Pining for resistance: exploring potential traits associated with lodgepole pine resistance to dothistroma needle blight

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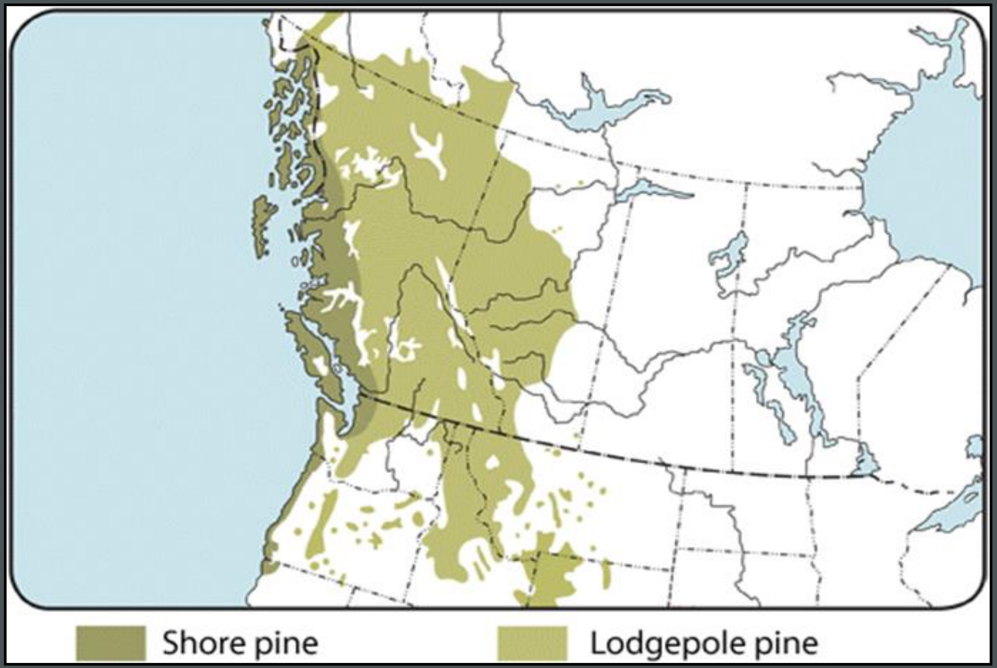
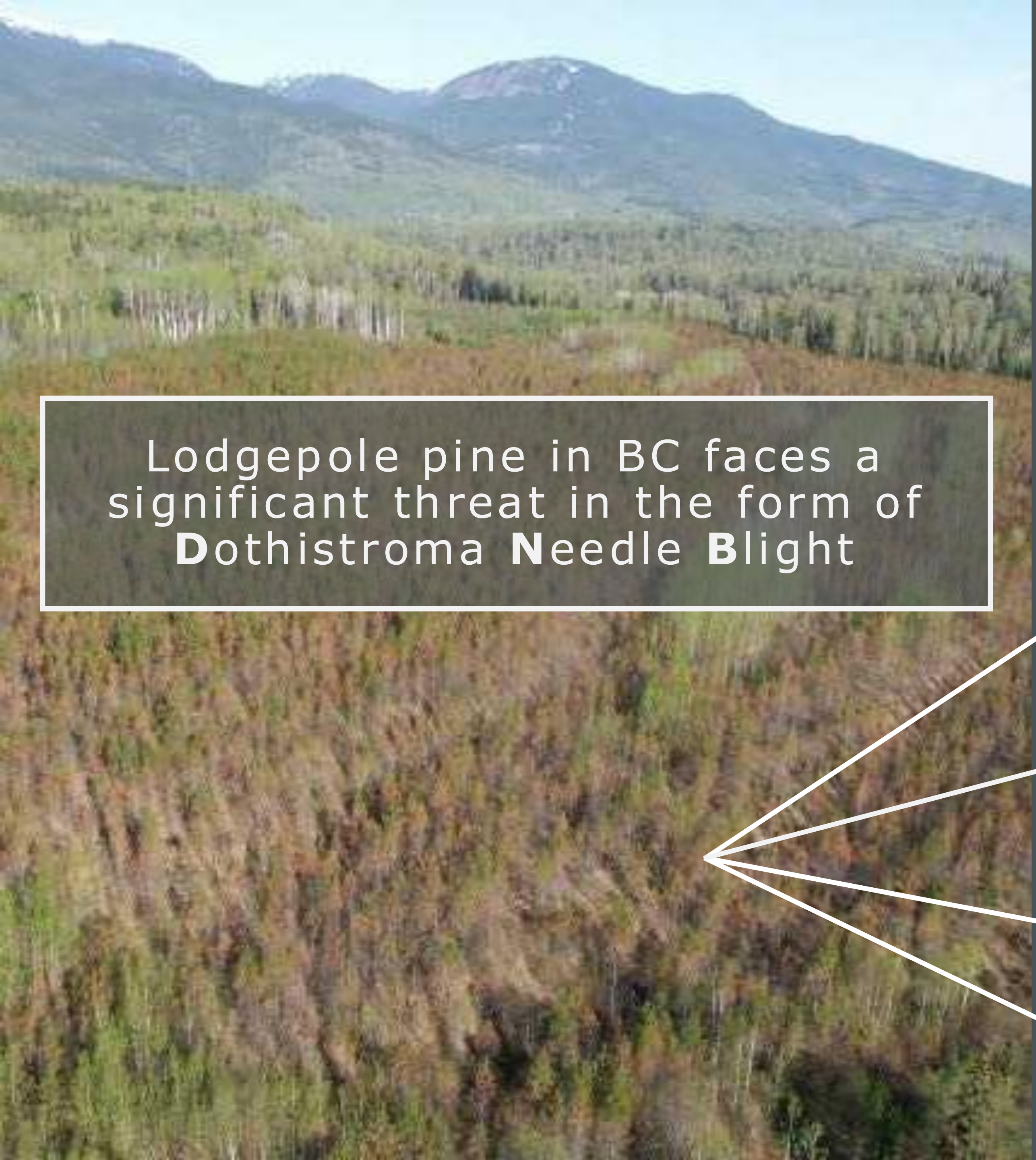


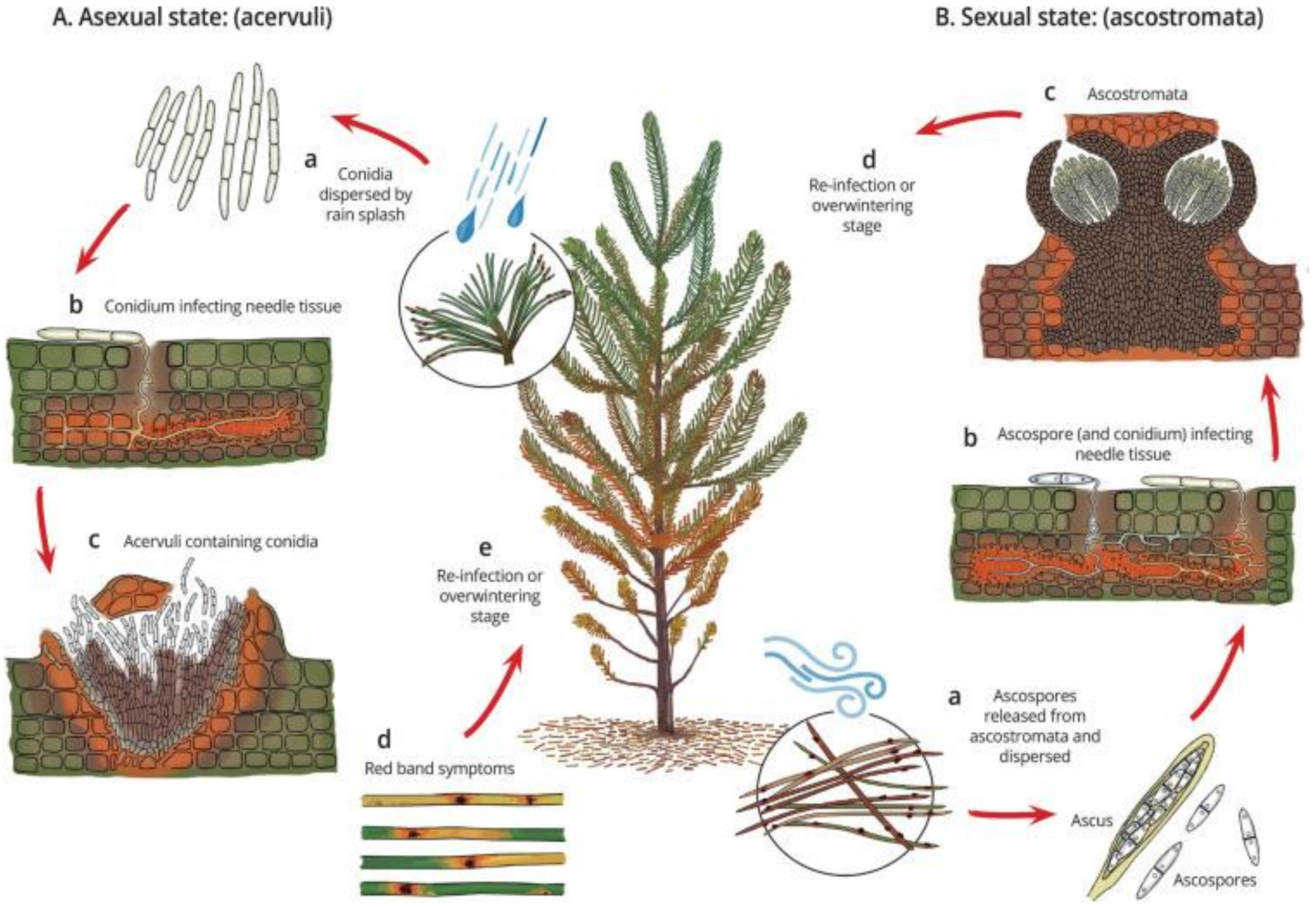
Lodgepole pine is the preferred choice for reforestation in BC

- Typically grows in pure, even-aged post-fire forests
- High utility and value to pulp and lumber industries
- Experiences high initial survival rates, rapid early growth, and it tolerates a wide range of site conditions
- Data from 2010 suggested that nearly **half** of all trees planted annually in BC belonged to this species

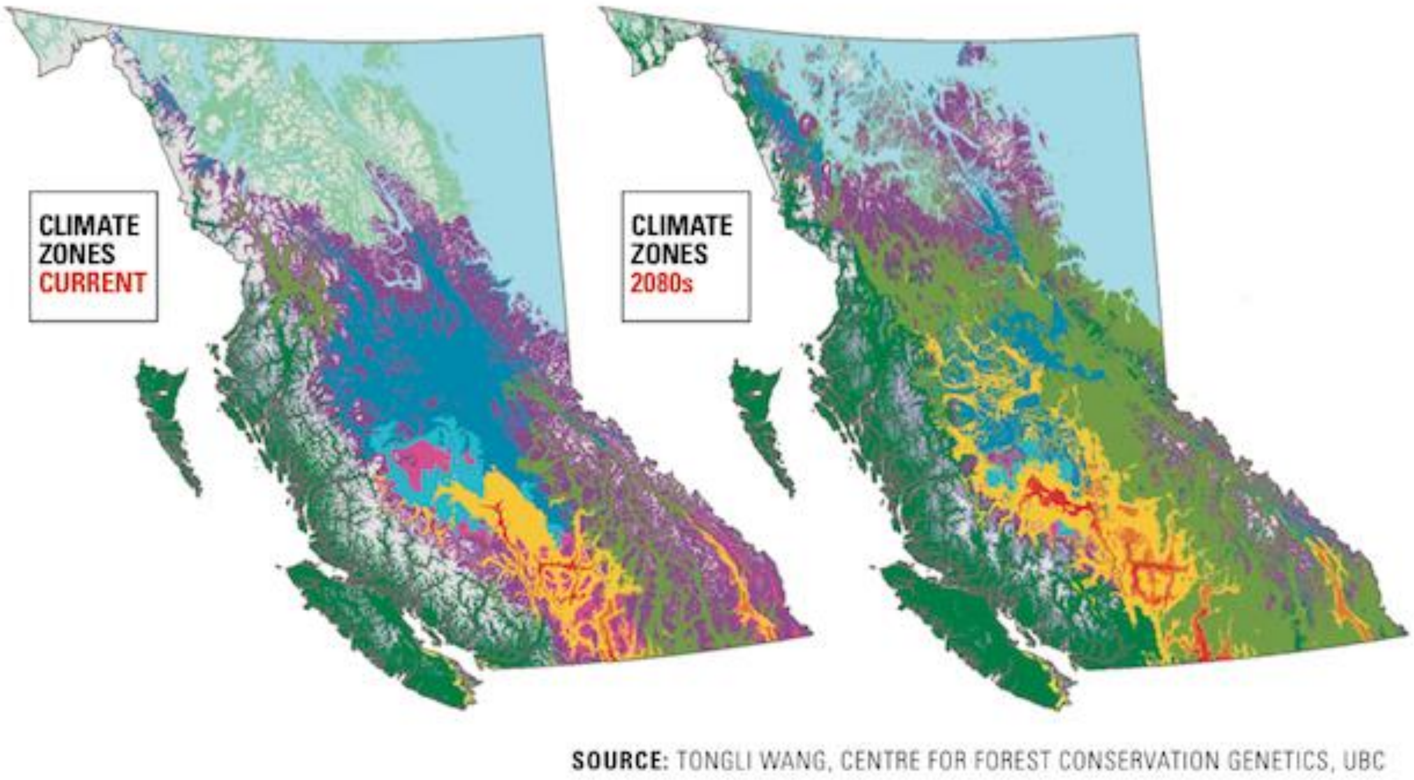


Lodgepole pine in BC faces a significant threat in the form of **D**othistroma **N**eedle **B**light

- Also known as red band needle blight
- In BC, it is caused by the **native** pathogen, *Dothistroma septosporum*
- One of the most **destructive** foliar diseases of commercial timber trees in the world
- Substantial economic impacts ranging in the **millions** of dollars in some countries



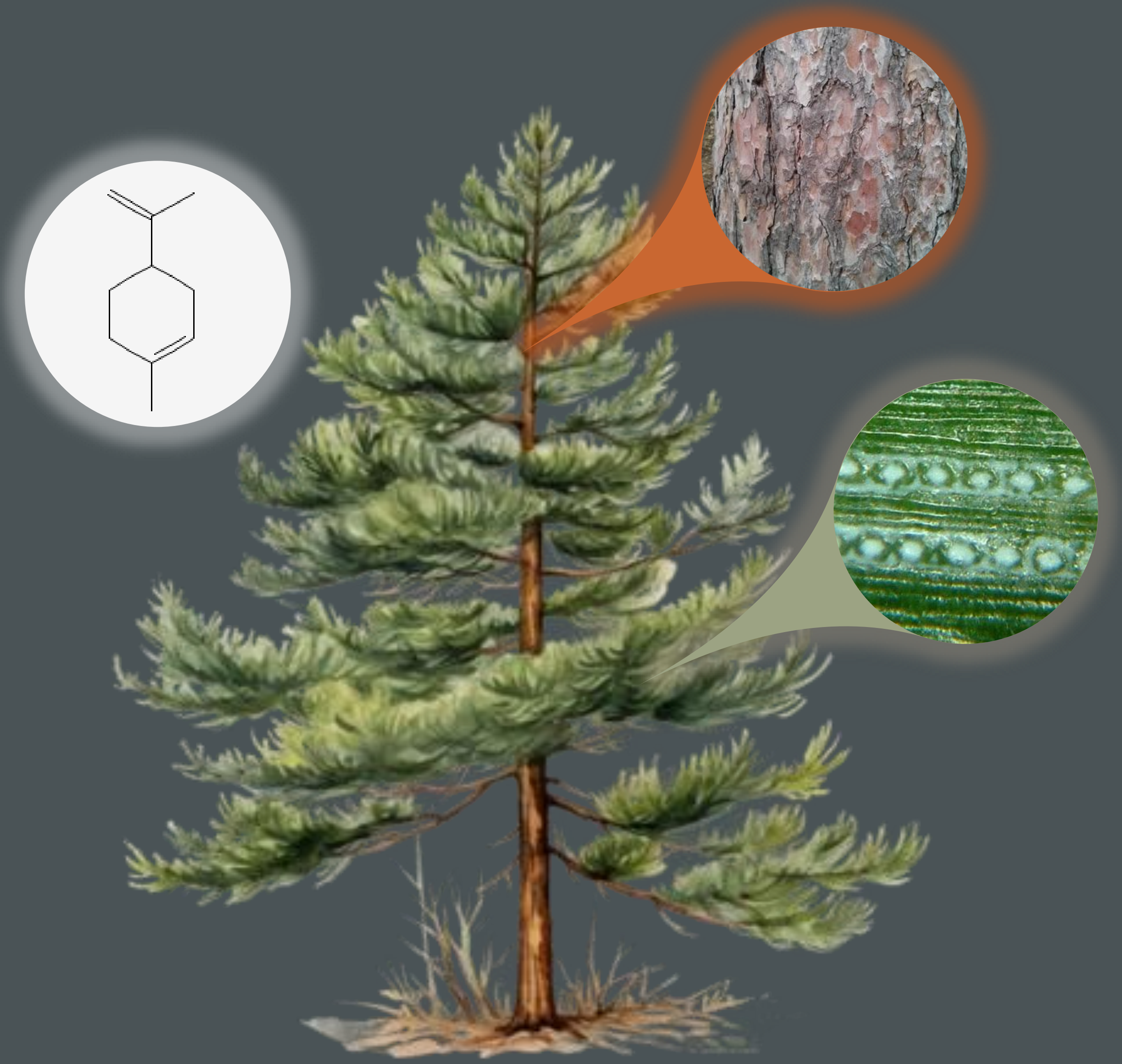
The presence of free water on needles is the most important factor for successful development and spread



Climate change has exacerbated the damage of DNB to susceptible pine populations

Increased precipitation and summer minimum temperatures due to expansion of the light green zone

- Positively associated with area affected by DNB and spore dispersal



Trees have evolved defense mechanisms to tolerate or resist external stresses

Encompass a range of physiological, biochemical, and molecular processes

- Physical barriers, chemical defenses, and induced immune responses

Pre- and post-infection

- Bark, epicuticular wax
- Defense chemicals (terpenes)

Defense chemicals have been associated with plant resistance

Play roles in photoprotection, reducing oxidative stress, and alleviating water stress

Vary with genetics and environmental factors

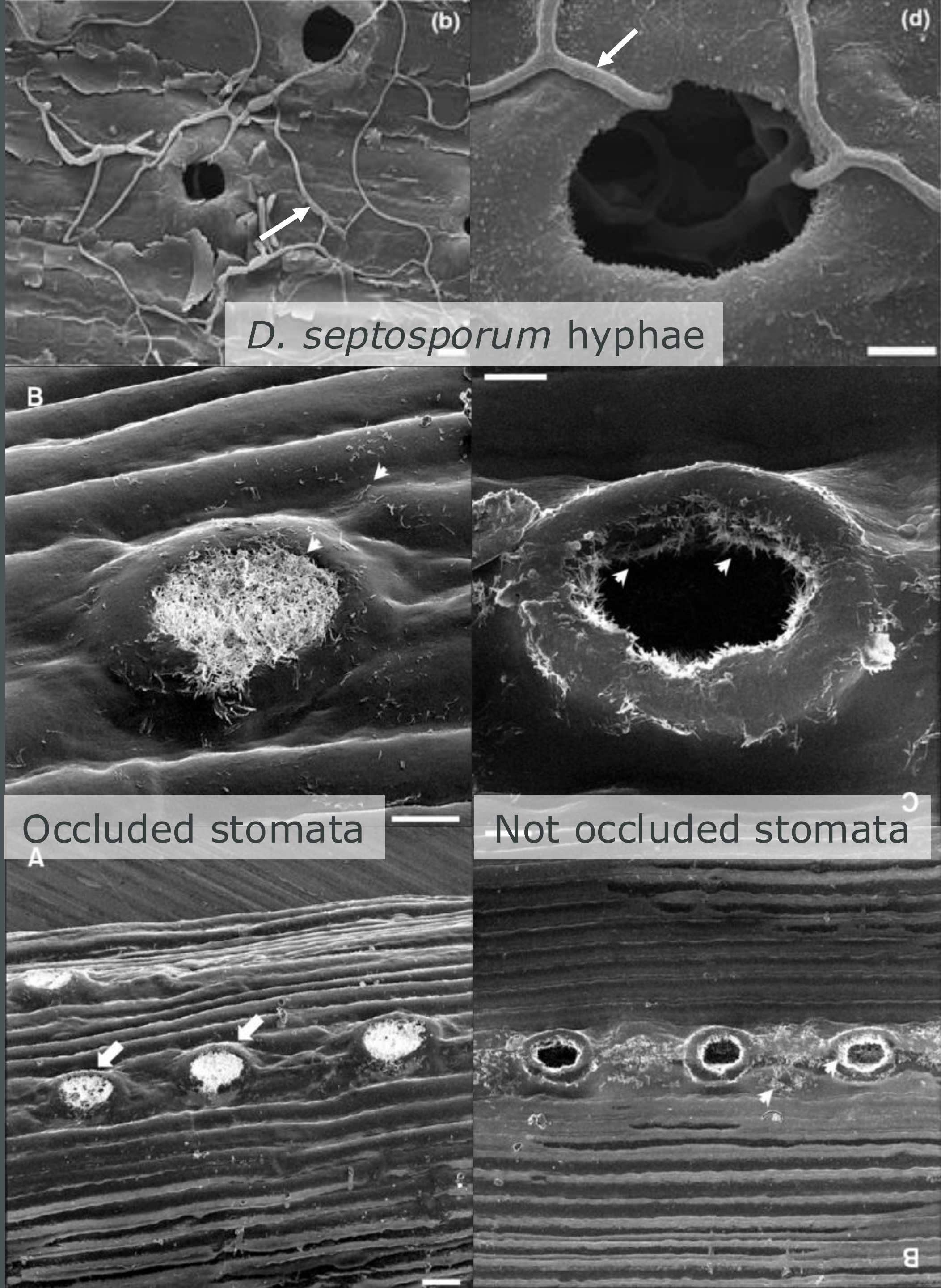
Positive correlation between outbreak history of DNB and levels of defense chemicals (Owen, 2018)

(E,E)-farnesol found to have inhibitory effects on *D. septosporum* (Owen, 2018)

Kabir et al., 2015, Forest Pathology

Epicuticular wax may contribute to tree resistance

- Protective layer for foliage of conifers
- Greater stomatal occlusion in resistant phenotypes
- Differences in wax concentration between resistant and susceptible phenotypes
- Effectiveness may differ with tree-pathogen system



Kim et al., 2012, Applied Microscopy

Morphological characteristics of needles may contribute to tree resistance

- Stomatal size
- Stomatal density
- Size of needles

Productivity traits may contribute to enhanced resistance

- Yearly growth
- Total height
- Biomass

Identifying resistance traits to produce resistant breeding stock is a promising strategy

- Identify lodgepole pine breeding families associated with reduced disease levels
- Determine traits underlying their enhanced resistance
- Provide possible resistance biomarkers for rapid screening of additional families

What traits are associated with DNB-resistant lodgepole pine seedlings?

Objective:

Identify traits predictive of resistant and susceptible seedlings:

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Phytochemical (41)
- Monoterpenes
- Sesquiterpenes
- Total

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Objective:

Identify traits predictive of resistant and susceptible seedlings:

Phytochemical (41)	**Anatomical** (5)
• Monoterpenes • Sesquiterpenes • Total	• Needle length & width • Needle surface area • Stomatal density • Epicuticular wax concentration

What traits are associated with DNB-resistant lodgepole pine seedlings?

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Identify traits predictive of resistant and susceptible seedlings:

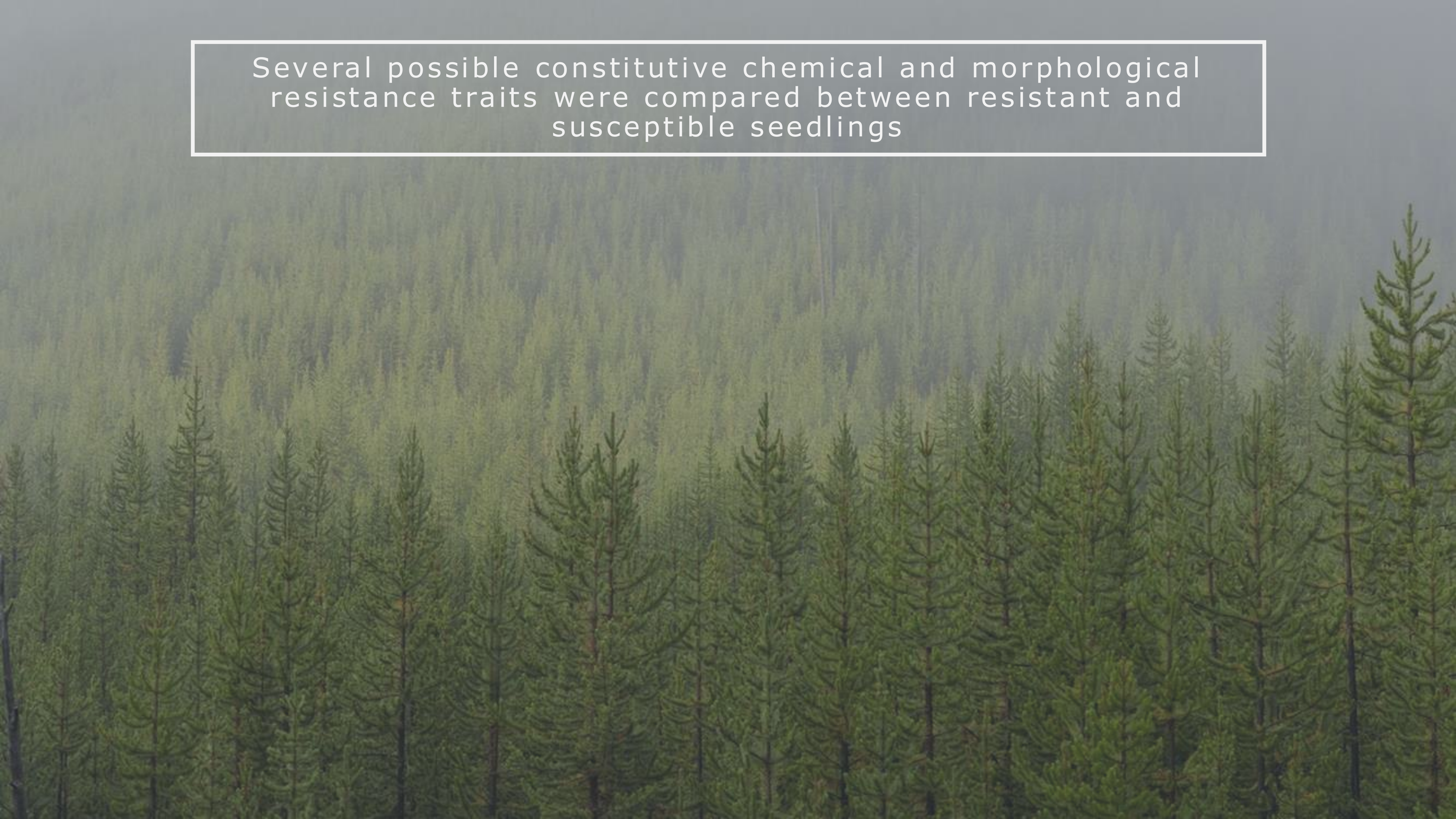
Phytochemical (41)
- Monoterpenes
- Sesquiterpenes
- Total

Anatomical (5)
- Needle length & width
- Needle surface area
- Stomatal density
- Epicuticular wax concentration

Productivity (7)
- Aboveground biomass: total, needle, and stem
- Needle biomass proportion
- Seedling height
- Incremental growth

Seedlings acquired from the Ministry of Forests

- 20 seedlings × 11 families = 220 seedlings
  - 5 putative resistant families
  - 6 putative susceptible families
- Potted one-gallon pots and grown in the EFL greenhouse at UNBC
- Healthy needles collected in mid-July for analysis



Several possible constitutive chemical and morphological resistance traits were compared between resistant and susceptible seedlings

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Putative resistant and susceptible lodgepole pine families

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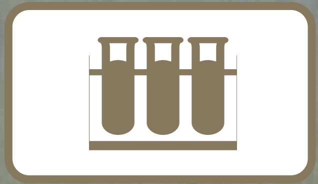

Remove and weigh epicuticular wax

Several possible constitutive chemical and morphological resistance traits were compared between resistant and susceptible seedlings

Putative resistant and susceptible lodgepole pine families

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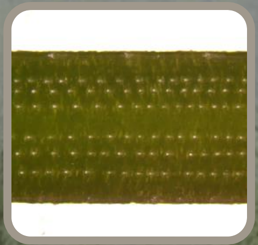
Remove and weigh epicuticular wax

Calculate stomatal density



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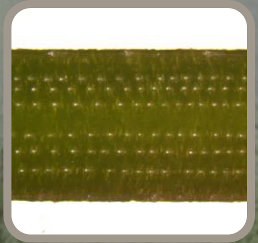
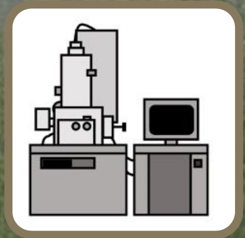


Capture SEM images for analysis of stomatal size and occlusion



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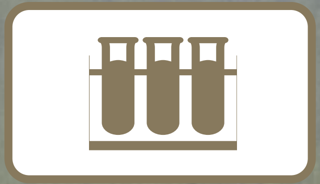

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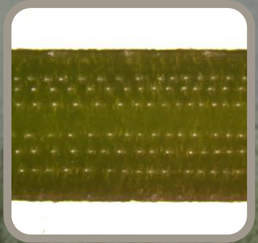

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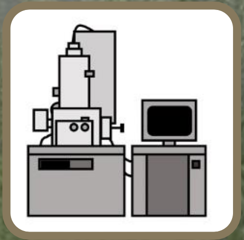
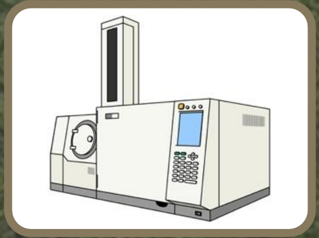


Analyze mono and sesquiterpenes contained in needles



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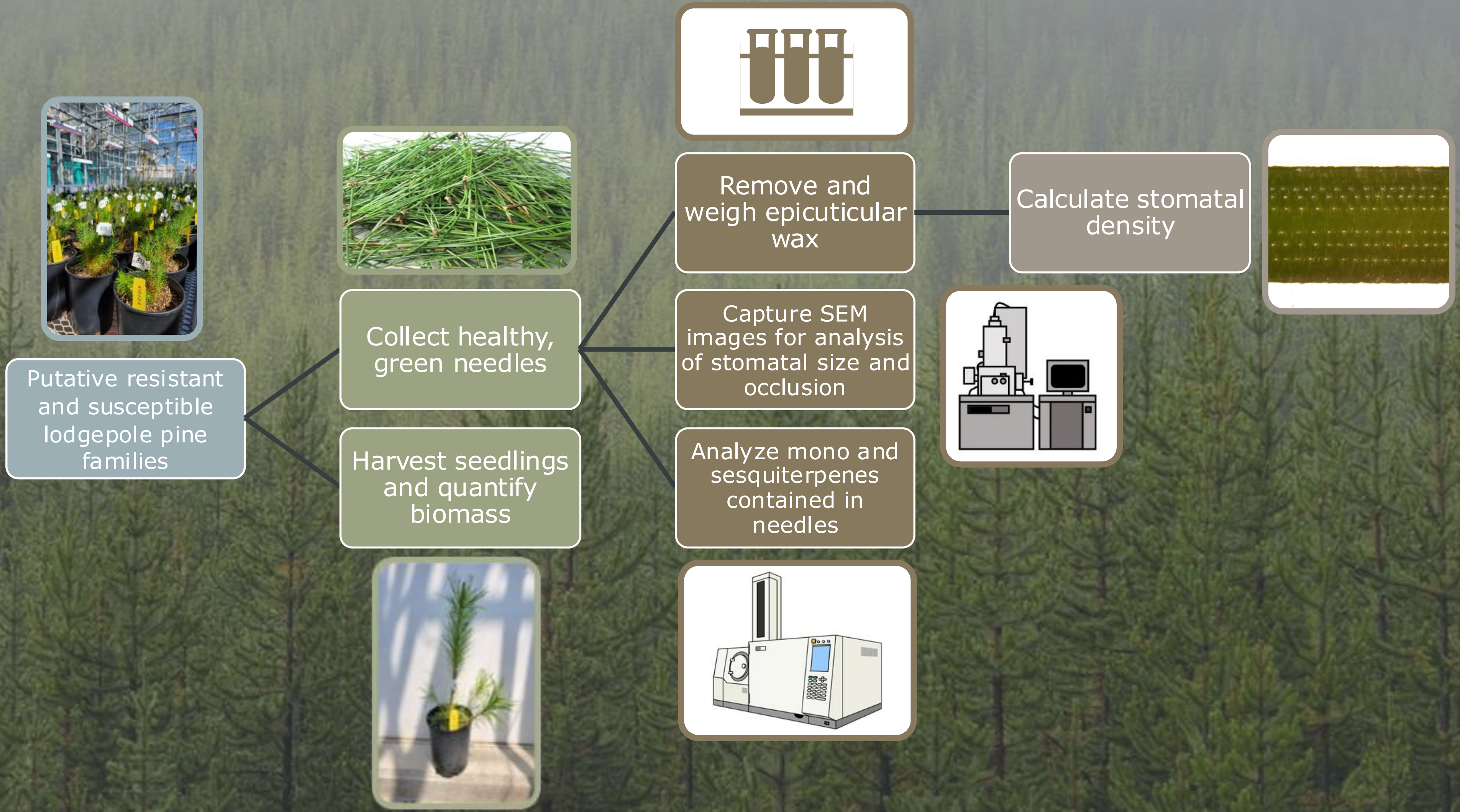

40 terpenes were identified through GC-MS

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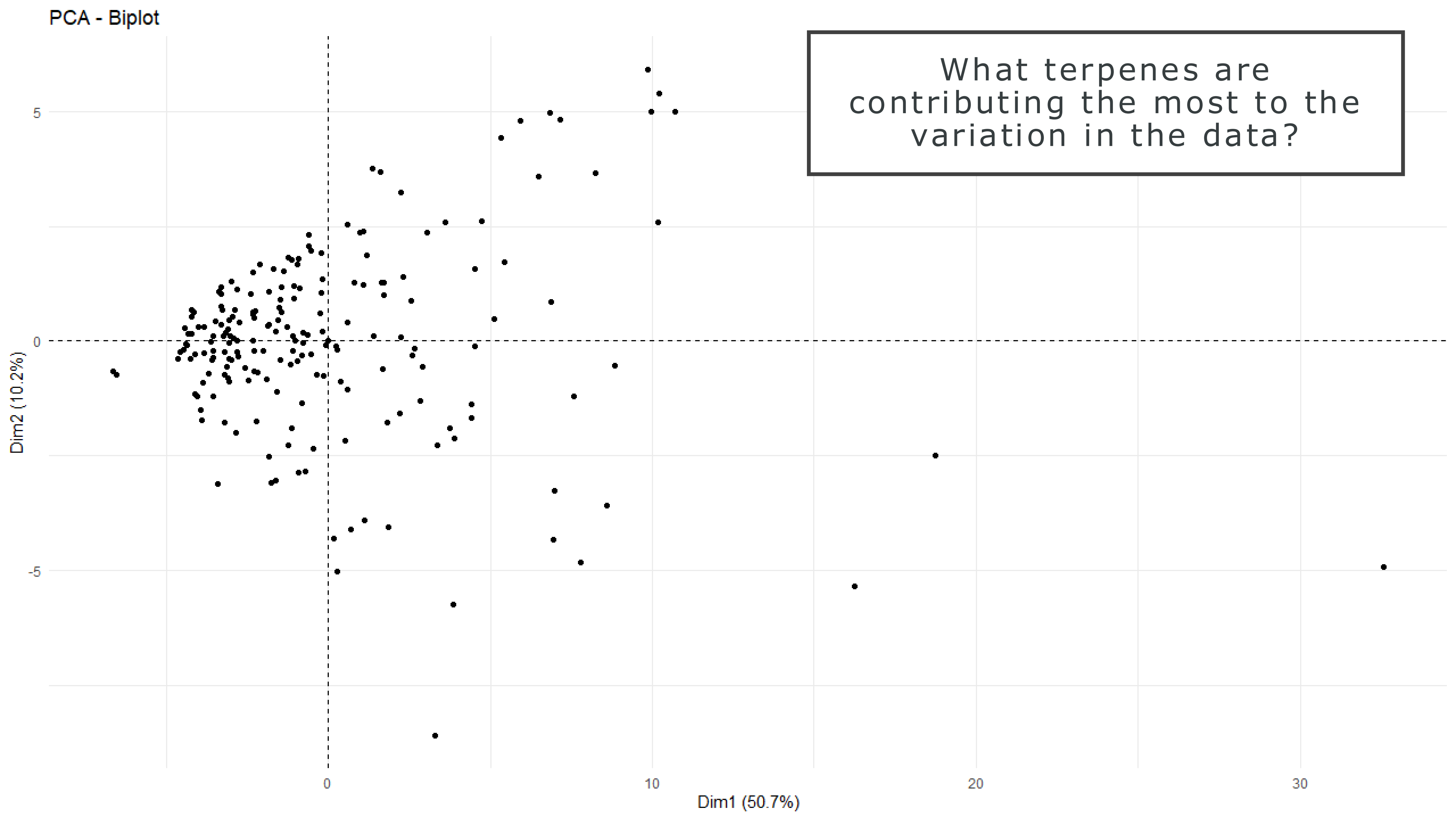
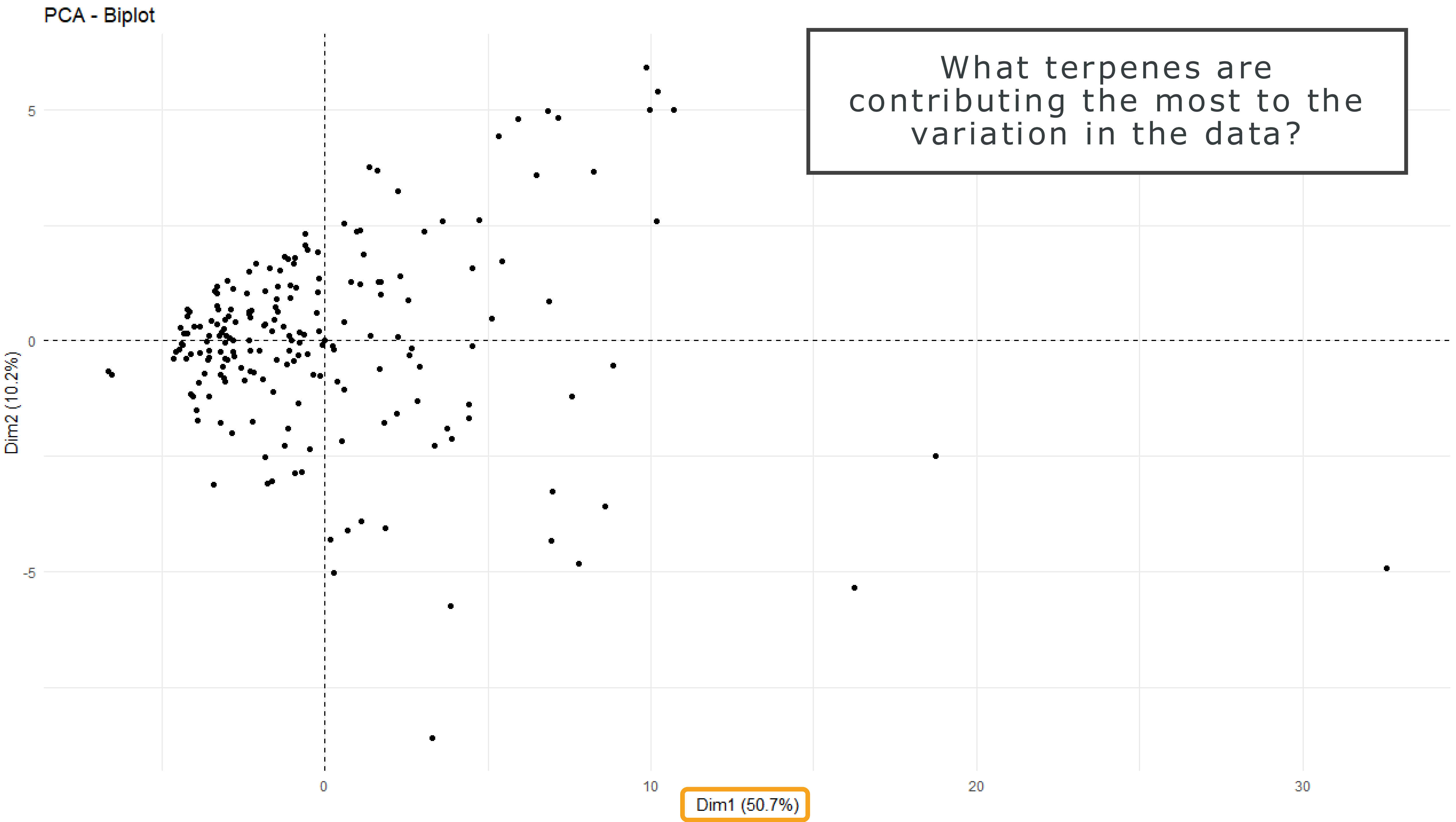
27 monoterpenes			
3-Carene	α-Phellandrene	α-Pinene	α-Terpinene
α-Terpineol	β-Myrcene	β-Ocimene	Bornyl acetate
β-Phellandrene	β-Pinene	β-Terpinene	Camphene
cis-2-Menthenol	D-Limonene	endo-Borneol	Geranyl acetate
Isoterpinolene	Linalool	Linalyl butyrate	neo-allo-Ocimene
Sabinene	Sabinene hydrate	Terpinolene	Tricyclene
trans-β-Ocimene	γ-Terpinene	γ-Terpineol	

40 terpenes were identified through GC-MS

27 monoterpenes				13 sesquiterpenes	
3-Carene	α-Phellandrene	α-Pinene	α-Terpinene	(Z,Z)-α-Farnesene	α-Cadinol
α-Terpineol	β-Myrcene	β-Ocimene	Bornyl acetate	β-Elemene	Cadina-1(10),4-diene
β-Phellandrene	β-Pinene	β-Terpinene	Camphene	Caryophyllene	Epi-cubebol
cis-2-Menthenol	D-Limonene	endo-Borneol	Geranyl acetate	Farnesol	Germacrene D
Isoterpinolene	Linalool	Linalyl butyrate	neo-allo-Ocimene	Germacrene D-4-ol	T-Cadinol
Sabinene	Sabinene hydrate	Terpinolene	Tricyclene	T-Muurolol	γ-Cadinene
trans-β-Ocimene	γ-Terpinene	γ-Terpineol		γ-Elemene	



What terpenes are contributing the most to the variation in the data?



What terpenes are contributing the most to the variation in the data?

Terpene	PC1
B.Myrcene	0.207031
Sabinene	0.200547
Camphene	0.196001
B.Terpinene	0.195495
D.Limonene	0.193485
Tricyclene	0.190369
Isoterpinolene	0.190318
B.Phellandrene	0.187916
a.Pinene	0.187834
a.Phellandrene	0.186082
a.Terpineol	0.185024
cis.2.Menthenol	0.184646
Linalool	0.182065
neo.allo.Ocimene	0.178367
a.Terpinene	0.177382
endo.Borneol	0.173645
Germacrene.D	0.173271
Germacrene.D.4.ol	0.168968
Epicubebol	0.168
Geranyl.acetate	0.16014
y.Terpinene	0.152971
Terpinolene	0.15099
X3.Carene	0.149404
y.Cadinene	0.148504
Linalyl.butyrate	0.146691
Bornyl.acetate	0.144199
T.Cadinol	0.142808
Farnesol	0.14032
Cadina.1.10..4.diene	0.138293
a.Cadinol	0.123136
Caryophyllene	0.117586
X.Z.Z..a.Farnesene	0.110243
Sabinene.hydrate	0.109119
B.Elemene	0.106876
Isopentanal	0.103638
T.Muurolol	0.097513
B.Ocimene	0.08817
trans.B.Ocimene	0.084201
y.Terpineol	0.057192
B.Pinene	0.048384
y.Elemene	0.029011

β-Myrcene
Sabinene
Camphene
β-Terpinene
D-Limonene
Tricyclene
Isoterpinolene
β-Phellandrene
α-Pinene

80th percentile of the loadings

Selected the top 9 terpenes for further analysis

Terpene	PC1
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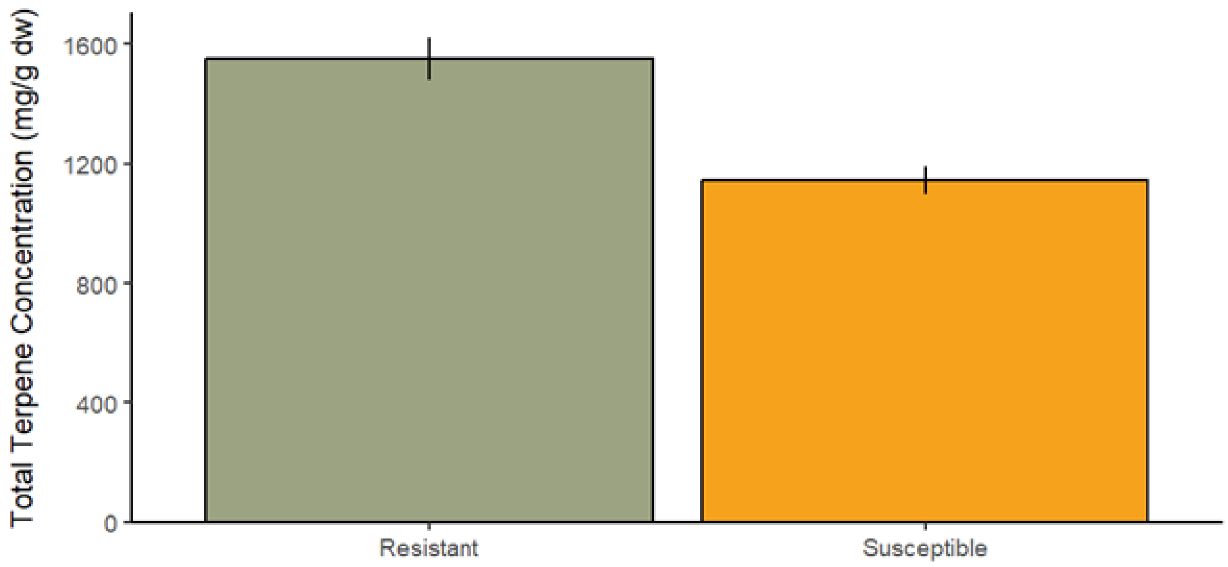
80th percentile of the loadings

Selected the top 9 terpenes for further analysis

The difference in total terpene concentration between resistant and susceptible seedlings becomes significant when using the top 9 terpenes



There **is** a significant difference in terpene concentration between resistant and susceptible seedlings, even after accounting for variability among families.

The variation in terpene profile is related to whether seedlings are resistant or susceptible

Permutational multivariate analysis of variance (PerMANOVA)

- On the terpene concentration profiles using the subset of 9 terpenes
- $p = 0.001$ and therefore:

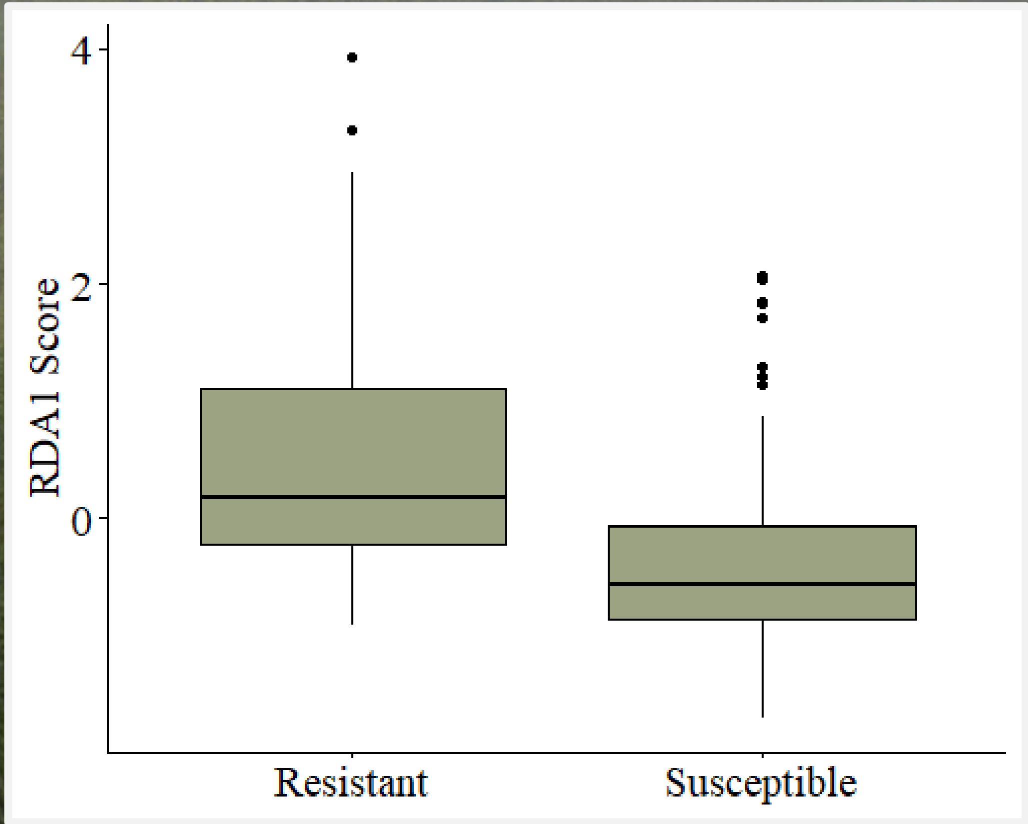
The variation in terpene profile **is** significantly related to whether seedlings are resistant or susceptible.

Terpene profile is informative for predicting whether seedlings will be resistant or susceptible

Redundancy analysis (RDA)

- **18%** of the variation can be explained by whether a seedling is resistant or susceptible
- The rest of the variation comes from other unknown or unmeasured influences

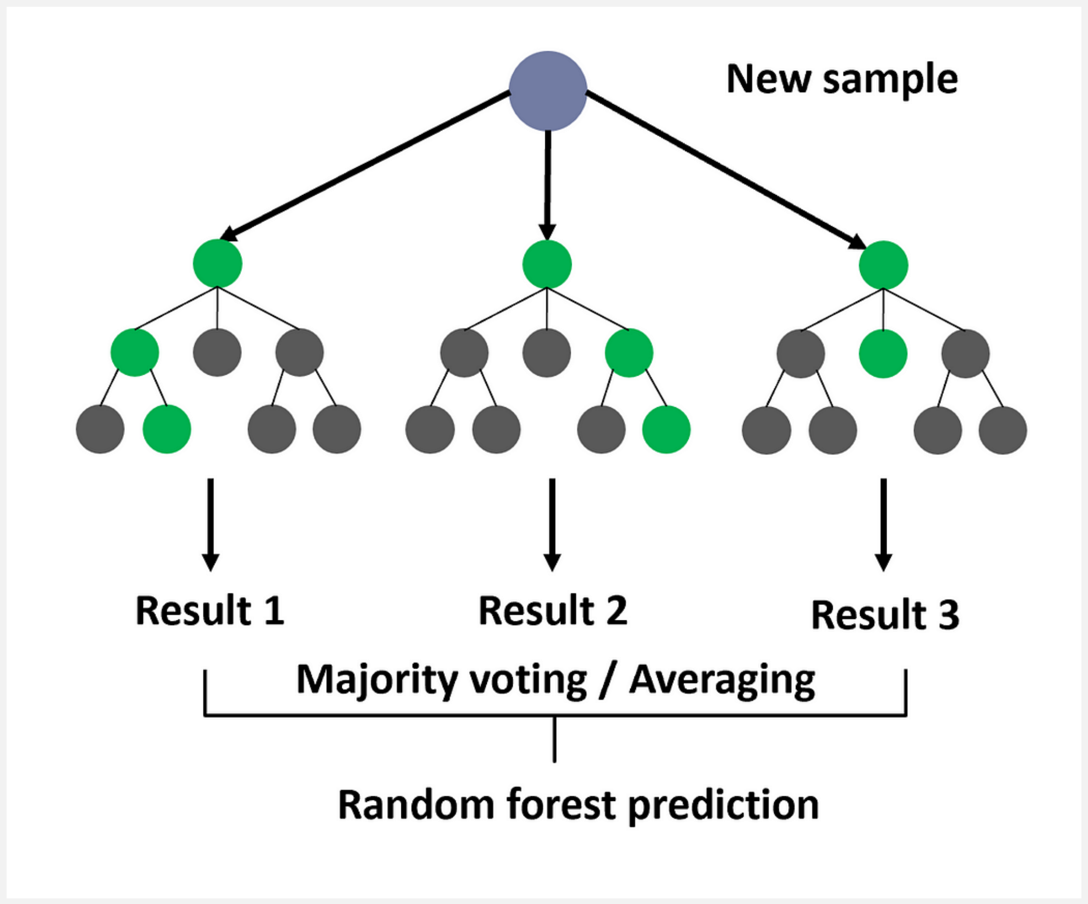
RDA1 loadings → further analysis



What are the **most** informative traits for predicting resistant vs. susceptible seedlings?

Random Forests

- Terpene profiles (RDA results)
- Needle surface area
- Epicuticular wax concentration
- Stomatal density
- Needle biomass
- Seedling height and incremental growth

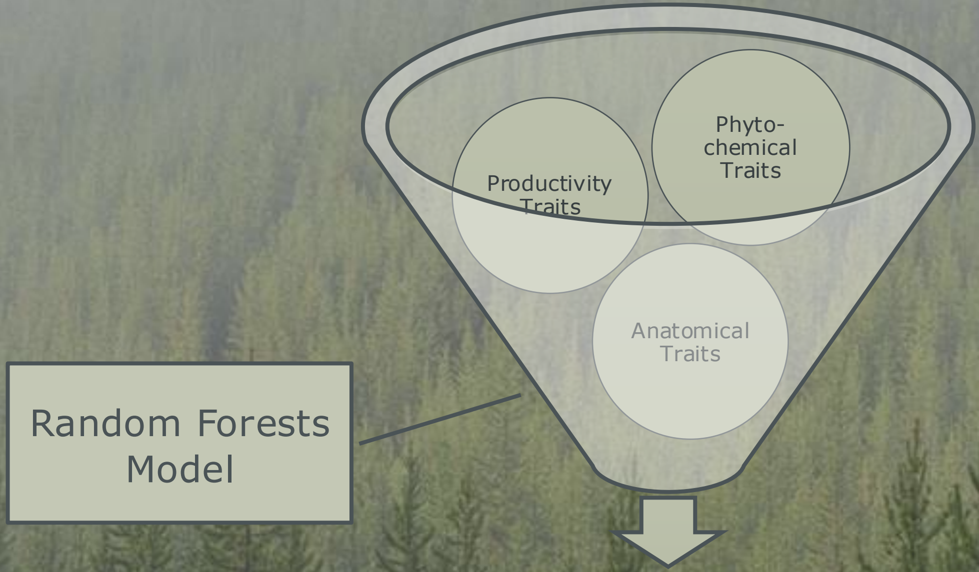


The model trained on the top three bioindicator traits performs the best

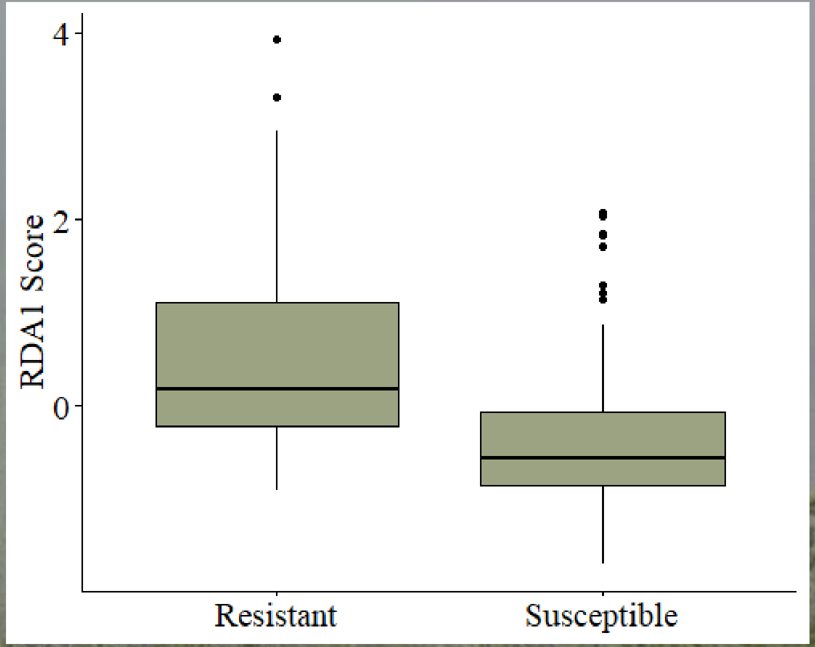
- Random Forest model evaluated using Leave-One-Family-Pair-Out Cross-Validation (LOFPO-CV)
- Best predictive accuracy achieved with the top two traits:

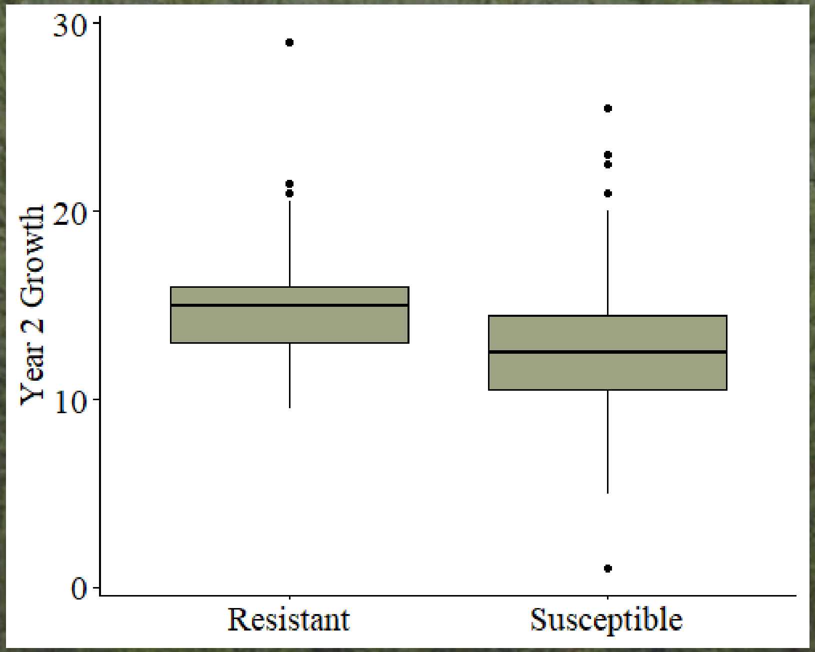
1. Terpene profile
2. Year 2 growth

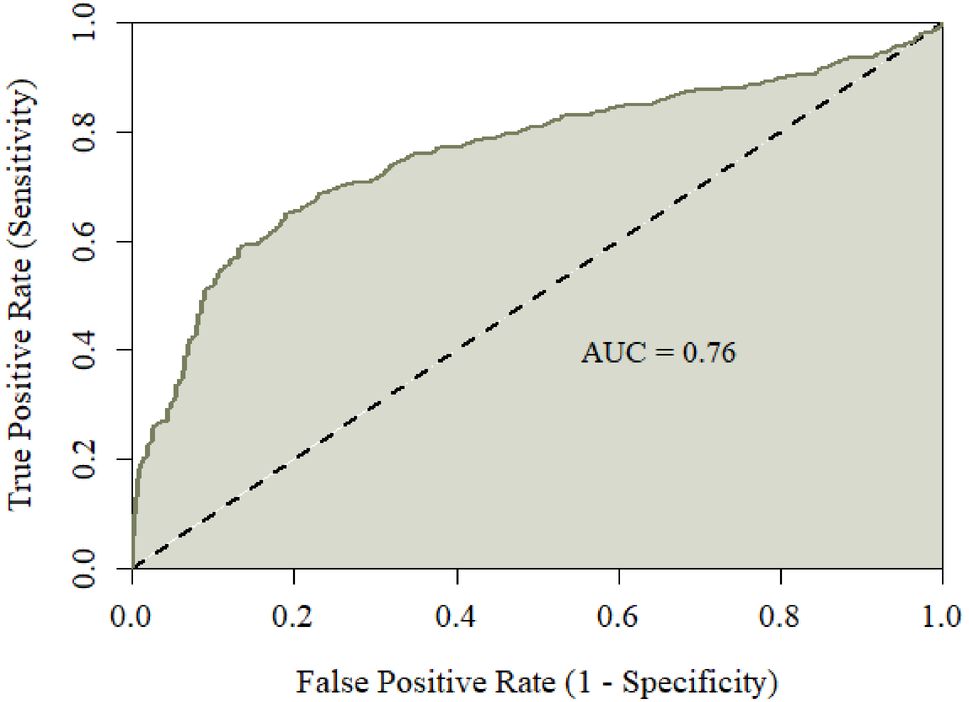
Terpene profile and year 2 growth appear to be most informative for predicting resistance status



Trait (descending importance)
Terpene Profile
Year 2 Growth
Proportion of Needle Biomass
Stomatal Density
Total biomass
Average Needle Surface Area
Year 1 Growth
Total Seedling Height
Epicuticular Wax Concentration



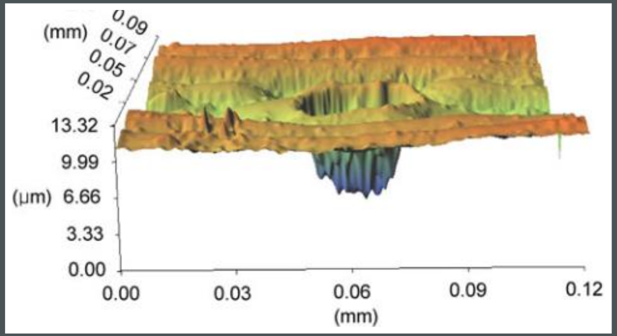




The final random forests model demonstrates moderate predictive power

- **Sensitivity:** the proportion of susceptible seedlings that were correctly predicted
- **Specificity:** the proportion of resistant seedlings that were correctly predicted
- Area under the curve (AUC): 0.75
  - The model is 75% effective at predicting whether a seedling is resistant or susceptible.

The addition of new traits may improve the model



Kim et al., 2011, Microscopy and Microanalysis

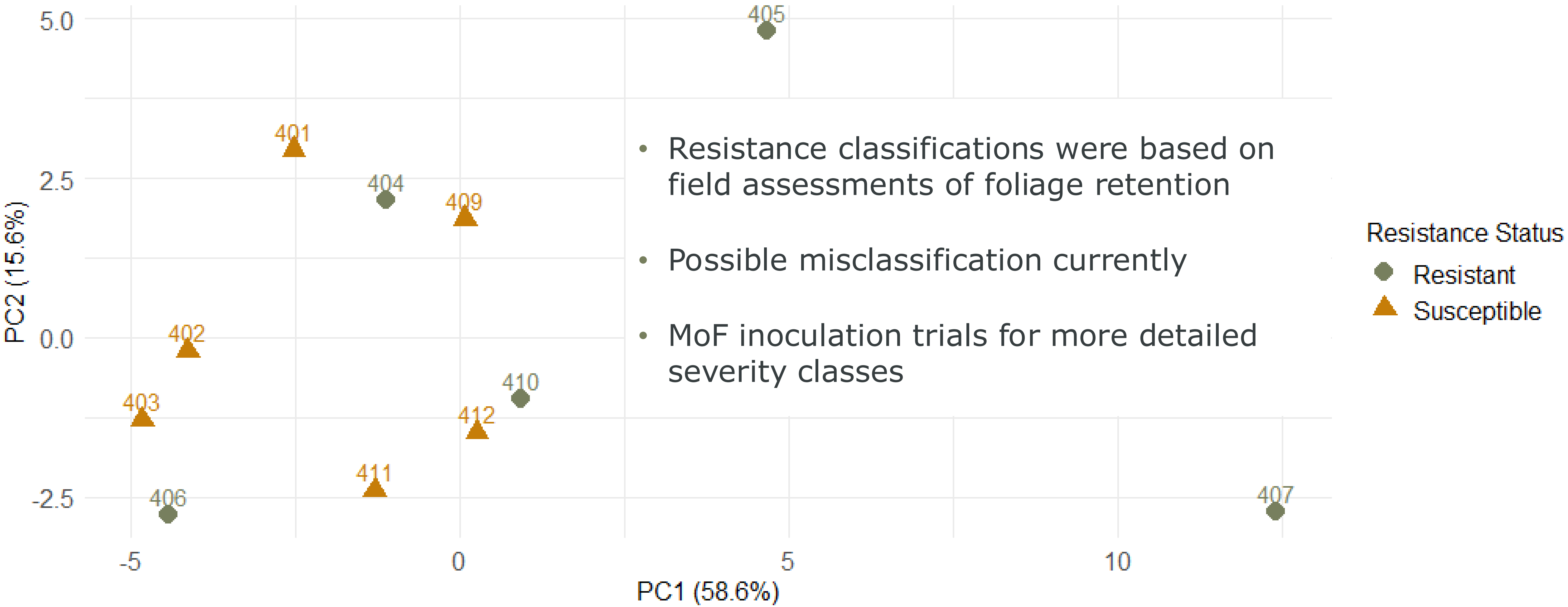
SEM-measured traits:

- Epistomatal chamber diameter and area
- Stomatal occlusion by epicuticular wax
- All similar between resistant and susceptible seedlings

Future research could incorporate traits such as:

- Spectral properties
- Needle morphology
- Resin canal density
- Post infection phytochemicals
- Dothistroma lesion length

A more continuous variation in resistance status is likely



- Resistance classifications were based on field assessments of foliage retention
- Possible misclassification currently
- MoF inoculation trials for more detailed severity classes

Summary

- Lodgepole pine is an important species for BC’s forest industry
- It faces a significant threat from DNB, especially in areas that will become warmer and wetter due to climate change
- Identifying defense traits to produce resistant breeding stock is a promising strategy
- Results are promising so far:
  - The RF model is 75% effective at predicting whether a seedling is resistant or susceptible.

What’s next?

- Inoculation trials to confirm resistance status in these families
- Investigate additional traits to capture more variation between resistant and susceptible families and potentially enhance the predictive accuracy of the RF model
- Validate the model using new seedlings from the same families

Acknowledgements

Supervisor: **Jonathan Cale**, Ph.D.

Committee members:

- **Nick Ukrainetz**, Ph.D., RPF
- **Alex Woods**, M.Sc., RPF
- **Kalindi Morgan**, Ph.D.

Collaborator: **Nadir Erbilgin**, Ph.D.

Research assistant: **Karra McFarlane**

SEM: **Northern Analytical Laboratory Services**

EFL Greenhouse Curators:

- **Doug Thompson**
- **Kennedy Boateng**, Ph.D.