Southern Forest Nursery Management Cooperative

Rapid Determination of Freeze Damage to Loblolly Pine Seedlings

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Southern Forest Nursery Management Cooperative 2021 Advisory 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

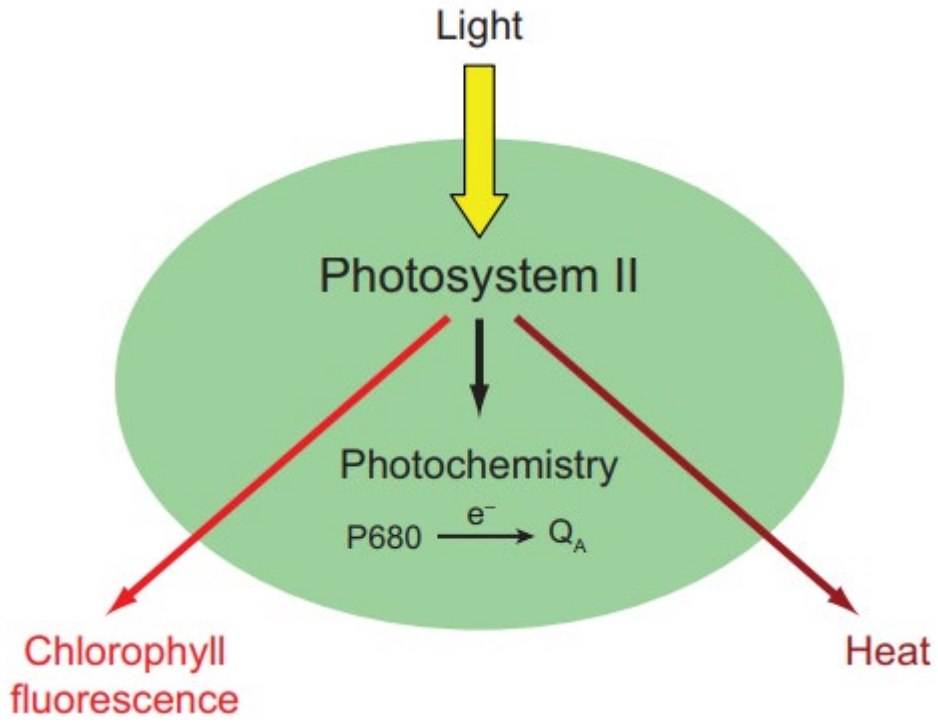
- Why we need a rapid, low-cost tool to evaluate freeze damage.
  - Visual damage, either tip dieback or stem and root discoloration, can take more than 2 weeks prior to appear.
  - Significant reductions in survival can occur by outplanting seedlings that are unknowingly damaged by freeze events.
  - 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_vF_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.

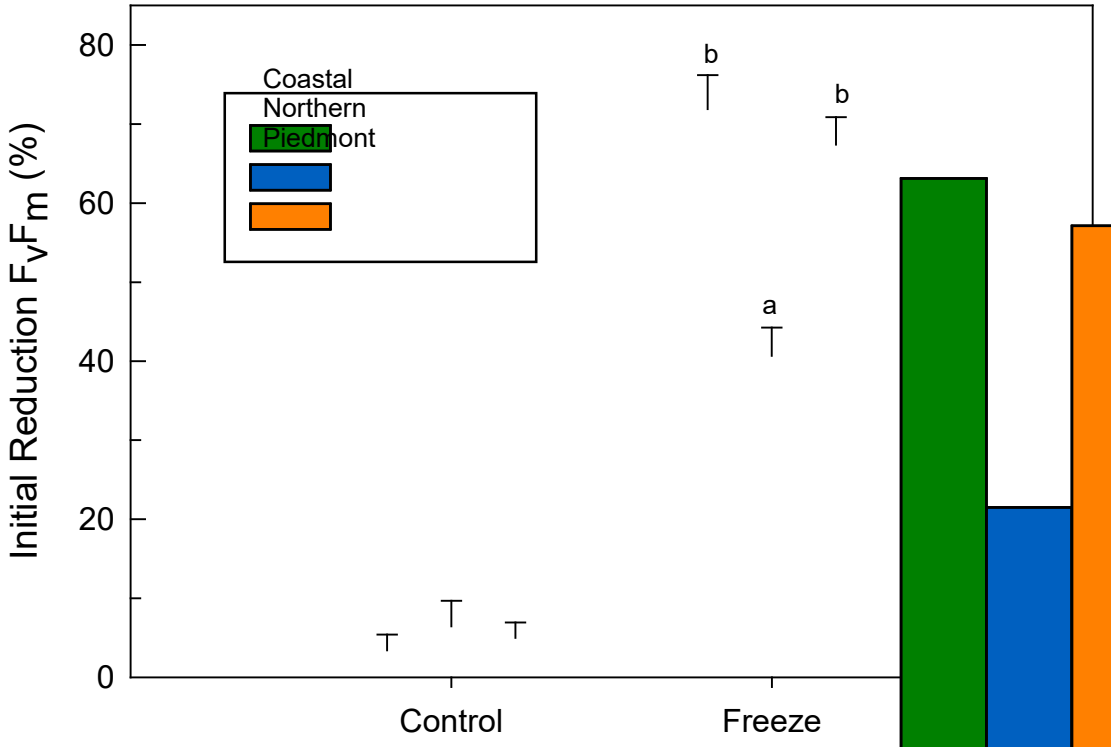
Results

- 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

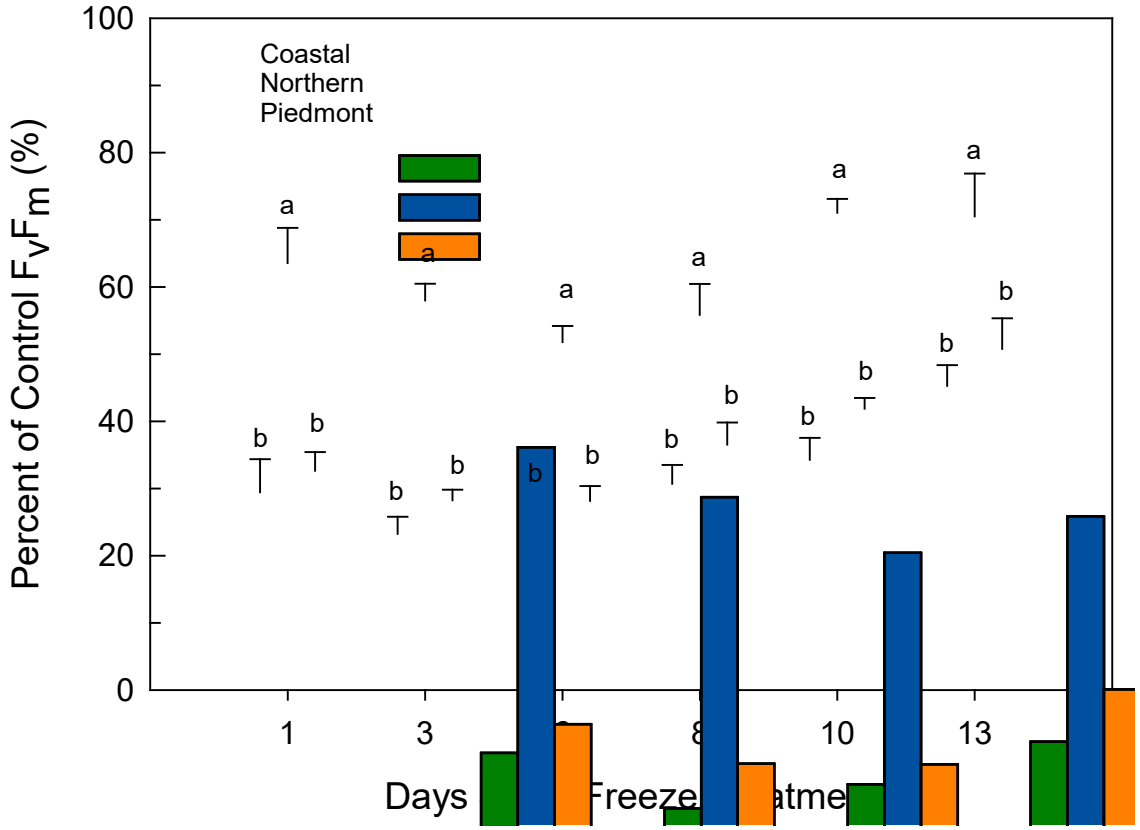
Results

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



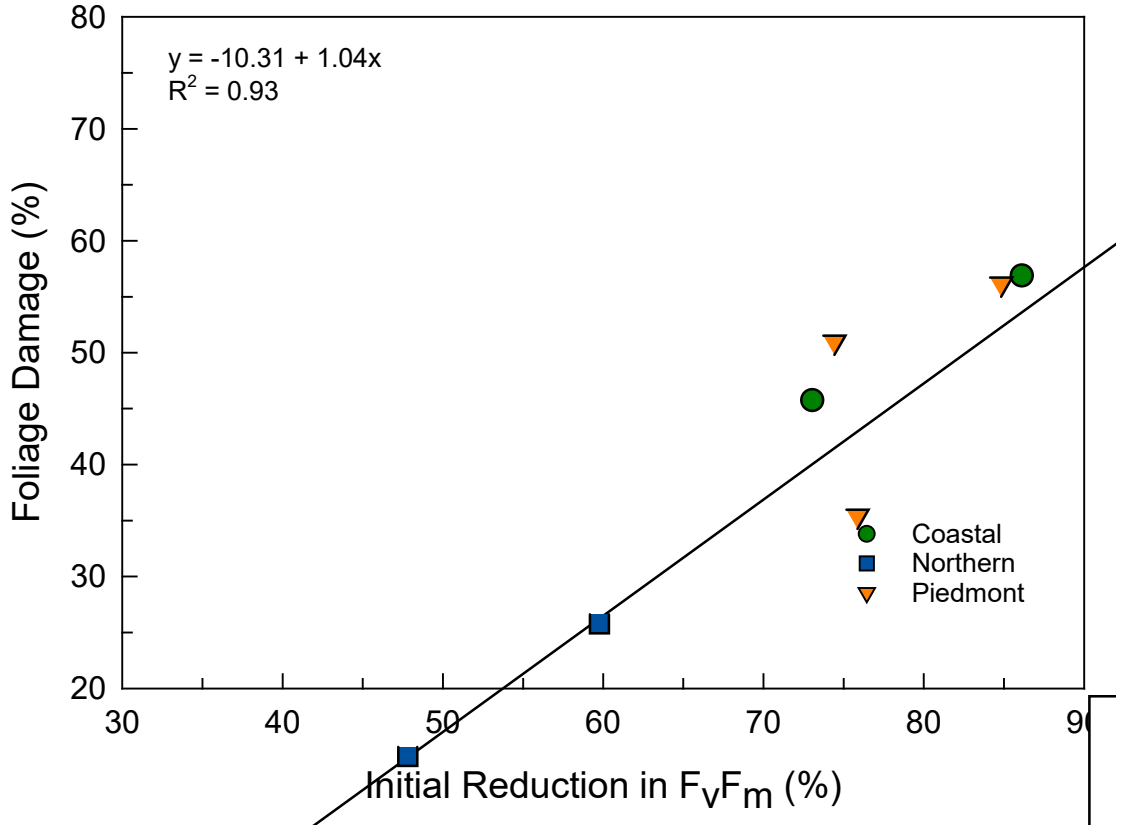
Results

- 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).



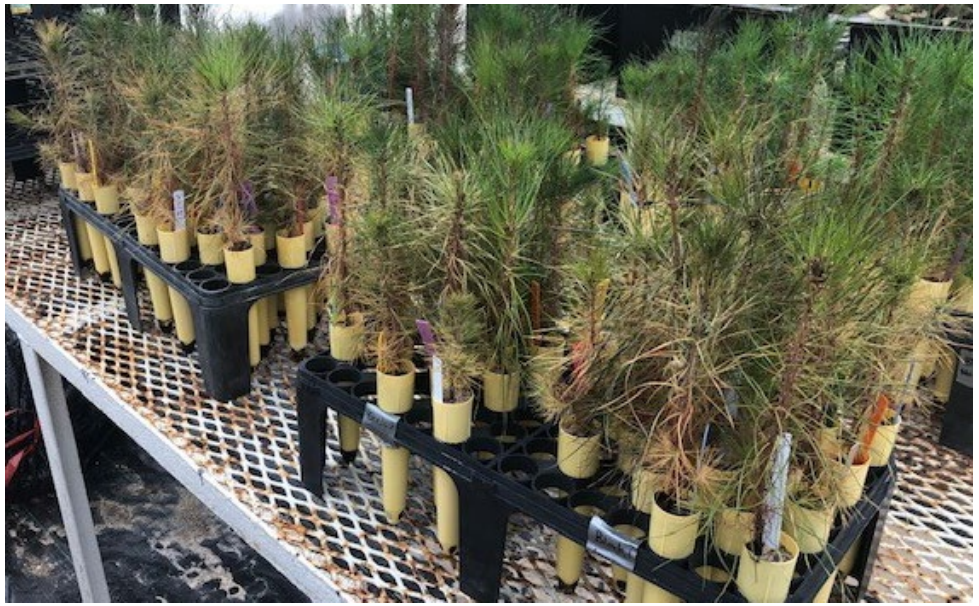
Results

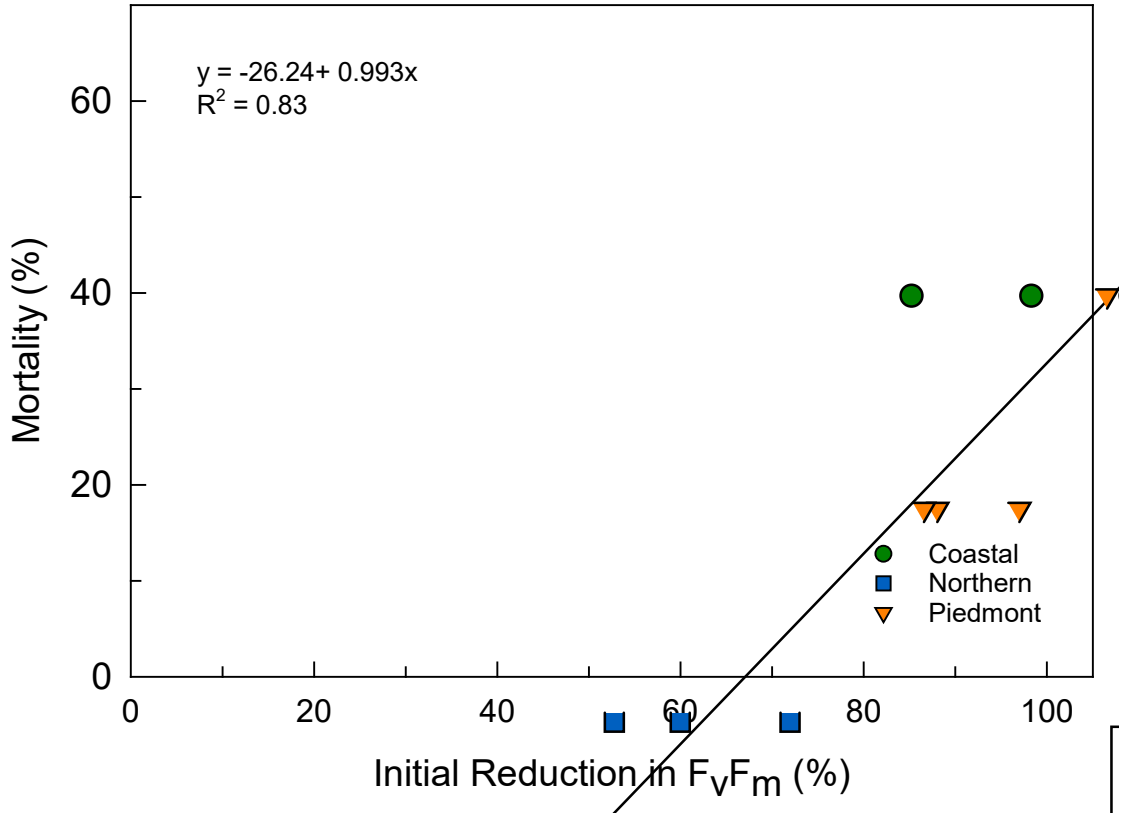
- Across all genetic families there was a strong linear relationship between initial reduction in F_vF_m and percent foliar damage 14 days post-freeze.



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.

Southern Forest Nursery Management Cooperative

RESEARCH REPORT 21-03

RAPID DETERMINATION OF FREEZE DAMAGE TO LOBLOLLY PINE SEEDLINGS

by

Tom Stokes, Ryan Nadel, Mike Aspinwall, Kitt Payn, Nina Payne, and Scott Enebak

INTRODUCTION

Uncontrollable weather conditions, such as a freeze event, can result in significant seedling mortality regardless of quality of seedlings, site preparation or planting (Cameron and Lowerts 2007). Sudden freeze events can be damaging to forest seedling nursery crops, especially in the southeastern U.S. when sudden freeze events occur immediately preceded by above-normal warm temperatures (Zheng et al. 2012, Krasowski et al. 1993, South et al. 1993). While cold acclimation occurs with reduced photoperiod (sunlight) and lower temperatures, southern pines can de-acclimate within hours of unseasonable warm weather (Krasowski et al. 1993). This is of particular concern with increasing warm winters and periodic hard freezes in the southeastern U.S. A challenge in determining the sudden impact of a freeze event on forest seedlings is that it may take more than 2 weeks afterward for visible signs of damage to appear (Cameron and Lowerts 2007). Visible damage may include tip dieback or winter desiccation and stem and root discoloration (Semerci et al. 2020, South 2006). Significant reductions of survival can occur by outplanting seedlings that are unknowingly damaged by freeze events (South 2006). Since sudden freeze events preceded by above normal warm temperatures can occur during lifting season, decisions of whether seedlings were damaged in the nursery become more time sensitive.

To reduce the time-period in determining if seedlings have suffered irreversible freeze damage, following a freeze event, a rapid determination for nursery seedling health is needed. One such method may be the use of chlorophyll fluorescence that has been used to evaluate a plant's ability to tolerate low temperatures but can also determine the extent of damage caused by those stresses (Murchie and Lawson 2013, Maxwell and Johnson 2000, Groom and Baker 1992). Measurements of chlorophyll fluorescence can provide information on the state of the photosynthetic efficiency in photosystem II (PSII) which can frequently be the first sign of stress in the plant (Maxwell and Johnson 2000). More specifically, examining the ratio of; 1) variable fluorescence to 2) maximum fluorescence (F_vF_m) will provide a measure of the maximum efficiency of PSII, thus giving information of seedling damage to PSII by cold temperatures (Maxwell and Johnson 2000).

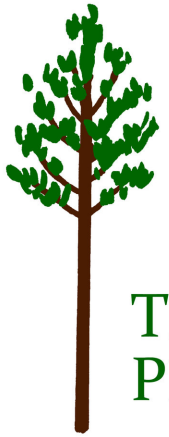
The objective of this study was to determine if chlorophyll fluorescence could be used as a tool to measure and evaluate the extent of freeze damage directly after a controlled freeze event. Our goal was to predict eventual seedling damage (mortality, growth reduction, etc) by correlating the reduction in PSII (F_vF_m) caused by a freeze event to the resulting severity of freeze injury or mortality in loblolly pine seedlings and to investigate the recovery time and loss of growth caused by the resulting freeze injury.

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.

Future Work

- We have secured seed from 3 provenance containing 5 families each from the North Carolina State Tree Improvement Cooperative.
- Seed will be grown in containers at one of our member nurseries until study initiation in the winter of 2022
- We purchased a handheld CF meter that is affordable for nurseries to utilize.
  - Cost < $3,000
- Additional work
  - This year we will look at different freezing levels on initial reductions in FvFm.



TREE IMPROVEMENT PROGRAM NC STATE UNIVERSITY

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Questions