Variation in Tolerance of Several Loblolly Pine Families to *Leptographium terebrantis* and *Grosmannia huntii*

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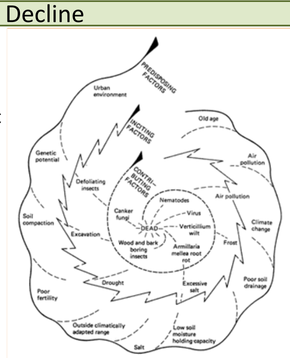
Introduction

Forest Decline

- Out-of-season leaf discoloration
- Reduction in diameter increment
- An evident loss of tree vigor
- Dieback and death
- Chain reaction hypothesis (1967)
  - Predisposing factors
  - Inciting factors
  - Contributing factors



Spiral Death Theory

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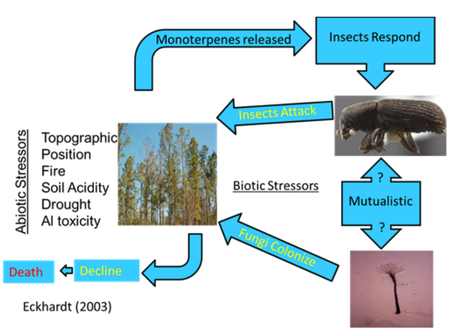
Introduction

Pine Decline Cycle



Eckhardt (2003)

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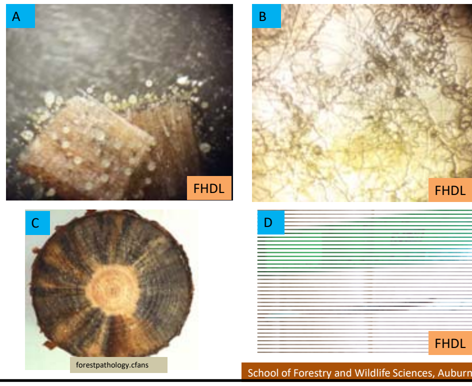
Introduction

Ophiostomatoid Fungi



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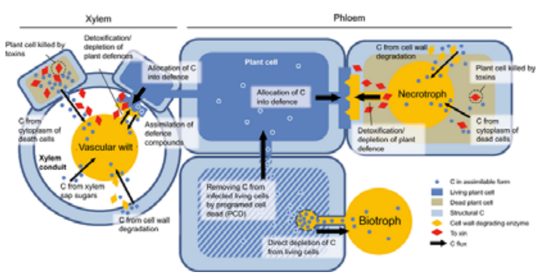
Fungal Invasion



Oliva et al. 2014

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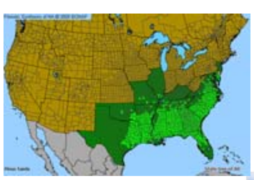
Introduction

Why should we care ?



Importance
- 29 million acres
- Half of standing pine volume
- $30 billion and 110,000 jobs
- All purpose marketable forest products

Problem
- Premature decline and mortality
- Potential threat to forest production industry

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Disease Tolerance Study

Motivation

➢ <u>Published reports</u>

- *Pinus taeda* is relatively more susceptible to fungi among the southern pines (Matusick et al. 2010)
- Intra-specific variation in tolerance of *P. taeda* to ophiostomatoid fungi (Singh et al. 2014)
- Resistance to other disease like fusiform rust has been incorporated in breeding (20 - 25% infection reduction) (Liu et al. 1999)

➢ <u>Research gap</u>

- Need for further screening of more commonly out-planted *P. taeda* families

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Disease Tolerance Study

Objectives

- To determine intra-species variation in tolerance of loblolly pine to *Leptographium terebrantis* and *Grosmannia huntii*
- To determine the yearly performance of connector families

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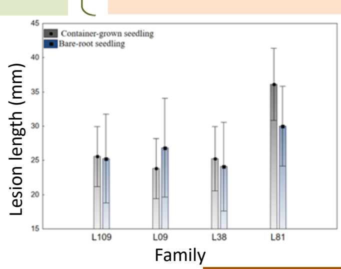
Disease Tolerance Study

Methodology

Forest Companies

Plum Creek, The Westervelt Company, Rayonier, ArborGen, and Weyerhaeuser



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Forest Health Dynamics Laboratory | **Disease Tolerance Study**

Methodology

Forest Companies: Plum Creek, The Westervelt Company, Rayonier, ArborGen, and Weyerhaeuser

2017 → 29 Families

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Forest Health Dynamics Laboratory | **Disease Tolerance Study**

Methodology

1. Planting In ProMix BX potting medium
2. Randomized Complete Block Design
3. Field Acclimatization

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Forest Health Dynamics Laboratory | **Disease Tolerance Study**

Methodology

Artificial Stem Inoculation

- Fungi
  - *L. terebrantis*
  - *G. huntii*
- Controls
  - Wound
  - Wound + Media

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Disease Tolerance Study

Methodology

Harvesting and Measurements

- Height and RCD
- Allowing dye movement through stem
- Measurements of lesion and occlusion
- Data analysis
  - Mixed model
  - Tukey HSD at $\alpha = 0.05$

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Disease Tolerance Study

Results

Effect	P-value (lesion length)	P-value (occlusion length)
Family	0.001	0.045
Treatment	<0.0001	<0.0001
Family*Treatment	0.991	0.259

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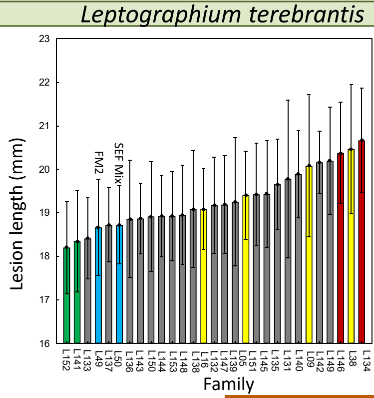
Disease Tolerance Study

Leptographium terebrantis



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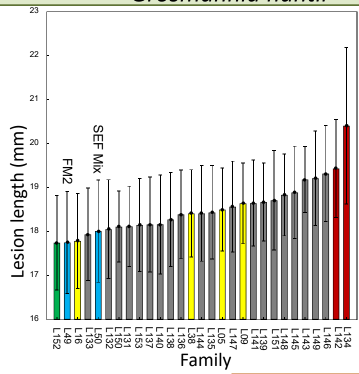
Grosmannia huntii



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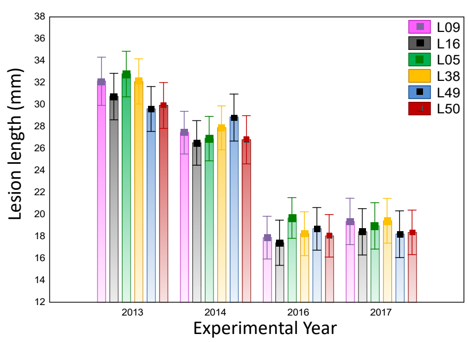
Disease Tolerance Study

Connector Families - Yearly Performance



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Disease Tolerance Study

Conclusion

- Intra-specific variation in tolerance to *L. terebrantis* and *G. huntii*
- Great potential for selection of tolerant families to mitigate potential problem due to decline
- Genotype and environment (GXE) interaction more likely
- Relative susceptibility of the families remain same

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Drought Study

The Role of *Leptographium terebrantis* and *Grosmannia huntii* Invasion in Driving Drought-Related Decline in Loblolly Pine

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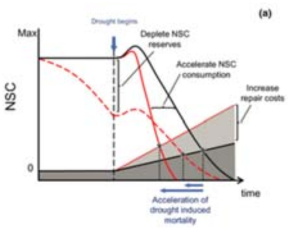
Drought Study

Motivation

- Forests of the southeastern USA have experienced severe droughts
- Drought - inciting factor to Pine Decline (PD)
- Fungi associated with PD
  - block vascular tissue
  - alter plant physiology

➢ <u>Research gap</u>

Previous studies focused on only one factor (biotic or abiotic)



Oliva et al. 2014

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Drought Study

Objectives

- To determine the combined effect of the drought and ophiostomatoid fungi on loblolly pine
- To understand how loblolly pine families susceptible and tolerant to ophiostomatoid fungi will respond to *G. huntii* and *L. terebrantis* under drought conditions

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Forest Health Dynamics Laboratory — **Drought Study**

Methodology

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Forest Health Dynamics Laboratory — **Drought Study**

Methodology

- Watering Treatments
  - Normal Moisture (70% FC)
  - Moderate Drought (50% FC)
  - Severe Drought (25% FC)
- Artificial Inoculation Treatments
  - Controls: NW, W, and WM
  - Fungi: GH and LT

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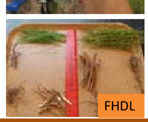
Forest Health Dynamics Laboratory — **Drought Study**

Methodology

Measurements

- Height and RCD
- Seedling volume
- Needle greenness
- Water potential
- Relative water content
- Lesion length, width and depth
- Occlusion length, width and depth
- Number of new bud break
- Seedling volume
- Aboveground and belowground biomass



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Drought Study

Results

Measurement	Watering(W)	Family (F)	Inoculation (I)	W x Fam	Fam x I	W x I
Lesion Length	**<0.0001**	**<0.0001**	**<0.0001**	0.8886	**0.0030**	**<0.0001**
Lesion L/Ht	**<0.0001**	**<0.0001**	**<0.0001**	0.4859	0.2426	**0.0013**
Lesion width	**0.0152**	0.0722	**<0.0001**	0.1006	0.2960	**0.0132**
Occlusion L	0.1776	**0.0407**	**<0.0001**	0.3042	0.0407	**0.0007**
Occlusion L/Ht	**0.0030**	**<0.0001**	**<0.0001**	0.2130	0.1945	**0.0287**
Fine root yield	**<0.0001**	**<0.0001**	0.3199	0.6530	0.7444	**0.0012**
Bud increment	**0.0334**	0.0937	**0.0036**	0.1810	0.1733	**0.0363**

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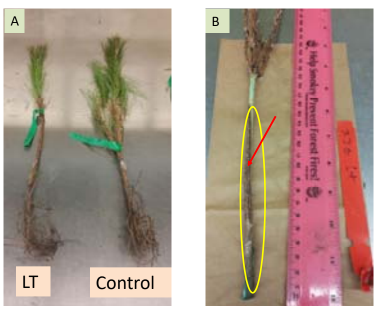
Drought Study

Results



Source: FHDL

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Drought Study

Results

Source: FHDL

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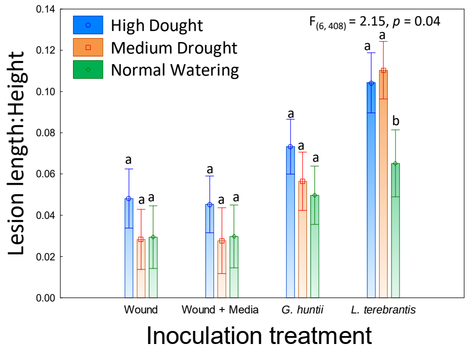
Drought Study

Results



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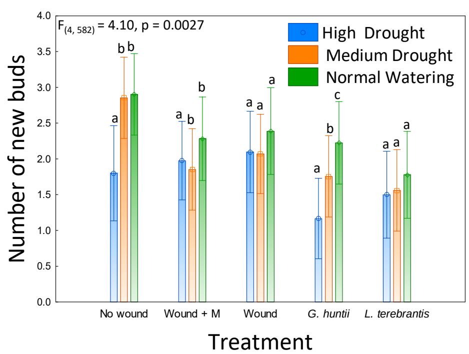
Drought Study

Results



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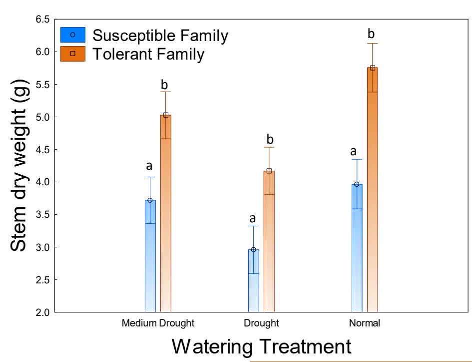
Drought Study

Results



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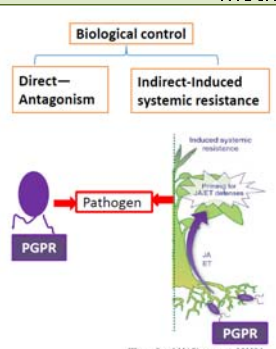
Conclusion

- Tolerant families tended to be more productive under severe droughts
- Virulence of two fungi increase under drought through different mechanisms
- Drought and fungal interactions impact lesion size, fine root formation, and new bud-break
- Trade off between adaptive traits and seedling growth
- Interactions put health of *P. taeda* at risk

Driving Towards Biocontrol of Ophiostomatoid Fungi By Plant Growth-Promoting Rhizobacteria

Motivation



(Photo, Corné M.J Pieterse, et al 2009)

- Root-colonizing bacteria
- PGPR strains have shown both plant growth promotion and control of plant pathogens
- Specific PGPR have shown ISR to fusiform rust in *P. taeda*
- PGPR is being considered in forestry

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PGPR Study

Antibiosis Study

Objective

- To understand if specific strain of Plant Growth Promoting Rhizobacterial (PGPR) will inhibit the growth of blue-stain fungi

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PGPR Study

Methodology

- PDA plates with holes at equidistant
- TSA on holes

Dual agar

PGPR

- 29 PGPR (10^8 CFU ml^{-1})
- Fungal inoculation

- Measurement
- Inhibition index

Inhibition

$$I = \frac{C - T}{C} \times 100$$

Where, I = inhibition index, C = growth in control, T = growth in treatment

Strong (I≥20%)
Medium (10% < I <20%)
Weak (I≤10%)

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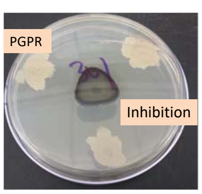
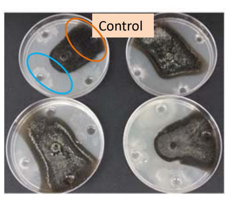
PGPR Study

Results





- Fungi on the control edge reached the end of the plate
- All of the tested strains inhibited growth compared to the control
- No sporulation around the inhibition zone
- *L. procerum* (29 strong)
- *L. terebrantis* (23 strong, 5 medium)
- *G. huntii* (28 strong, 1 weak)

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PGPR Study

Induced Systemic Resistance Study

Objective

- To understand if PGPR strains will induce the systemic resistance of loblolly pine to blue-stain fungi

Hypothesis

- PGPR will induce the resistance of the loblolly pine to *L. terebrantis* and *G. huntii*

Individual PGPR → Biological control
Individual PGPR → Plant growth promotion

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PGPR Study

Methodology

1. • Seedling planting
   • Acclimatization
2. • PGPR - *Serratia marcescens* (90-166), *Bacillus pumilus* (INR 7, and SE-34)
3. • Artificial inoculation of fungi (*G. huntii* and *L. terebrantis*)
4. • Lesion and biomass
   • Biological control efficacy

Biological control efficacy $= (C - T\ /C)$ x 100
Where, C= Lesion or biomass in control
T= Lesion or biomass in treatment

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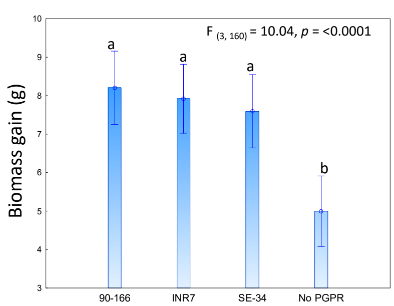
PGPR Study

Results



Growth promotion efficacy

- 90-166: 30 %
- INR7: 29 %
- SE-34: 28 %

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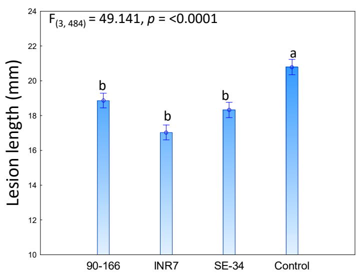
PGPR Study

Results (Rep - 1)



Biological efficacy

- 90-166: 13 %
- INR7: 22 %
- SE-34: 13 %

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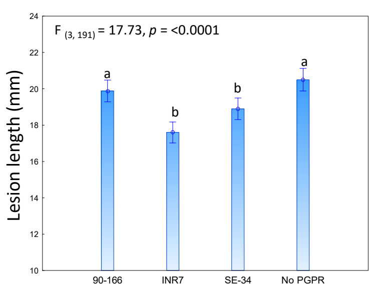
PGPR Study

Results (Rep -2)



Biological efficacy

- INR: 14 %
- SE-34: 7 %

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PGPR Study

Conclusion

- Most of the studied PGPR strains have the ability to inhibit the growth of the fungi *in vitro*
- Fungal sporulation is inhibited
- PGPR may produce some metabolites which inhibit the growth of the fungi
- Specific strains of PGPR promote plant growth
- PGPR strains may reduce the fungal impact

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Intra-Species Variation in Response of Mature Loblolly Pine to Root-Infecting Ophiostomatoid Fungi

Motivation

- Previous studies
  - Intra-specific variation in tolerance of *P. taeda* to ophiostomatoid fungi (Singh et al. 2014)
  - Considerable variation in susceptibility to pitch canker within mature *Pinus* species (Rice et al. 2007)
- Research gap
  - Screening conducted in seedling stage
  - Performance of mature trees in an ecological scenario

Objective and Hypothesis

Objective

To determine the intra-species variation in tolerance of mature *P. taeda* families to root-infecting ophiostomatoid fungi

Hypothesis

Intra-species variation in tolerance of *P. taeda* to ophiostomatoid fungi is independent of the plant growth stage

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Mature Root Inoculation Study

Methodology

Summer 2015 and Spring 2016 in 3 locations

- Eufaula AL (2 sites), Georgetown GA, and Cordele GA

Four families of *P. taeda*

- Two susceptible and two tolerant to ophiostomatoid fungi

25 *P. taeda* trees per family (17 year old)

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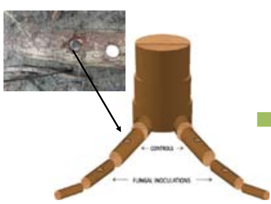
Mature Root Inoculation Study

Methodology



Matusick et al. 2010

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Mature Root Inoculation Study

Methodology

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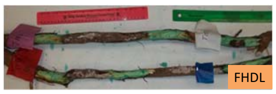
Mature Root Inoculation Study

Methodology



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Mature Root Inoculation Study

Results

Time	Variable	DF	Lesion Area	Lesion Length
Summer	Root Diameter	1	**0.01**	**0.01**
2015	Family	3	**<0.0001**	0.31
	Treatment	1	0.08	0.1
	Family * Treatment	3	0.85	0.46
Spring	Root Diameter	1	**0.03**	0.86
2016	Family	3	**<0.0001**	**<0.0001**
	Treatment	1	**0.002**	**<0.0001**
	Family * Treatment	3	**0.03**	0.32

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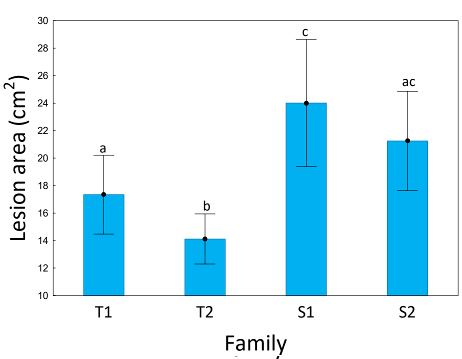
Mature Root Inoculation Study

Results - Summer 2015



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Mature Root Inoculation Study

Results - Spring 2016

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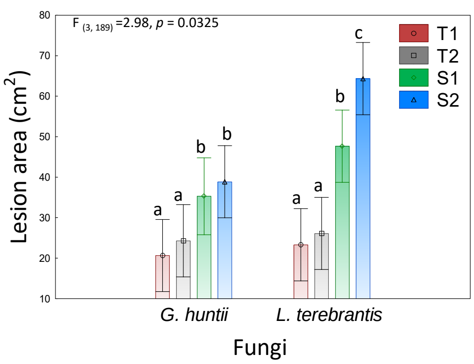
Mature Root Inoculation Study

Results - Spring 2016



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Mature Root Inoculation Study

Conclusion

- Intra-specific variation in tolerance of mature families to ophiostomatoid fungi
- Infection is proportionally larger in mature trees than seedlings
- Families had same patterns of tolerance independent of the tree growth stage
- Results support seedling screening studies
- Shows reliability of seedling screening studies
- Great potential for selecting fungi tolerant *P. taeda* families from current planting stock

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Acknowledgements

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