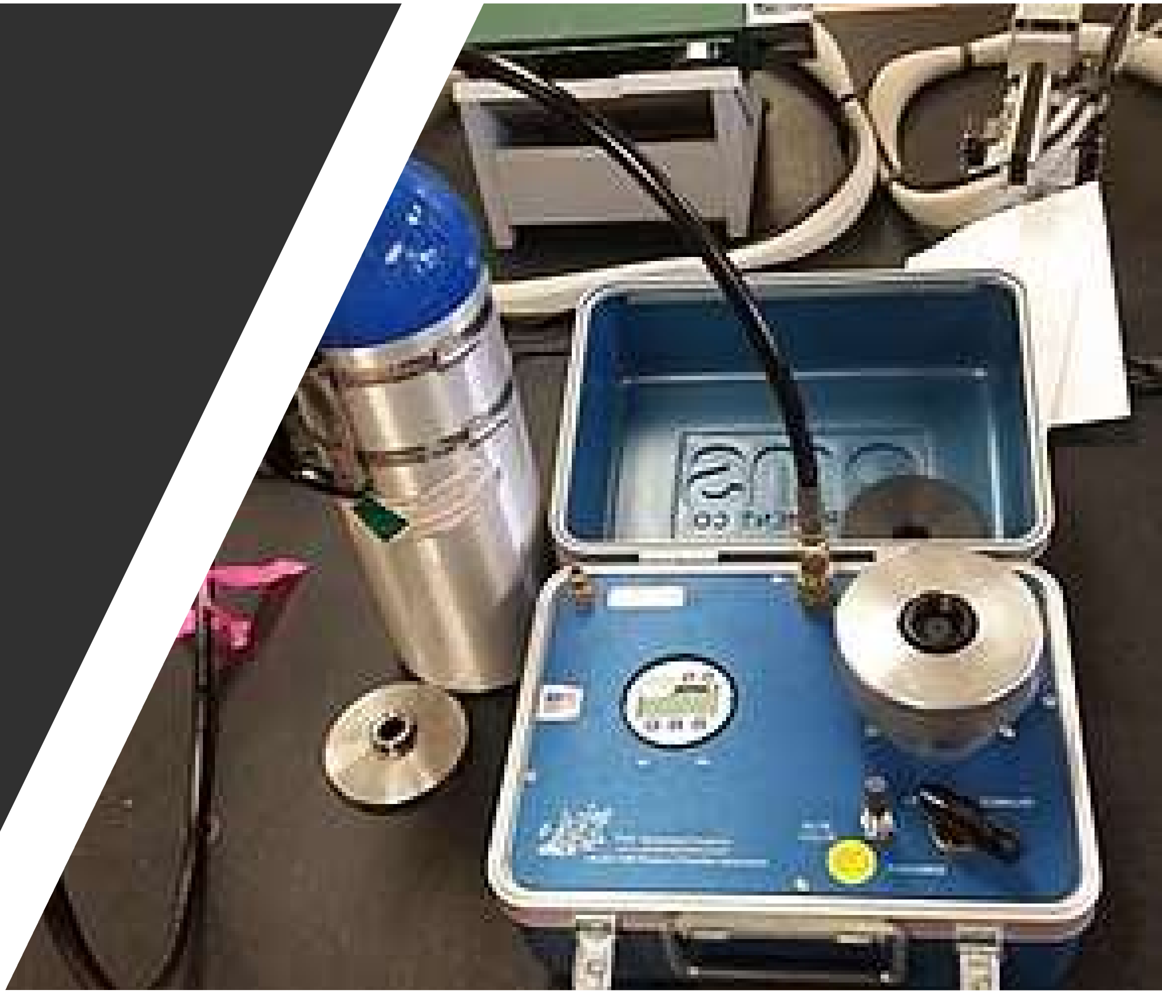
Methods

- 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



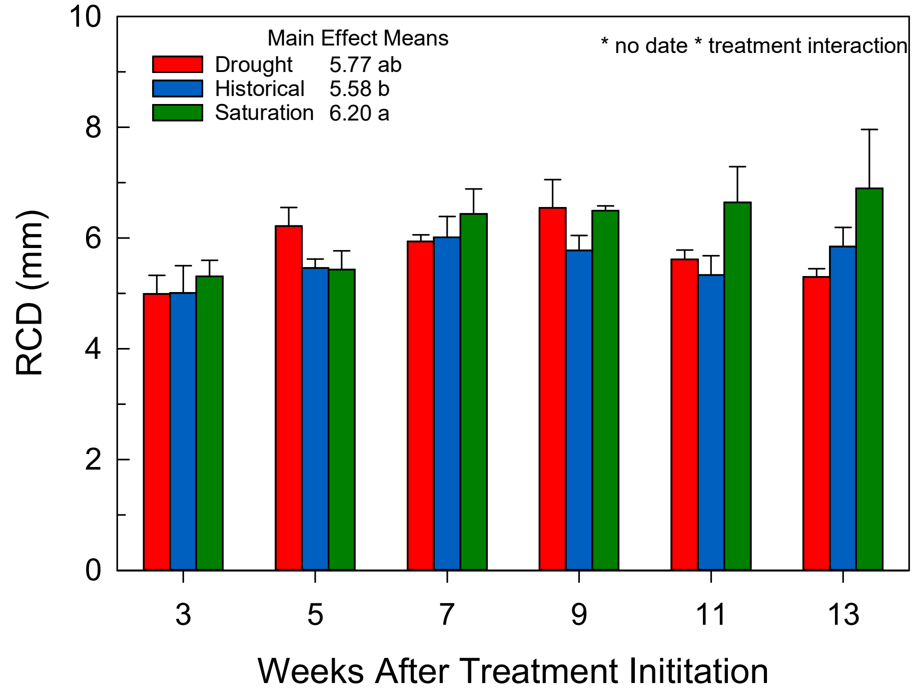
Methods

- 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%

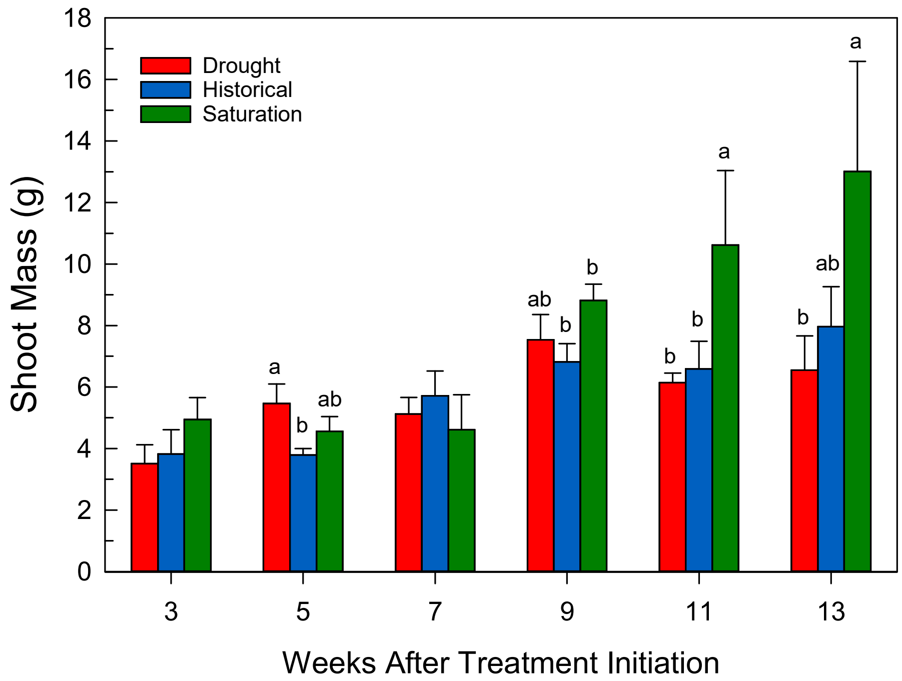


6 months post treatment

Treatment	RCD (mm)	RCD Growth (mm)
Saturation	12.06 ± 0.42 a	5.16 ± 0.67
Historical	10.07 ± 0.59 b	4.22 ± 0.47
Drought	9.18 ± 0.42 b	3.88 ± 0.28
p-value	**0.023**	0.133

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

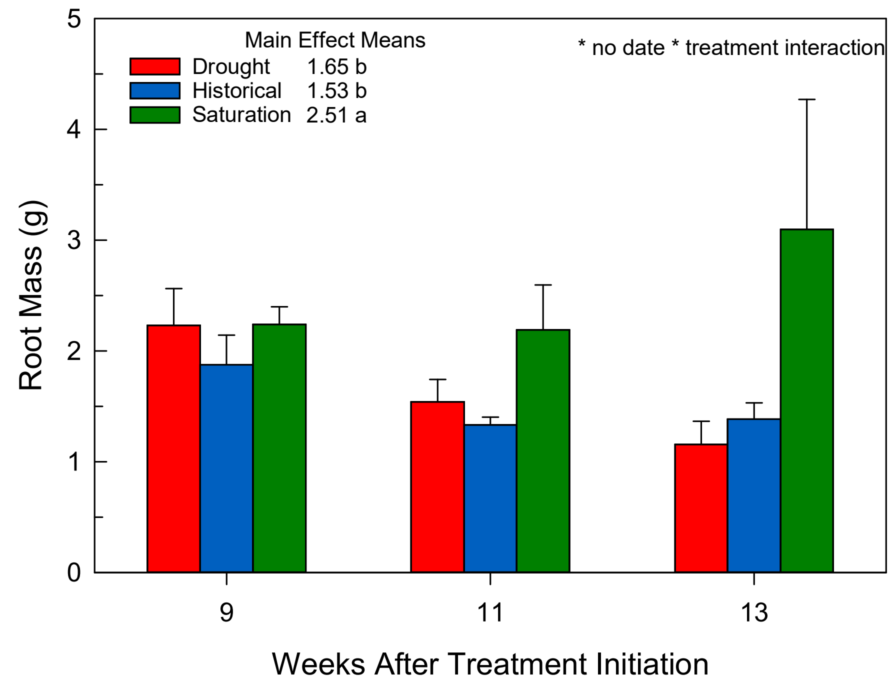
Treatment	Shoot Mass (g)	Shoot Mass Growth (g)
Saturation	51.09 ± 3.69	38.08 ± 3.63
Historical	42.76 ± 5.57	34.79 ± 6.38
Drought	33.92 ± 2.27	27.36 ± 1.58
p-value	0.088	0.249

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

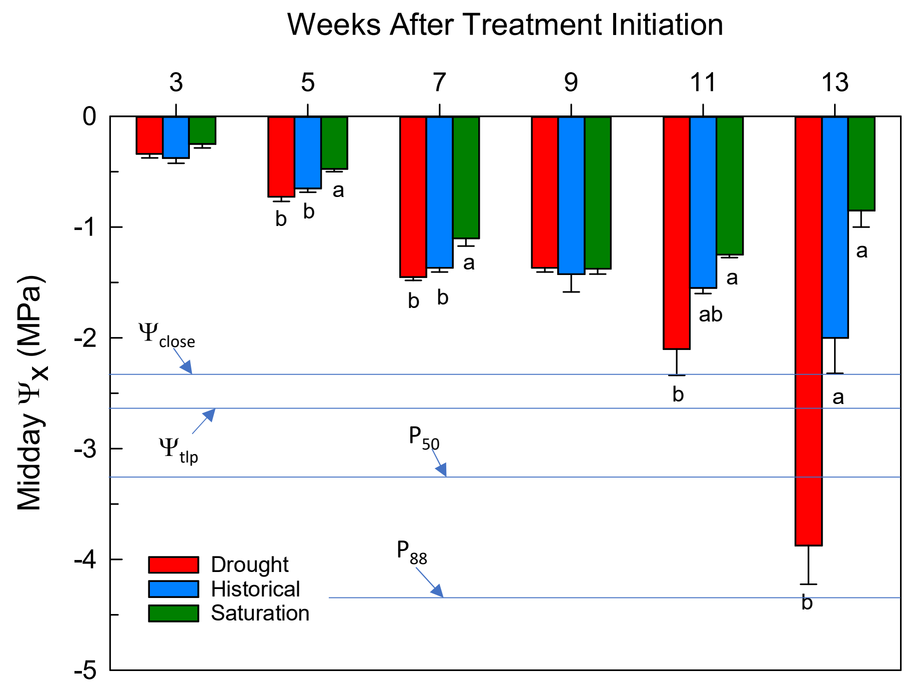


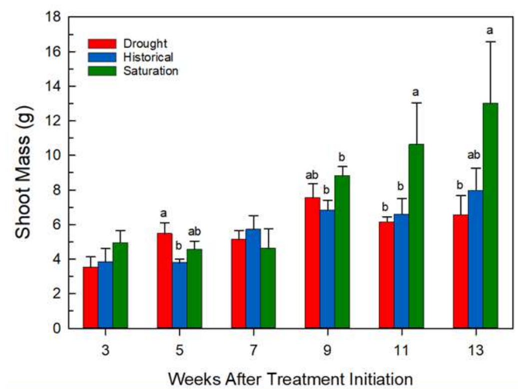
6 months post treatment

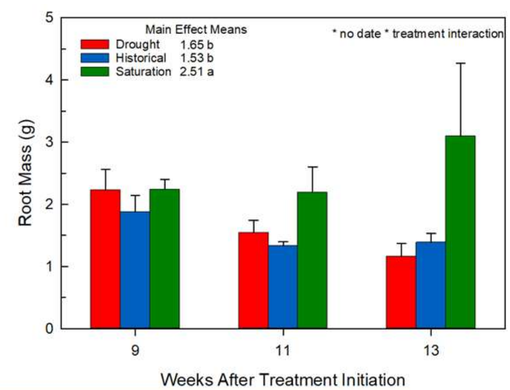
Treatment	Root Mass (g)	Root Mass Growth (g)
Saturation	7.20 ± 0.59	4.10 ± 0.63
Historical	5.61 ± 0.64	4.22 ± 0.69
Drought	5.02 ± 0.74	3.86 ± 0.85
p-value	0.178	0.902

Results

- Treatment phase:
  - By week 13, Ψ_x in the drought treatment was decreased by 355% compared to the saturation treatment.
  - Shown is an estimate of Ψ_{tlp} (-2.6 MPa). When Ψ_x declines below Ψ_{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, Ψ_{close} has been estimated at -2.3 MPa. (90% stomatal closure).
  - Stomatal closure possibly resulted in reductions of root and shoot biomass.

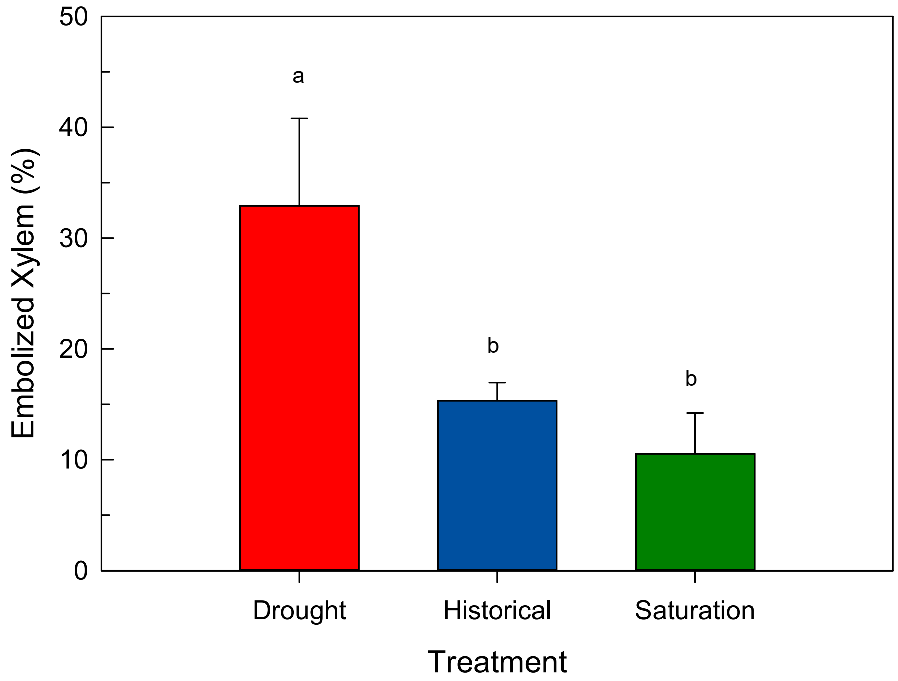






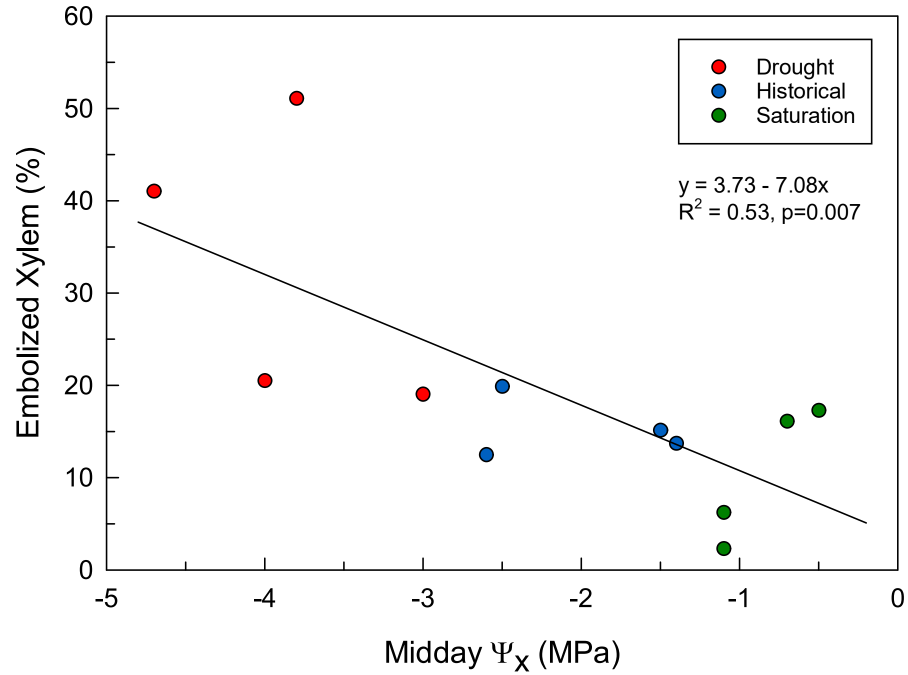
Results

- Six months post treatment:
  - Precent 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 Ψ_x at the end of the treatment phase.



Management Implications

- Under drought conditions, drought hardened seedlings had reduced size, growth and Ψ_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

Future Work

- 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

Questions?

Rapid Determination of Freeze Damage to Loblolly Pine Seedlings

Tom Stokes

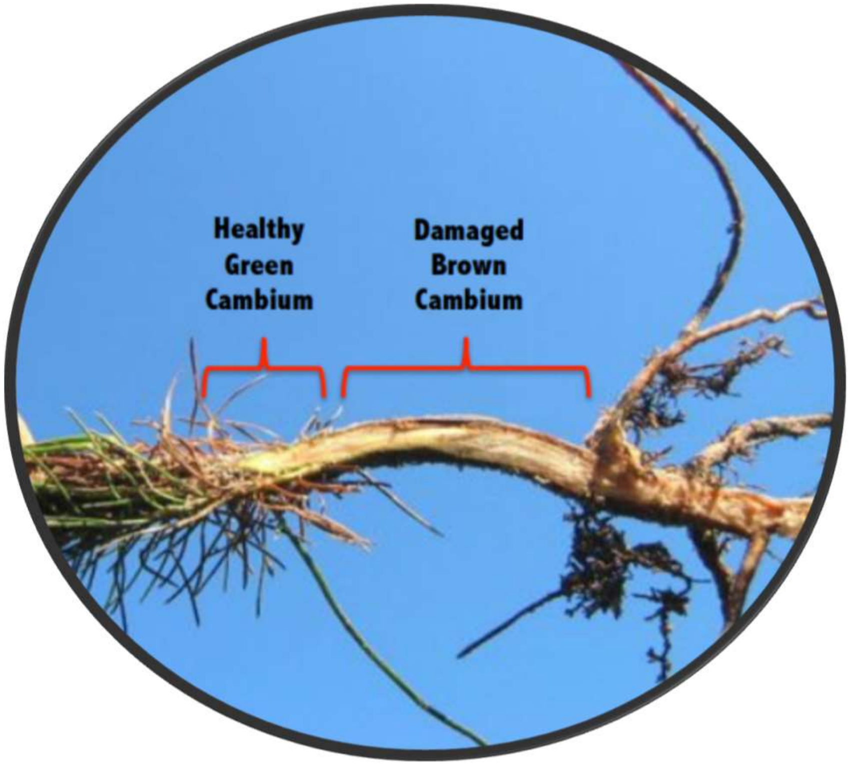
Southern Forest Nursery Management Cooperative 2021 Contact Meeting

Introduction

- Uncontrollable weather conditions, such as a freeze event, can result in significant seedling mortality regardless of seedling quality, site preparation or planting.
- Of particular concern is sudden freeze events immediately preceded by above normal warm temperatures.
- Southern pines can de-acclimate within hours of unseasonable warm weather.

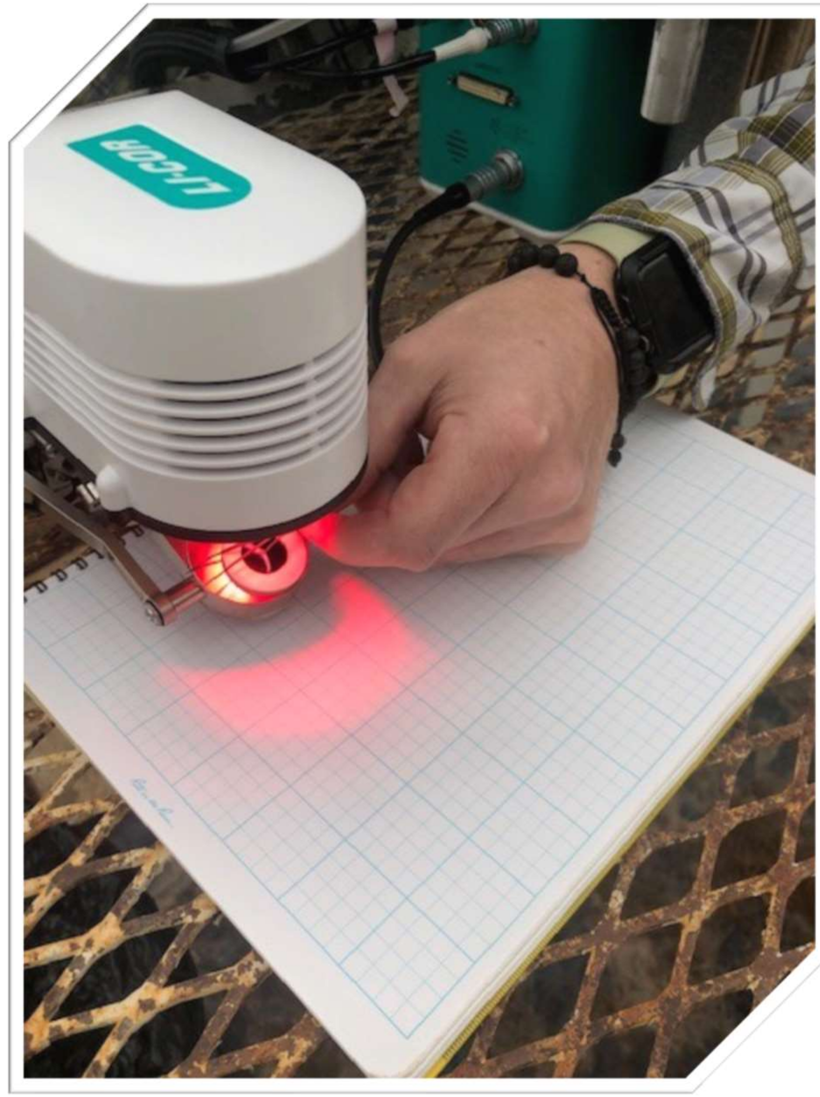
Introduction

- Why we need a rapid, low-cost tool to evaluate freeze damage.
  - Visual damage, either tip dieback or stem and root discoloration, can take more than 2 weeks prior to appear.
  - Significant reductions in survival can occur by outplanting seedlings that are unknowingly damaged by freeze events.
  - With increasing warm weather and sudden freeze events happing during lifting season, decisions of whether seedling were damaged in the nursery become more time sensitive.



Introduction

- Chlorophyll fluorescence can not only evaluate a plant's ability to tolerate low temperatures but can also determine the **extent** of damage caused by freeze stress.



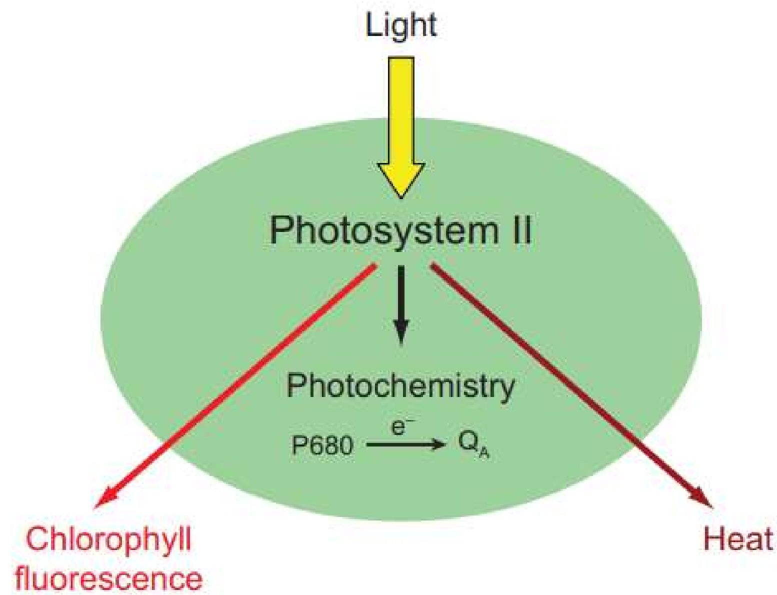
Introduction

- What is chlorophyll fluorescence?

  - Unused absorbed light that enters PSII can be lost either to heat or chlorophyll fluorescence.

  - Cold temperatures can disrupt all major components of photosynthesis.
    - Membrane damage
    - Oxidative stress
    - Electron capture
    - Quantum yield of electron transport

  - This damages causes PSII to become less efficient.



Introduction

- How do we measure chlorophyll fluorescence?
  - Using a chlorophyll fluorometer with pulsating intense light, we measure the ratio of variable fluorescence to maximum fluorescence (F_vF_m).
  - F_vF_m provides a measure of the maximum efficiency of PSII, thus giving information of seedling damage to PSII by cold temperature stress.
  - Unstressed seedlings loblolly pine can have F_vF_m of **0.60 to 0.80.**
  - Damage to PSII by stress **reduces** F_vF_m.

Objective

- Determine if chlorophyll fluorescence could be used as a rapid tool to measure and evaluate the extent of freeze damage immediately after a controlled freeze event in loblolly pine seedlings.
- To predict eventual seedling damage (mortality, growth reduction, etc.) by using the immediate reductions in $F_v F_m$ caused by the freeze event.

Methods

- Study Design
  - Nine genetic families representing three provenances (Coastal, Piedmont and Northern) of one-year old containerized seedlings.
  - 2 seedlings from each family randomly assigned to 4 replications of 2 treatments (freeze and control)

Maternal Parent	Paternal Parent*	# of Genetic Families	MWT (°F)
Coastal Provenance	Coastal pollen mix	3	15.4 - 21.4
Northern Provenance	Northern pollen mix	3	5.1 – 8.0
Piedmont Provenance	Piedmont pollen mix	3	9.3 – 10.6

*The pollen mix for each provenance comprised of pollen from 20 trees common to that region

Methods

- Treatments
  - Control – no freeze
  - Freeze – controlled freeze
    - Seedlings were placed in chest freezer at 50°F for 1 hour.
    - Temperature was adjusted to 5°F at a rate of 9°F per hour
    - Seedlings were brought back to 50°F at a rate of 9°F per hour

Methods

- Measurements
  - Initial measurements of heights and RCD.
  - Measurements of F_vF_m
    - Just prior to control freeze
    - 1, 3, 6, 8, 10 and 13-days post freeze.
  - Visual assessment of foliar damage 14 days post freeze
  - Mortality assessment 41 days post freeze
  - Outplanted after 41 days post freeze to monitor growth.

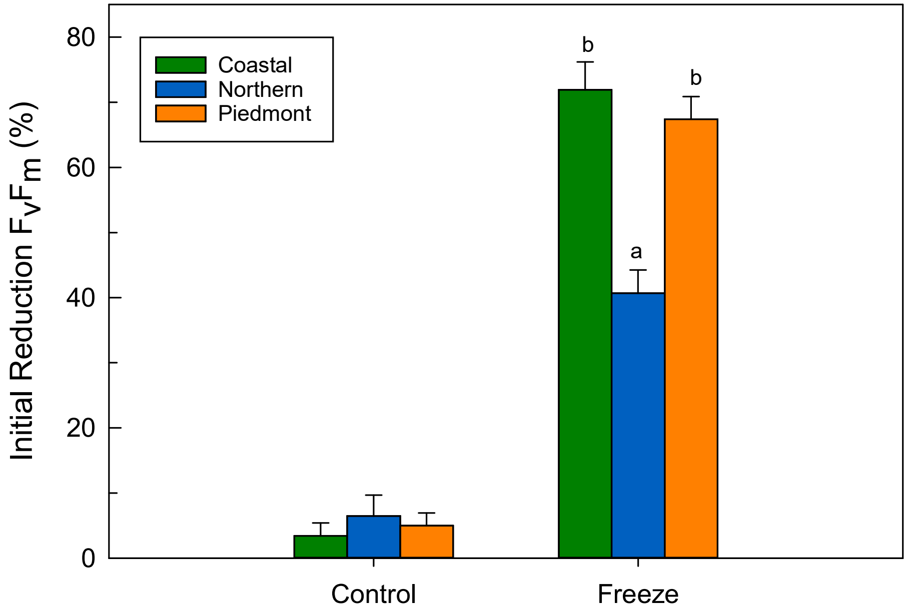
Results

- Average initial RCD and height was 4.22 mm and 20.8 cm, respectively, across both treatments
- Piedmont provenance had slightly smaller RCD.
- Average initial F_vF_m was 0.602 across both treatments.

	Initial RCD (mm)	Initial HGT (cm)	Initial F_vF_m
Provenance			
Coastal	4.26 a	20.0	0.599
Northern	4.36 a	21.0	0.600
Piedmont	4.04 b	21.3	0.616
Treatment			
Control	4.17	21.1	0.593
Freeze	4.27	20.5	0.611

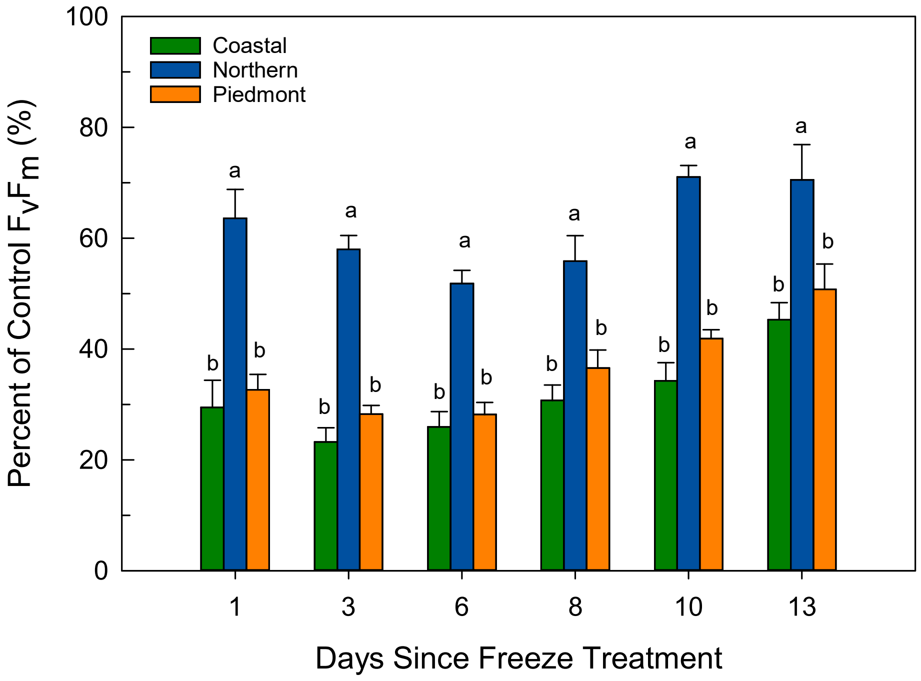
Results

- The experimental freeze reduced F_vF_m from 41 to 72%.
- The Northern provenance sustained less damage than both the Coastal and Piedmont provenances.



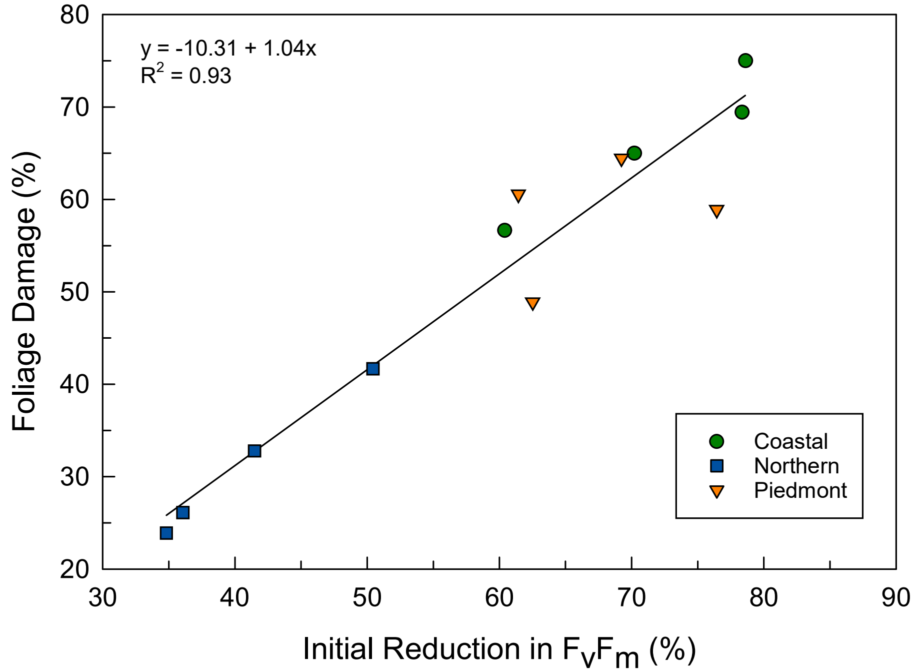
Results

- All provenances tended to show reductions in F_vF_m until 6 days after the experimental freeze then started showing signs of recovery.
- On average across the 13 days post-freeze, F_vF_m of the Northern provenance was 62% of control (F_vF_m =0.350).
- Average F_vF_m in the Coastal and Piedmont provenances were 34% of control (F_vF_m =0.203).



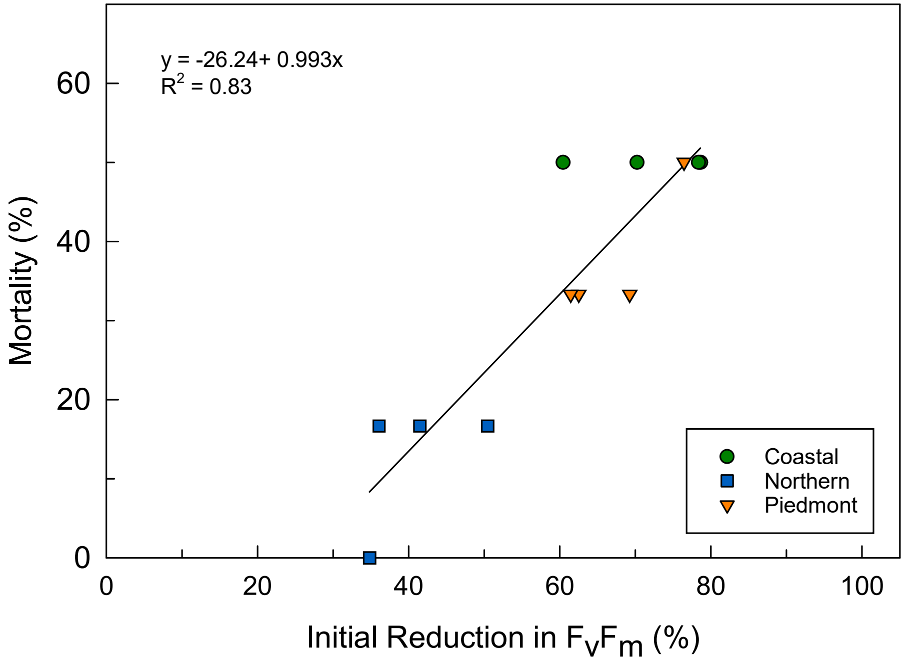
Results

- Across all genetic families there was a strong linear relationship between initial reduction in F_vF_m and percent foliar damage 14 days post-freeze.



Results

- Across all genetic families, initial reduction in F_vF_m was related to mortality 41 days post-freeze.



Results

- Outplanting growth and survival
- Control seedlings had 100% survival after outplanting while seedlings in the Freeze treatment had 0% survival.
    - Temperature fell below freezing 4 days after outplanting followed by warm sunny days.
    - We wanted to push seedlings to make sure chlorophyll fluorescence would pick up damage, so freezing to 5°F was extreme.
    - Damage caused by this extreme freeze along with the freezing temperatures after outplanting was likely too much to overcome with the added stress of outplanting.

Management Implications

- Loblolly pine seedling freeze damage changes the photochemistry in PSII which is detectable with chlorophyll fluorescence.
- Future freeze damage was related to immediate reductions in F_vF_m caused by the experimental freeze.
- Mortality, before outplanting, was related to immediate reduction in F_vF_m caused by the experimental freeze.
- Results suggest that chlorophyll fluorescence may be used as a rapid, low-cost tool to quicky assess freeze damage in loblolly pine seedlings so that nurseries have the tools needed to make informed decisions on seedling quality.

Future Work

- More realistic freeze common to the region (we need some survival and some mortality).
  - Low 20's°F?
  - Multiple (3 nights) freeze with warming during day?
- Determine recovery time?
  - We need to determine how long survivable seedlings need to recover before outplanting.
  - What is the maximum time nurseries have to make the decision to sale seedlings?

TREE IMPROVEMENT PROGRAM NC STATE UNIVERSITY

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Questions

FUSIFORM RUST CONTROL FUNGICIDES

Tom Stokes

Southern Forest Nursery Management Cooperative 2021 Contact Meeting

Fusiform Rust

- *Cronartium quercuum* f. sp. *fusiforme* the causal agent of Fusiform rust is still of major concern to many loblolly and slash growers.
- Both genetic and cultural options are available to reduce the risk of this disease, but the most effective control is the use of fungicides
- The Nursery Coop in 1980 was instrumental in the registration of Bayleton® with the incidence of rust fell from 2.5 to 0.1% due to the use of this compound. Fungicide usage fell from 4 to < 1 lb/ac/yr due to the reduced number of applications.
- The Nursery Coop continued to look for alternative chemistries to assist with Fusiform control and was instrumental in the registration of Proline® in 2011.

Proline® as a seed treatment

- As a seed treatment, current labelled rate is 10 fl oz./50 lb of seed.
- These labelled rates have been tested and shown to provide optimum activity.

Greenhouse Trials:

2019 Active Ingredients tested for foliar spray in greenhouse

Fungicide	Manufacturer	Active Ingredient	Rate Tested
BanBanner Max II®	Syngenta	Propiconazole – 14.3%	6 fl oz. per 100 gallons of water
Mural®	Syngenta	Azoxystrobin – 30%	3 oz. per 50 gallons of water
Proline®	Bayer Cropscience	Prothioconazole – 41%	5 fl oz. per acre

2020 Active Ingredients tested for foliar spray in greenhouse

Fungicide	Manufacturer	Active Ingredient	Rate Tested
Protect® DF	Nufarm	Mancozeb – 75%	4 fl oz. per 1000 ft^2
Hurricane®	Syngenta	Fludioxonil – 32% Mefenoxam – 16%	¾ oz. in 100 gallons of water
Proline®	Bayer Cropscience	Prothioconazole – 41%	5 fl oz. per acre

2021 Active Ingredients tested for foliar spray in greenhouse

Fungicide	Manufacturer	Active Ingredient	Rate Tested
Protect® DF	Nufarm	Mancozeb – 75%	4 fl oz. per 1000 ft^2
Proline®	Bayer Cropscience	Prothioconazole – 41%	5 fl oz. per acre

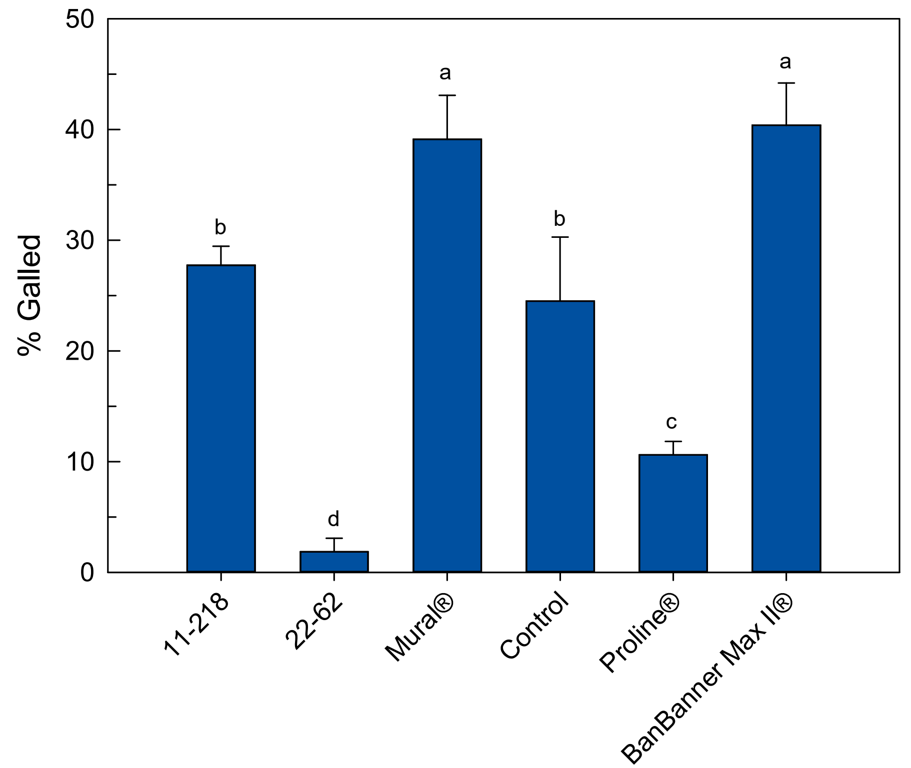
Seedling Treatment - Greenhouse

- Fungicide treatments applied to seedlings at Auburn Laboratories at 2 weeks post germination.
- Seedlings sent to Asheville, NC Rust Lab.
- Seedlings challenged with rust spores at 3 weeks post germination.
- 3 and 6 month evaluations made by NC Rust Center.


Fusiform Rust

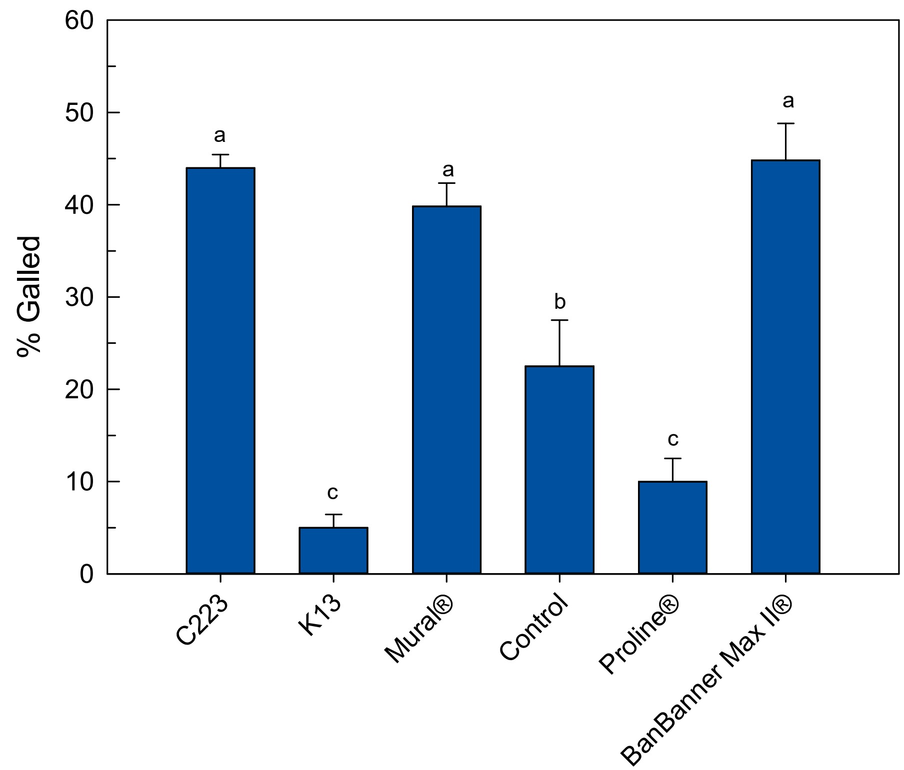
2019 Loblolly Seedling Treatment Results

- Active ingredients Azoxystrobin + Benzovindiflupyr (Mural®) and Propiconazole (BanBanner Max II®) performed worse than untreated controls and therefore ineffective in reducing the incidence of Fusiform galls.



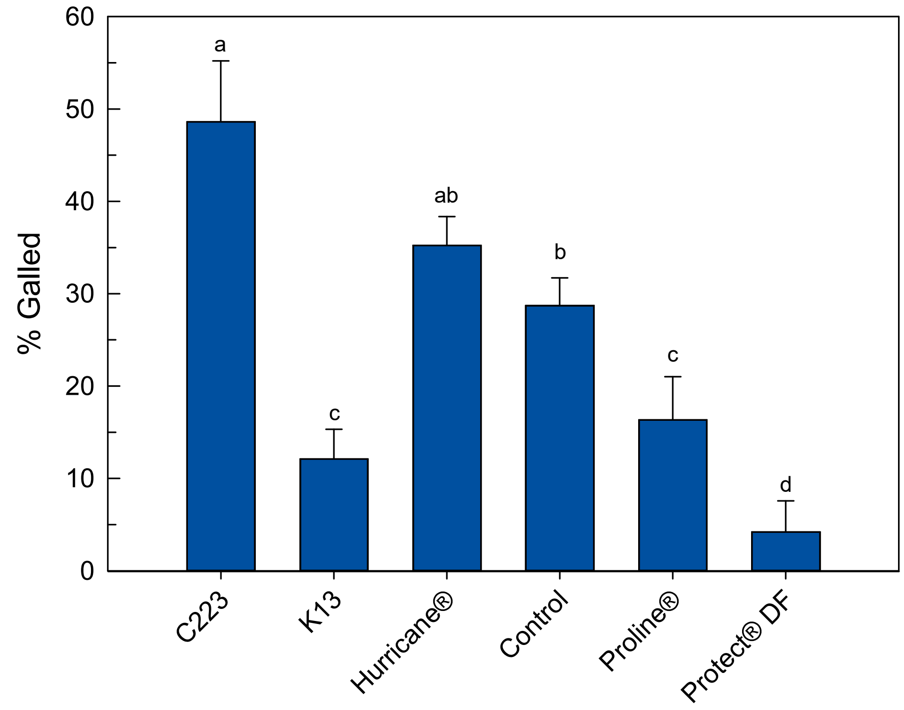
2019 Slash Seedling Treatment Results

- Active ingredients Azoxystrobin + Benzovindiflupyr (Mural®) and Propiconazole (BanBanner Max II®) performed worse than untreated controls and therefore ineffective in reducing the incidence of Fusiform galls.



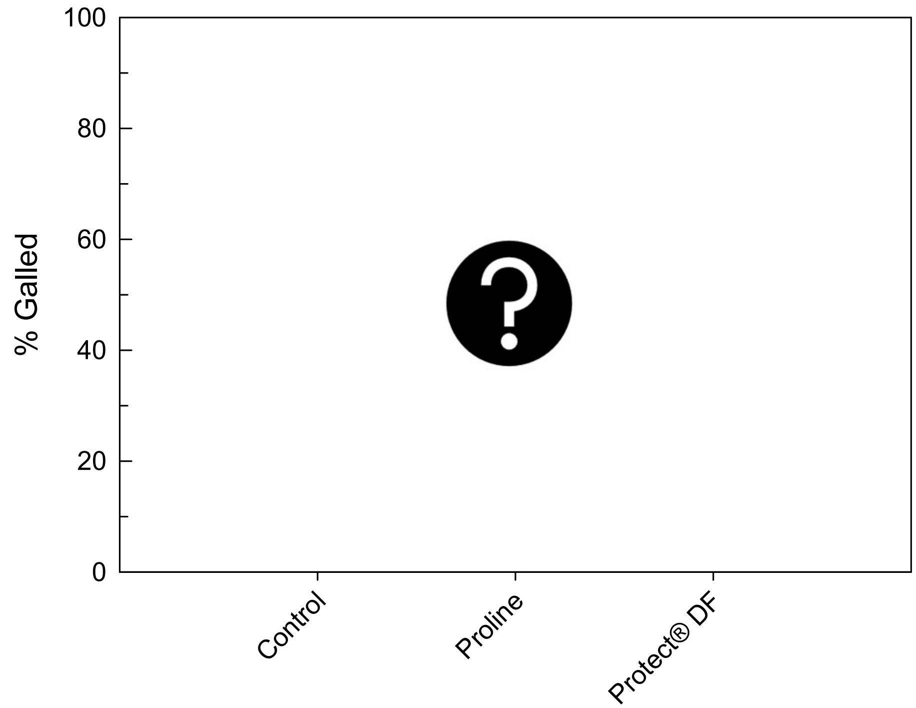
2020 Slash Seedling Treatment Results

- Active ingredients Mancozeb (Protect® DF) is effective in reducing the incidence of Fusiform galls on slash pine.



2021 Seedling Treatment Results

- Although Mancozeb (Protect® DF) was determined to be effective in slash pine, poor survival prevented an assessment of its performance on loblolly pine.
- We are currently repeating the treatment of Mancozeb (Protect® DF) in both slash and loblolly pine in 2021.



Field Trials:

Southern Forest Nursery Management Cooperative 2021 Contact Meeting

Active Ingredients tested as foliar spray

Fungicide	Manufacturer	Active Ingredient	Rate Tested
Compass®	Bayer Cropscience	Trifolxystrobin – 50%	3 oz. per acre
Stratego® 250EC	Bayer Cropscience	Propiconazole – 11.4% Trifolxystrobin – 11.4%	10 fl oz. per acre
Proline®	Bayer Cropscience	Prothioconazole – 41%	5 fl oz. per acre

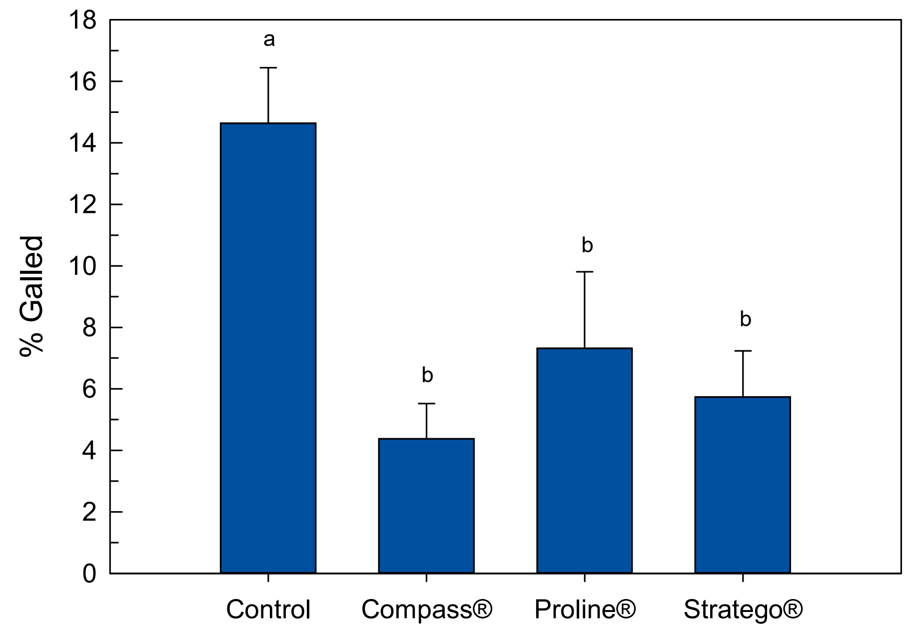
Field Trial – Study Design

- 2 proven fungicides in greenhouse study along with Proline® were tested operationally on several nursery blocks in 2019 and 2020.
- In 2019, treatments were applied to 2 seedlots of loblolly and slash pine. In 2020, treatments were applied to 1 seedlot of loblolly pine.
- Seedlings were sprayed on 5 occasions-
  - 1st spray commenced 21 days following sowing as all seed was treated.
  - Subsequent sprays were 14 days apart.
- Products
  - Control – no treatment
  - Proline® - operational control
  - Compass®
  - Stratego®
- At the end of each growing season, we assessed: seedling quality, number of rust galls and root morphology

2019 Loblolly Seedlot 1 Results

Treatment	Height (cm)	RCD (mm)	Shoot weight (g)	Root Weight (g)
Control	37.4	5.45	4.70	0.96
Compass®	38.7	5.71	5.71	1.06
Proline®	38.3	5.92*	5.41*	1.12*
Stratego®	38.4	5.65	5.25	0.96

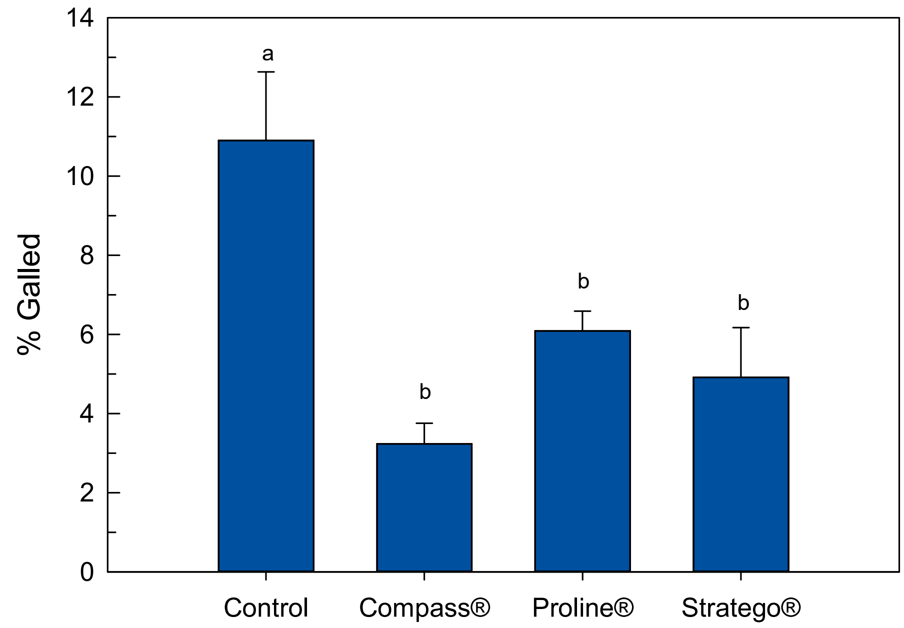
- All compounds tested were found to be effective in reducing Fusiform rust.



2019 Loblolly Seedlot 2 Results

Treatment	Height (cm)	RCD (mm)	Shoot weight (g)	Root Weight (g)
Control	38.2	5.91	4.92	0.83
Compass®	37.1	5.82	4.95	0.85
Proline®	37.3	6.20	5.78	1.02
Stratego®	38.8	5.91	5.33	0.85

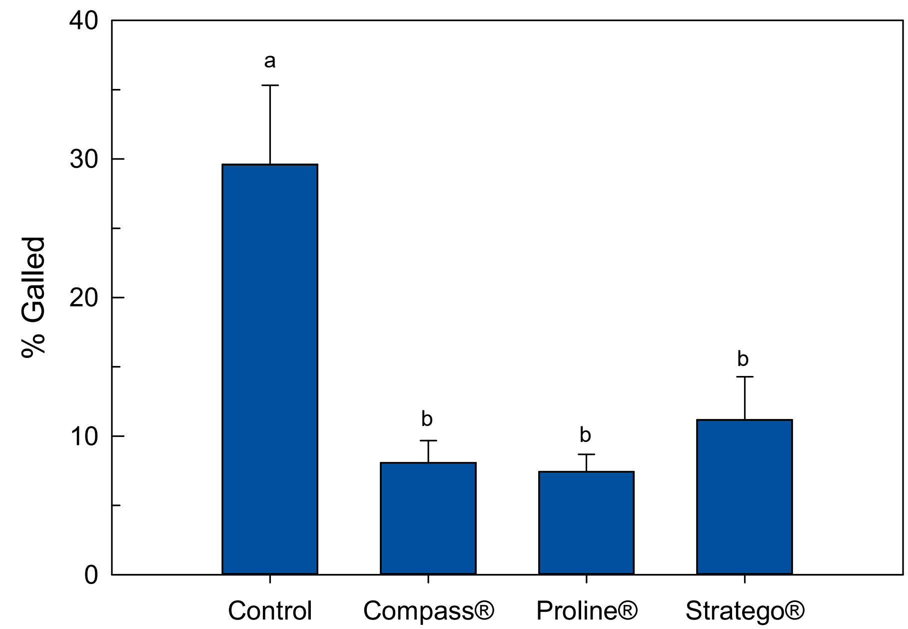
- All compounds tested were found to be effective in reducing Fusiform rust.



2019 Slash Seedlot 1 Results

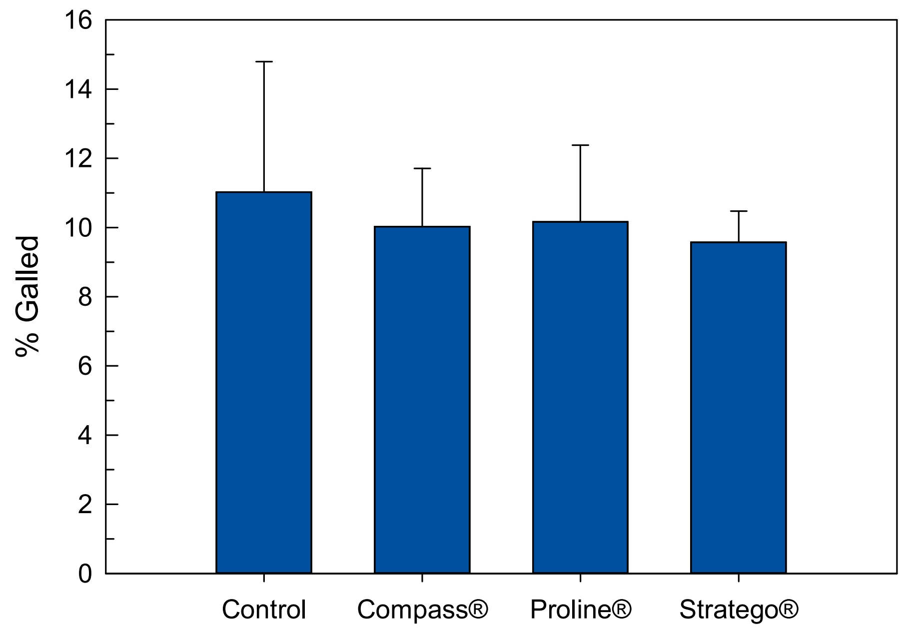
Treatment	Height (cm)	RCD (mm)	Shoot weight (g)	Root Weight (g)
Control	34.1	7.40	6.82	1.33
Compass®	34.5	7.45	7.11	1.32
Proline®	35.5	7.84*	7.54	1.48
Stratego®	34.8	7.95*	8.39*	1.32

- All compounds tested were found to be effective in reducing Fusiform rust.



2019 Slash Seedlot 2 Results

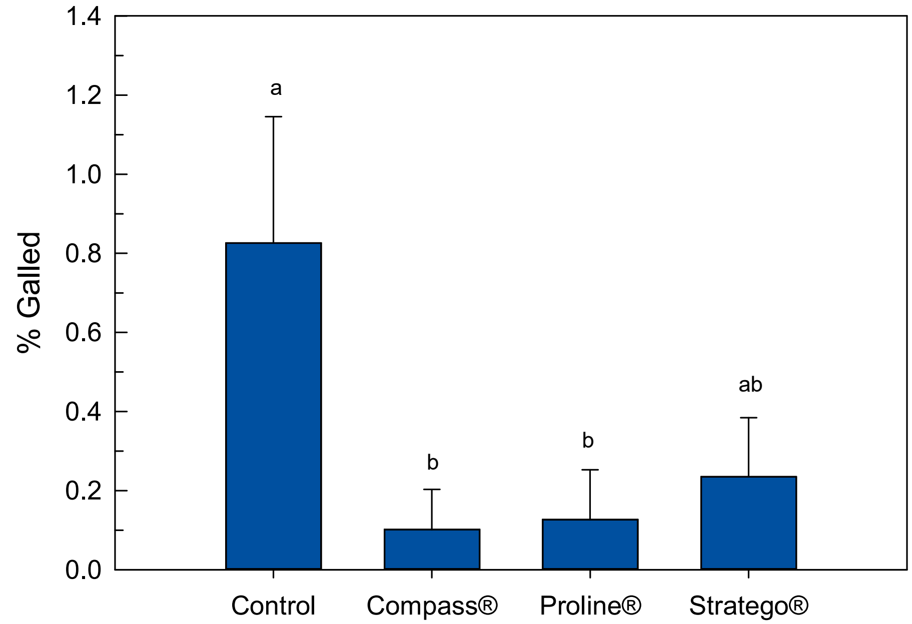
- We were unable to assess the efficacy of the fungicide treatments due to animal browsing (deer and squirrel) that resulted in low seedling survival.



2020 Loblolly Results

Treatment	Height (cm)	RCD (mm)	Shoot weight (g)	Root Weight (g)
Control	26.2	5.73	3.50	0.83
Compass®	27.1*	5.76	3.72	0.81
Proline®	27.3*	5.75	3.73	0.82
Stratego®	27.3*	5.87	3.73	0.86

- Compass and Proline were found to be effective in reducing Fusiform rust.
- Infection was extremely low in 2020, likely a result of environmental conditions needed for infection.



Field Trial Summary

- The new chemistries tested as a seedling control were found to be effective in reducing Fusiform rust.
- The active ingredients Trifolxystrobin (Compass®) and Propiconazoe + Trifloxstrobin (Stratego®) were found to be as effective as Prothioconazole (Proline®).
- New chemistries show promise to potential alternatives as a Fusiform rust seedling treatment after successful greenhouse and field trials.
- These chemistries, however, require registration prior to being used commercially.

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