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Phenolic defense capacity and seedling morphology among 15 loblolly pine families

Auburn University Forest Health Cooperative
Annual Advisory Meeting
Americus, Georgia, June 24-26, 2014

PRESENTATION OUTLINE

- Introduction
  - What is the problem?
  - Are there practical solutions?
- Hypothesis and objectives
- Methodology
- Results
- Summary
- Application

INTRODUCTION

Background

What is the problem?

- Isolated pockets of loblolly pine mortality.
  - Attributed to interaction among abiotic and biotic stressors with ophiostomatoid root disease as a common biotic stressor.
  - Symptoms include ophiostomatoid root disease, sparse and chlorotic crowns, low annual stemwood production, and root system deterioration.
  - Symptoms suggest there is a carbon balance problem.
    - Inadequate whole-tree carbon fixation.
    - Carbohydrate deficit in the root system.
    - Inadequate carbohydrate for production of defense compounds.

INTRODUCTION

Are there practical solutions?

- Change species from loblolly to longleaf pine.
- Improve C balance and increase defense capacity.
  - Management activities that maintain photosynthesis for adequate C allocation to defense.
  - Plant families that resist the pine decline disease complex.

Examples of chemical defense or disease resistance under genetic control.		
Plant	Pathogen	Reference
Glycine max (L.) Merr.	*Sclerotinia sclerotiorum* (Lib.) de Bary	Malenčić et al. 2010. Plant Physiology and Biochemistry 48: 903-908.
Eucalyptus grandis Hill x Maiden	*Puccinia psidii* Winter	Moon et al. 2007. Functional Plant Biology 34: 1010-1018.
Picea abies (L.) Karst.	*Heterobasidion annosum* (Fr.) Bref.	Danielsson et al. 2011. BMC Plant Biology 11: 154-169.
Pinus taeda L.	*Fusarium circinatum* Nirenberg & O'Donnell	Quesada et al. 2010. Genetics 186: 677-686.

HYPOTHESIS

The defense capacity of loblolly pine is related to heritable traits of C allocation.

1st justification– C allocation in loblolly pine is under some level of genetic control.

Range in dry weight allocation among 10 loblolly pine clones at age 6 years.[1]				
Foliage	Stem	Branches	Taproot	Lateral roots
12-19%	41-55%	15-21%	12-15%	3-5%

[1]Stovall et al. 2013. Allometry varies among 6-year-old *Pinus taeda* (L.) clones in the Virginia Piedmont. Forest Science. 59: 50-63.

HYPOTHESIS

The defense capacity of loblolly pine is related to heritable traits of C allocation.

2nd justification– Plants exhibit a hierarchy of C allocation.

Hierarchy of C allocation in forest trees.[2]					
Foliage	Maintenance respiration	Stem and branches	Root system network	Defenses	Reproduction
Priority sinks Relatively constant.		Secondary sinks Affected by resource availability and environmental conditions. (abiotic and biotic stressors)			

[2]Litton et al. 2007. Carbon allocation in forest ecosystems. Global Change Biology. 13: 2089-2109.

OBJECTIVE

Hypothesis

Defense is related to heritable traits of C allocation.

Ultimate goal

Identify a heritable morphological indicator that predicts defense capacity.

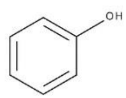
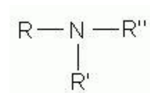
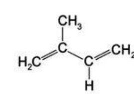
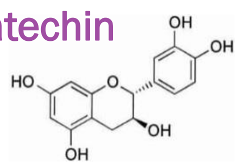
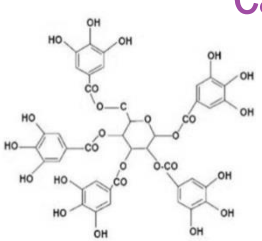
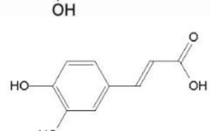
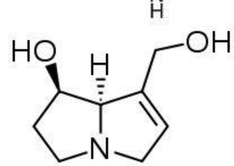
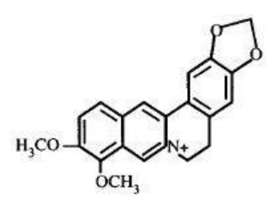
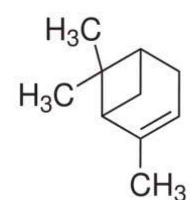
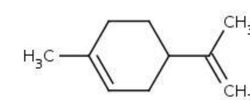
Objective

Evaluate relationships between defense capacity and morphology among 15 AUFHC loblolly pine families.

OBJECTIVE

Evaluate relationships between defense capacity and morphology among 15 AUFHC loblolly pine families.

Background

Three categories of chemical defenses in *Pinus.*		
Phenolics	Alkaloids	Terpenes
Functional group		
Phenol 	Organic amine 	Isoprene 
Examples		
Catechin   Gallotannin  Caffeic acid	 Piperidine  Pyrolizodine  Berberine	 Pinene  Limonene

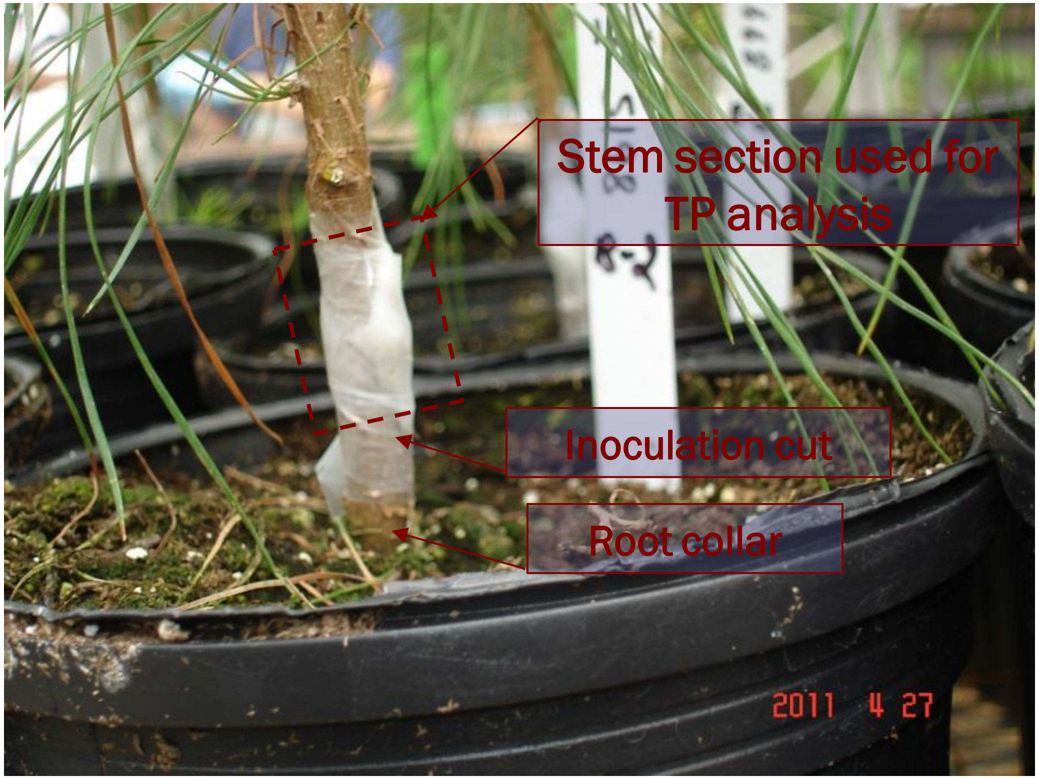
METHODOLOGY

- 20 container-grown seedlings of 15 loblolly pine families, potted in a peat vermiculite mix and placed in a greenhouse.
- Families were from those deployed by Arborgen, Weyerhauser, Plum Creek, and Rayonier.

- RCB split plot design: 10 blocks, subplot effect: N nutrition, whole plot effect: family.
- Two N treatment levels applied to broaden the range of C allocation patterns observed.
- Normal greenhouse culture with fertilization twice weekly.
- Harvested 28 weeks after potting.
- Measurements
  - RCD, TH, R:S, relative shoot volume growth rate by $(RCD / 2)^2 \times HT$ @ 3 week interval.
  - DW and fraction of total seedling DW: Foliage, stem & branches, woody roots, fine roots.
  - Stem total phenolic concentration (TP) by a modification of the phosphomolybdic-phosphotungstic acid method.

METHODOLOGY

Problem



- Stem inoculation with *Grossmannia huntii* 12 weeks after potting.

- Lesion lengths averaged 15 mm and did not differ by family.
  - Larger lesion lengths are reported in similar studies 20-55 mm *(Eckhardt et al. 2004, Matusick and Eckhardt 2010).*
  - Lesion lengths averaged 23 mm and differed by family *(Singh et al. 2014).*
- Problem did not affect family relationships between phenolic defense capacity and morphological traits.
- Stem total phenolic concentrations more representative of “baseline” or constitutive defense rather than induced defense.

RESULTS

Review

Ultimate goal

Identify a heritable morphological indicator that predicts defense capacity.

Families differ in morphological variables related to C allocation.

Families differ in phenolic compound production.

C allocation pattern influences defense compound production.

What we know.

RESULTS

Seedling variables

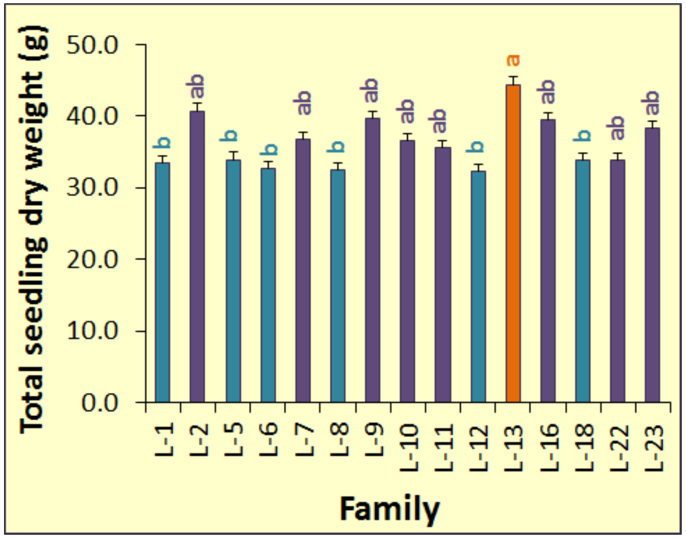
- N nutrition affected growth, morphology, and C allocation.

Main effect response to high N (HN: 1.7%) compared to normal N (NN: 1.2%).							
RCD	Shoot RGR	R:S	TDW	Percentage of total seedling dry weight			
				Foliage	Stem	Fine roots	Woody roots
↑	↑	↓	↑	↑	↑	↓	—

- No N x family interaction effects on growth, morphology, or C allocation.

- Families differed in all growth, morphology, and C allocation variables.

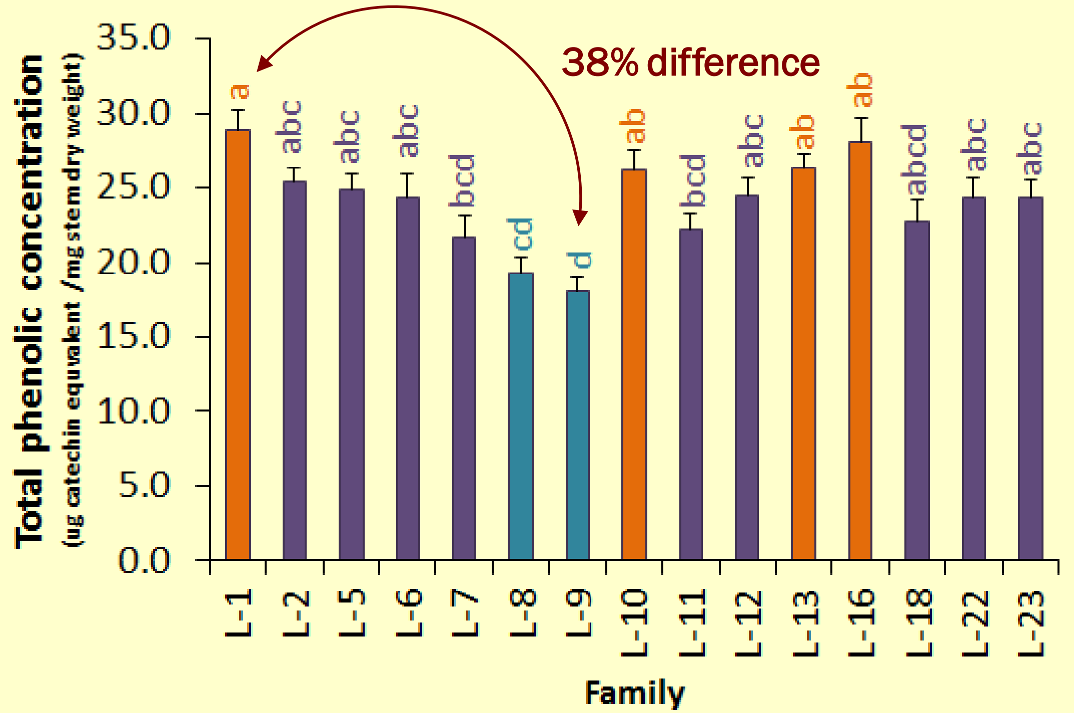
Total seedling dry weight of 15 loblolly pine families.



RESULTS

Stem total phenolic concentration

Mean stem total phenolic concentration of 15 loblolly pine families inoculated with *G. huntii* and grown in a greenhouse for 28 weeks.



- No N treatment effect on stem TP.
- Families differed in TP.
  - Up to a 38% difference among families.
  - Higher TP for families L-1, L-10, L-13, and L-16.
  - Lower TP for families L-8 and L-9.

RESULTS

TP x seedling variables

Regression analyses of TP by family.		
Independent variables (8)	n (10 NN and 10 HN)	Number of families
RCD	18-20	0
Shoot RGR		1
R:S		0
TDW		6
% foliage TDW		1
% stem TDW		1
% fine root TDW		1
% woody root TDW		2

- TP exhibited a significant relationship (R^2) with TDW for 6 families (40%).
- *Aspinwall et al. 2011.* Tree Physiology 21: 831-842: *"enhanced productivity of some loblolly pine clones may be accompanied by higher concentrations of total phenolics."*

RESULTS

TP x seedling variables

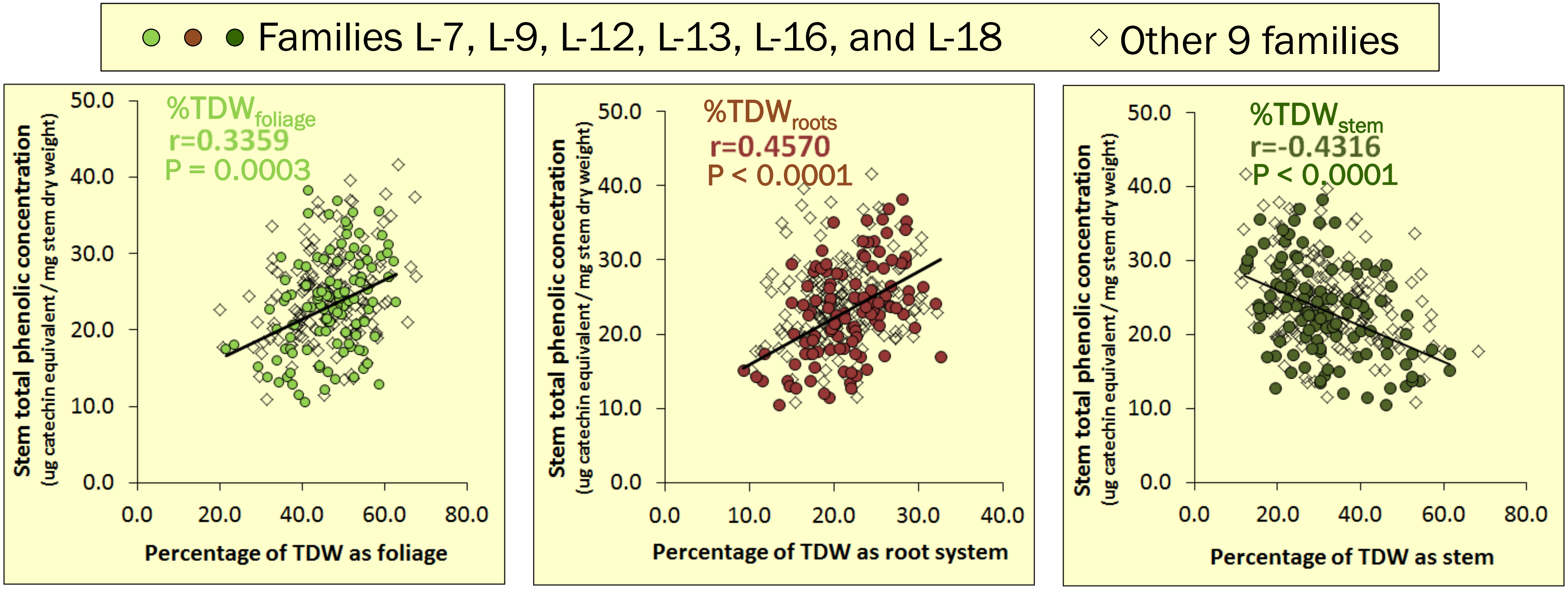
Significant correlations between TP and total seedling dry weight.						
Family	L-7	L-9	L-12	L-13	L-16	L-18
n	19	20	19	20	19	18
Correlation coefficient (r)	0.6762	0.6090	0.8024	0.6006	0.8316	0.6116
Pr > F	0.0016	0.0044	<0.0001	0.0065	<0.0001	0.0079

- For six families, r values were positive and relatively strong for TP x TDW.
- Whole seedling growth rate – shoot and root system – may be a predictor of phenolic defense capacity.

RESULTS

TP x seedling variables

Relationship between TP and percentage of total seedling dry weight by tissue



- As the percentage of TDW allocated to the foliage and root system increased, TP increased.
- As the percentage of TDW allocated to the stem increased, TP decreased.
- C allocation among foliage ($\%TDW_{foilage}$), root system ($\%TDW_{roots}$), and stem ($\%TDW_{stem}$)may be predictors of phenolic defense capacity.

RESULTS

Review

Ultimate goal

Identify a heritable morphological indicator that predicts defense capacity.

Four possible predictors of TP : (1) whole seedling growth rate, and C allocation to the (2) foliage, (3) root system, (4) and stem.

By Aspinwall et al. (2011), positive TP x growth relationships are likely driven by leaf area making leaf area a 5th possible predictor of TP.

What we know.

RESULTS

TP x seedling variables

- Eliminated $\%TDW_{roots}$ as a predictor of TP.
  - TP x $\%TDW_{roots}$ is likely an *indirect* effect of the *direct* effect of leaf area on root system growth.
- Leaf area rather than whole seedling growth rate as a predictor of TP.
  - Leaf area is the primary driver of whole seedling growth rate.
- Key predictors of TP are leaf area and $\%TDW_{stem}$.
  - Substituted foliage dry weight for leaf area.

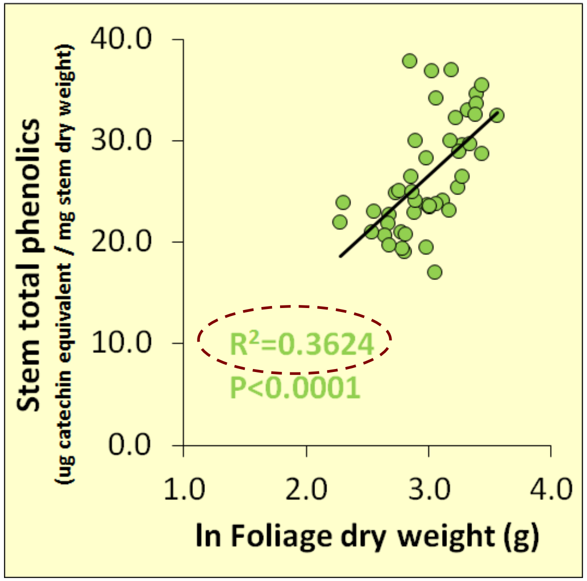
RESULTS

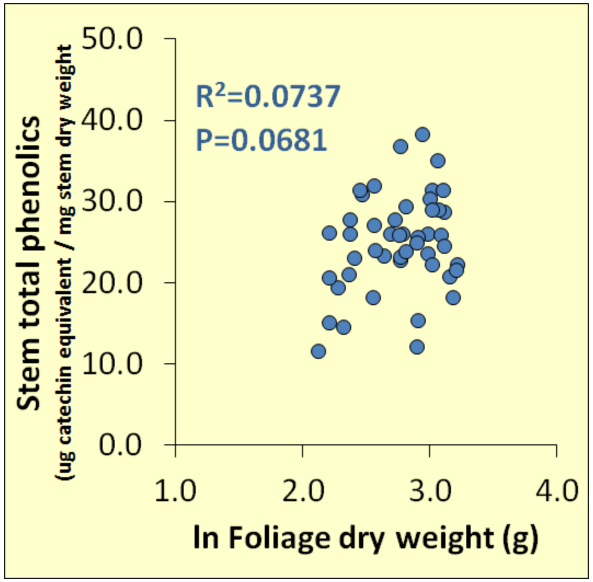
TP x seedling variables

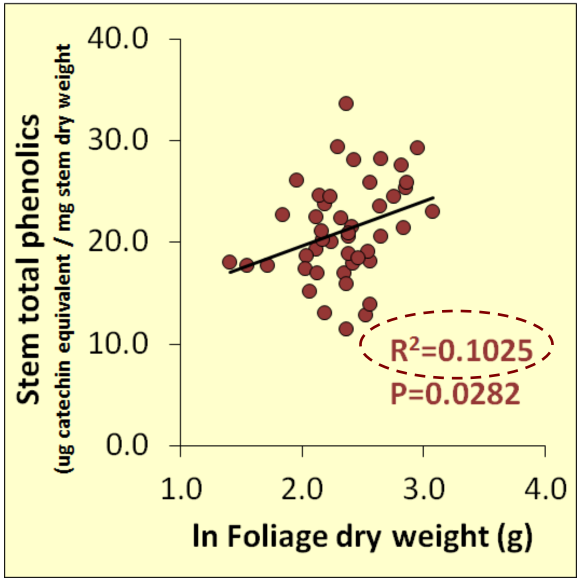
Relationship between foliage dry weight[1] and TP as $\%TDW_{stem}$ increased for NN seedlings

● $TDW_{stem} < 27\%$ ● $27\% < TDW_{stem} < 36\%$ ● $TDW_{stem} > 36\%$

[1] natural logarithm transformations to linearize the foliage dry weight data.







- Foliage dry weight (i.e., leaf area) explained more TP variation when $\%TDW_{stem}$ was low compared to when $\%TDW_{stem}$ was high.
- Low $\%TDW_{stem}$ seedlings produced more TP per unit of foliage than high $\%TDW_{stem}$ seedlings (slopes significantly different (P=0.0350)).

SUMMARY

Ultimate goal

Identify a heritable morphological indicator that predicts defense capacity.

- For families with genetic control of C allocation to foliage and stem, baseline phenolic defense may be predictable.
- An indicator of phenolic defense capacity will include measurements of dry weight allocation to the foliage and stem.
- Exclusionary clause… only in the absence of excessive hypersensitivity (e.g., possibly family L-1), and non-heritable influences on C allocation to the foliage and stem (e.g., excess N).

APPLICATION

Example

- To choose families for planting where pine decline is likely.

Families with ≥ 5 of the 10 NN seedlings in three stem C allocation categories [1].		
Low stem allocation, $TDW_{stem} < 27\%$	Medium stem allocation, $27\% < TDW_{stem} < 36\%$	High stem allocation, $TDW_{stem} > 36\%$
L-18	L-9, L-13	L-5, L-8, L-11

[1]and not more than 3 seedlings in another category.

L-18 MIGHT have a baseline higher defense capacity than L-5, L-8, and L-11.

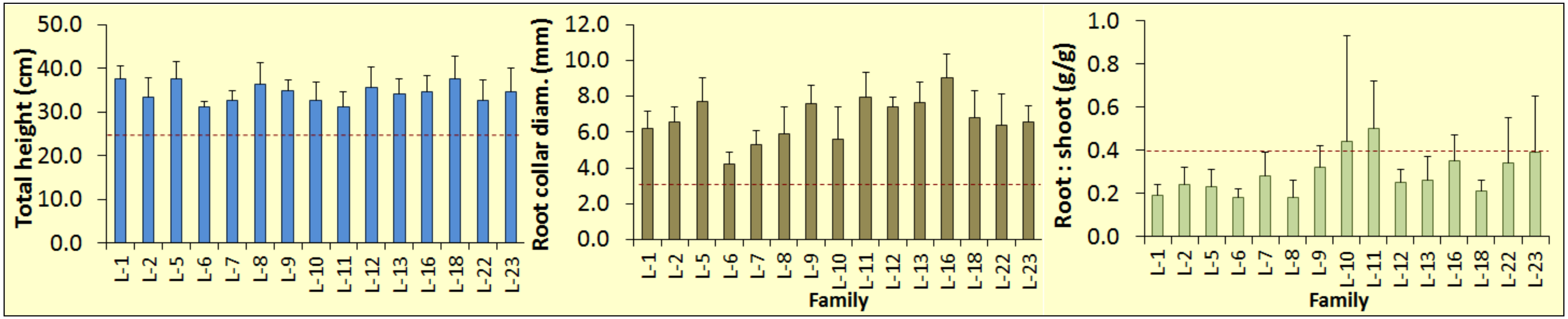
- More information is needed to apply research findings.

APPLICATION

Research needs

- Repeat the study to test relationship between TP and ophiosto-matoid infection.
  - What impact does phenolic defense have on pathogen spread?
- Repeat study with uniform seedlings and an inert potting medium.
  - One grower of all families?
  - Washed sand as a potting mix.

Pre-potting mean and standard deviation of total height, RCD, and R:S of 15 families (n=10).



- Develop heritable indicator of baseline phenolic defense capacity.
- Conduct a survey of pine decline among the 15 families.
  - Is pine decline incidence related to heritable C allocation pattern?

AKNOWLEDGEMENTS

Funding provided by the

Auburn University Forest Health Cooperative

and USFS Southern Research.

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Table 4. Coefficients of determination, probabilities of a greater *F*-value, and regression parameters of significant linear relationships between stem total phenolic concentration and the natural logarithm of foliage dry weight. Analyses were done for three quantiles of seedlings by level of nitrogen (N) treatment. Values of R^2, and differences between regression parameters were considered significant at an α level of 0.10.

Number of families	Level of N nutrition	Quantile definition	n[1]	R^2	Pr > F	s^2[2]	Slope	Y-intercept
15	NN[3]	SDW <27% of TDW	47	0.3624	<0.0001	20.0	11.1A[5]	-6.7B
		27%< SDW <36% of TDW	46	0.0737	0.0681	33.9	5.2AB	10.6AB
		SDW >36% of TDW	46	0.1025	0.0282	21.0	4.4B	10.8A
15	HN	SDW <26% of TDW	48	0.0665	0.0768	38.8	5.7	7.8
		26%< SDW <35% of TDW	48	NS[4]				
		SDW >35% of TDW	48	0.3145	<0.0001	24.7	8.5	1.7

- Low C allocation to the stem.
- Lots of C available for LA but N-limited.
- Limited N and excess fixed C allows extra C (above that needed for stem and LA) to be used for phenolics.

- Hi C allocation to the stem
- LA not maximum yet
- N being used for LA growth rather than secondary N compounds
- Extra C (above that needed for stem and LA) to be used for phenolics.

Figure 3. Relationship between stem total phenolic concentration and the natural logarithm (ln) of foliage dry weight for the **four families that had four or more of 10 NN seedlings with stem dry weights less than 26% of total dry weight, and four or more of 10 HN seedlings with stem dry weights greater than 37% of total dry weight** (L-12: Pr > $F < 0.0001$, $R^2 = 0.6815$, L-16: Pr > $F = 0.0001$, $R^2 = 0.6071$, L-18: Pr > $F = 0.0078$, $R^2 = 0.3660$, L-23: Pr > $F = 0.0635$, $R^2 = 0.1785$).