Production of defensive metabolites by *Pinus patula* X *Pinus tecunumanii* hybrids in response to *Fusarium circinatum* infection

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Introduction

- Pines respond to infection by fungal pathogens such as *Fusarium circinatum* through the activation of physical and chemical defence mechanisms.
- Such mechanisms include traumatic resin duct formation throughout the secondary phloem, and an upregulation in the production of certain phytochemicals, namely terpenes and phenolics.
- Some pine species or hybrids are more resistant to *F. circinatum* infection than others.
- The genetic and molecular mechanisms underlying this phenomenon are poorly understood.
- In this study, potential differences in phytochemical production between resistant and susceptible *Pinus patula* X *P. tecunumanii* hybrids were investigated.

Aims

- To examine phytochemical production in *P. pat* X *P. tec* hybrids in response to *F. circinatum* infection over time
- To determine the relationship between the higher resistance of low-elevation (LE) hybrids/higher susceptibility of high-elevation (HE) hybrids to *F. circinatum* infection and phytochemical upregulation

Methods

Compound production over time

One-year old LE hybrids were stem-inoculated with *F. circinatum* Control trees were treated with sterile ½ PDA

Lesion measurements and stem tissue samples were collected at 5 days, 14 days, and 28 days post-inoculation

Terpenes and phenolics were identified using GCMS and LCMS (gas- and liquid chromatography-mass spectrometry), respectively

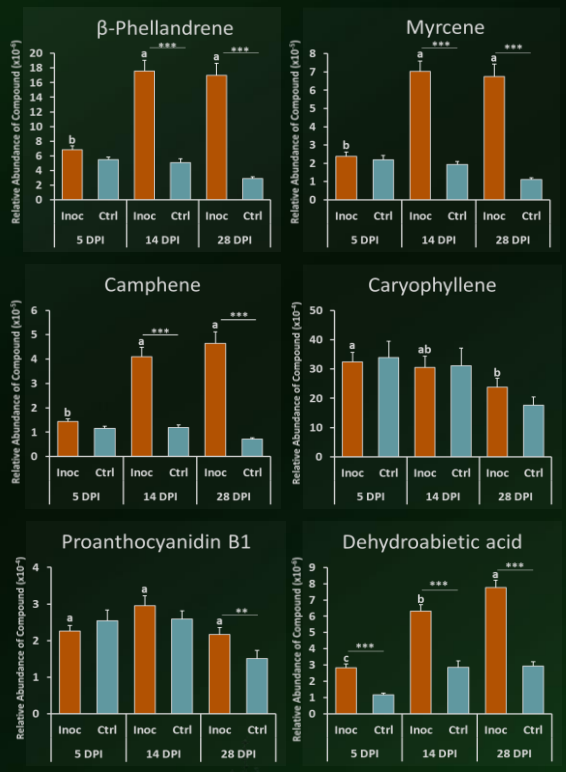
Compound production between hybrids

One-year old HE and LE hybrids were stem-inoculated with *F. circinatum*. Control trees were treated with sterile ½ PDA

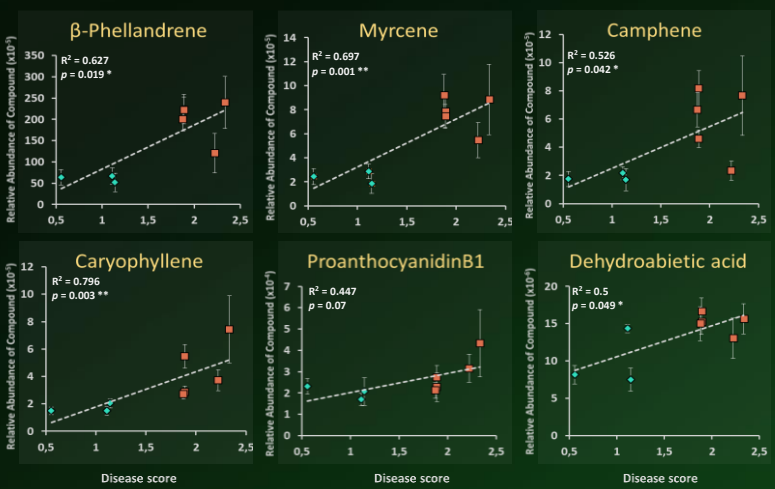
Infection was allowed to progress for 7 weeks before sampling

Terpenes and phenolics were identified using GCMS and LCMS respectively

Results



⇧ Relative abundance of 6 secondary metabolites produced LE genotypes across 5-, 14-, and 28 days post-inoculation. Letters above bars indicate differences between inoculated samples at the three DPIs. *'s Indicate significant difference between inoc. and ctrl.



⇧ Positive correlation between higher disease scores (with higher disease score = longer lesions, i.e., higher susceptibility to *F. circinatum* infection) and an increase in the production of individual secondary metabolites. Key: ◆ - LE hybrids ■ - HE hybrids

⇩ Individual secondary metabolites per compound group, and their abundance compared to other compounds in their group

	Secondary metabolite	HE hybrids	LE hybrids
Mono-(M) and Sesquiterpenes(S)		Proportion of total identified M and S	
	α-Pinene (M)	49%	58%
	β-Phellandrene (M)	38%	28%
	β -Pinene (M)	8%	9%
	Myrcene (M)	1%	1%
	Camphene (M)	1%	1%
	α-Terpinene (M)	1%	1%
	Caryophyllene (S)	1%	1%
	Longifolene (S)	1%	1%
	Germacrene-D (S)	<1%	<1%
Diterpene resin acids		Proportion of total identified diterpene resin acids	
	Dehydroabietic acid	35%	34%
	Neoabietic acid	26%	29%
	Levopimaric acid	10%	15%
	Abietic acid	12%	10%
	Isodextropimaric acid	10%	6%
	Isopimaric acid	7%	6%
Phenolics		Proportion of total identified phenolics	
	Catechin	45%	47%
	Unknown 336	17%	10%
	Matairesinol	6%	8%
	Pinosylvin	2%	8%
	Unknown 364	10%	7%
	Laricitrin 3-glucoside	5%	7%
	Dimethylstrobochrysin	7%	5%
	Unknown 378	3%	3%
	Proanthocyanidin B1	3%	3%
	- (-)-Nortrachelogenin	2%	2%

Conclusion

Overall, secondary metabolite production increased significantly only between 5 and 14 DPI, though not all metabolites followed this trend.

Unexpectedly, HE hybrids with higher susceptibility to infection also produced higher concentrations of secondary metabolites. Increased production could therefore be a marker of susceptibility, but is likely an insufficient defence mechanism, or potentially detrimental to the tree.

Acknowledgements

FABI CPHB tpcp

References

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- Mitchell RG, Wingfield MJ, Hodge GR, Steenkamp ET & Coutinho TA (2013). *New Forests* 44: 443-456.