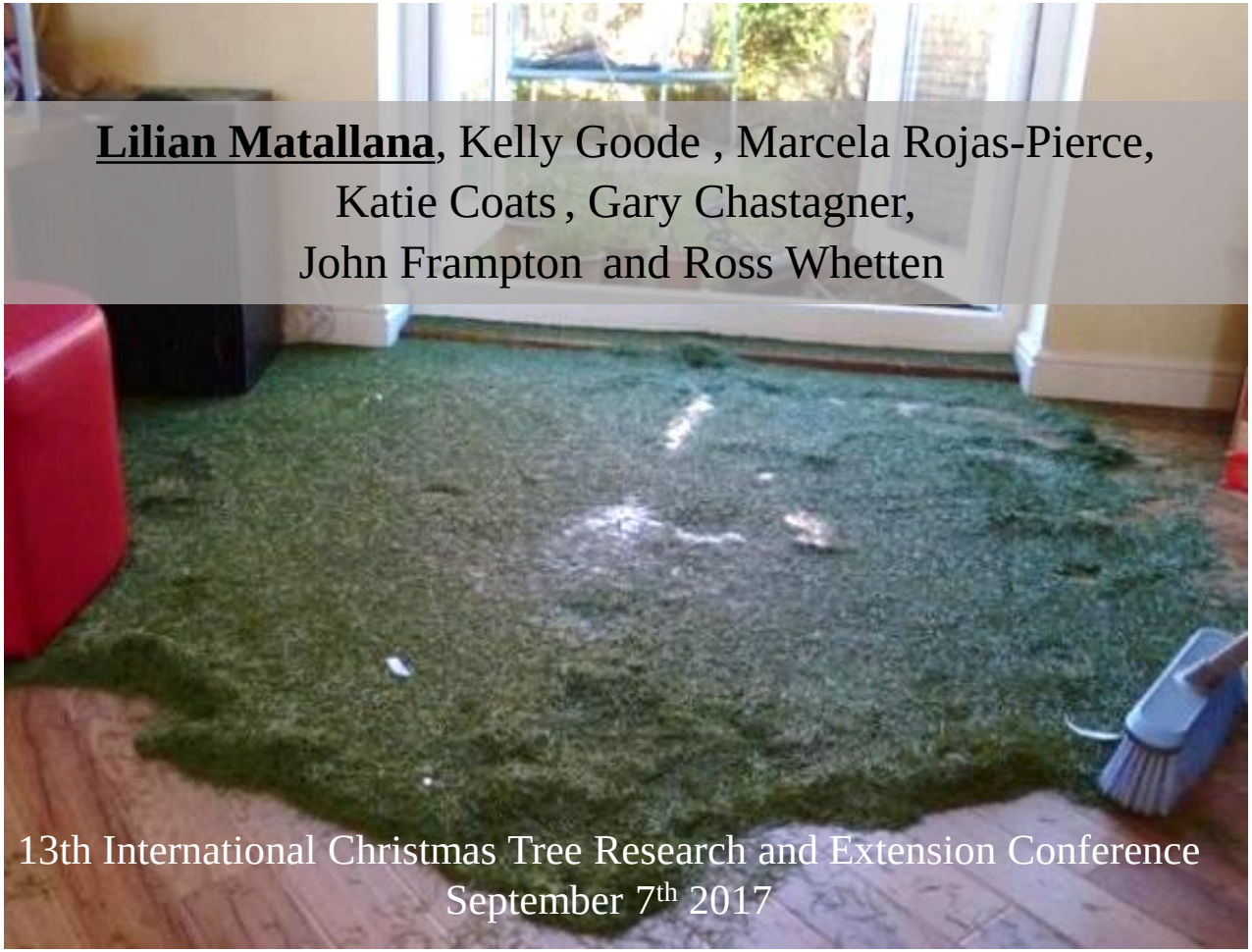
Christmas tree needle separation: a marriage that ends on the carpet



Lilian Matallana, Kelly Goode , Marcela Rojas-Pierce,
Katie Coats , Gary Chastagner,
John Frampton and Ross Whetten

13th International Christmas Tree Research and Extension Conference
September 7th 2017

North Carolina State University
Washington State University

The problem: “Needle separation”

Top 10 reasons to not buy a real Christmas tree

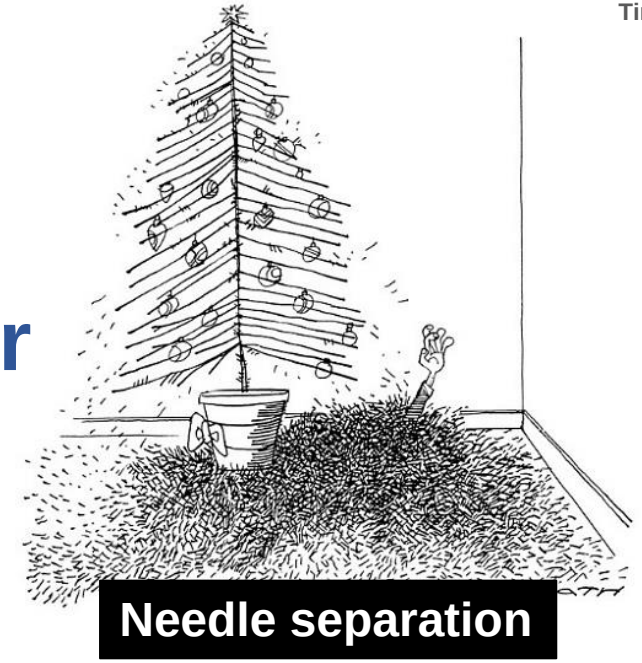
©NCTA 2001

“Messiness”

Needle retention

Good – Poor

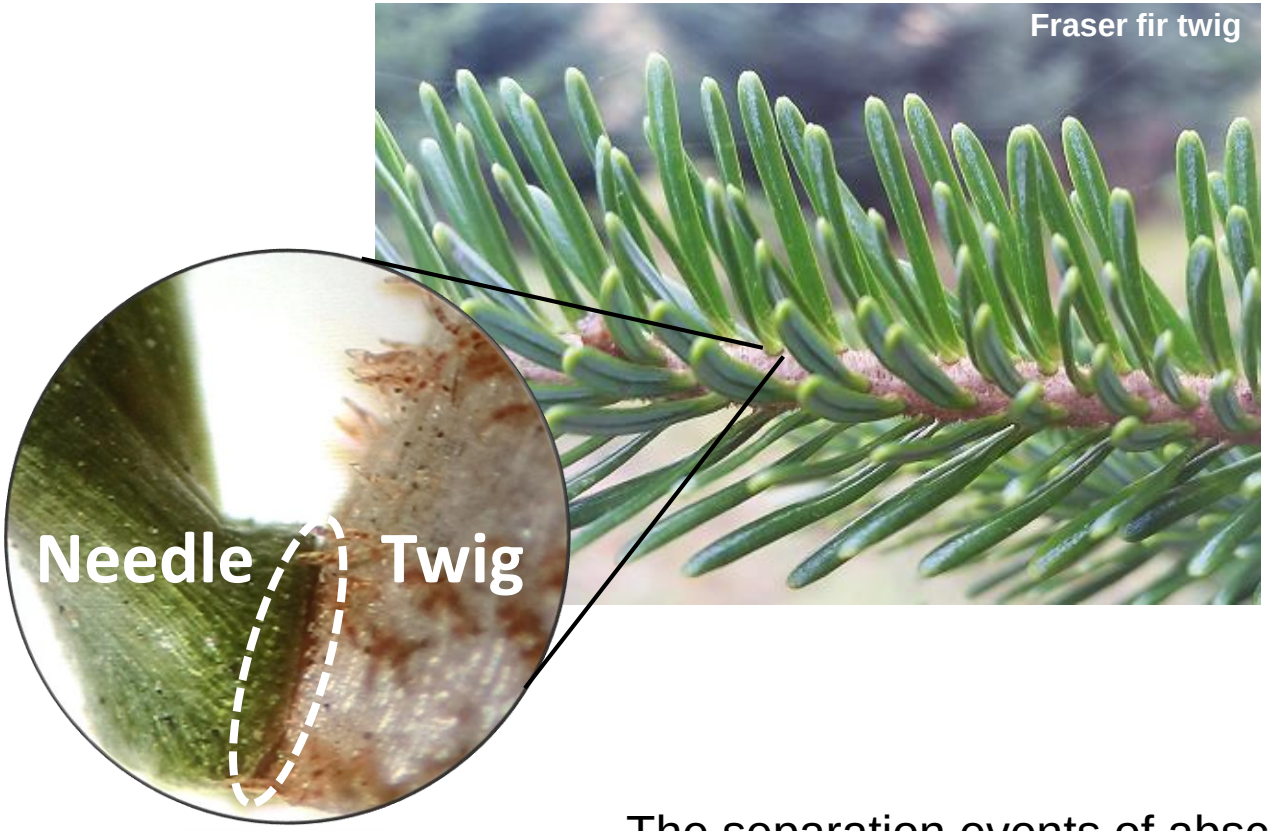
NR



Needle separation

Abscission

Needle separation = Needle abscission



Needle Abscission Zone (NAZ)

The term abscission is specific to the **plant world** and refers to a natural **physiological** process of **shedding of plant organs**.

The separation events of abscission occur within the **specific regions** called **abscission zones** (**AZs**).

Our approach

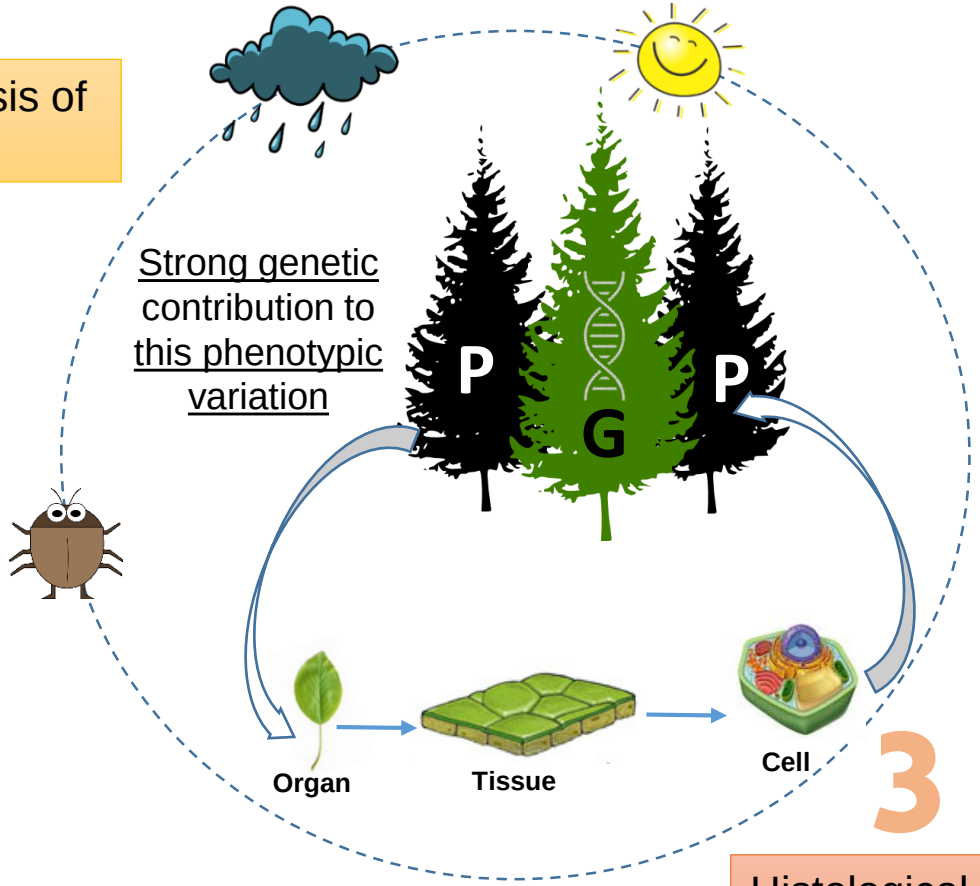
“Needle separation”

1

Collection and analysis of phenotypic data

2

Use genomic tools to analyze needle abscission.
(Lab protocol & Transcriptome data analysis)



3

Histological characterization of the AZ in Fraser fir

Our project

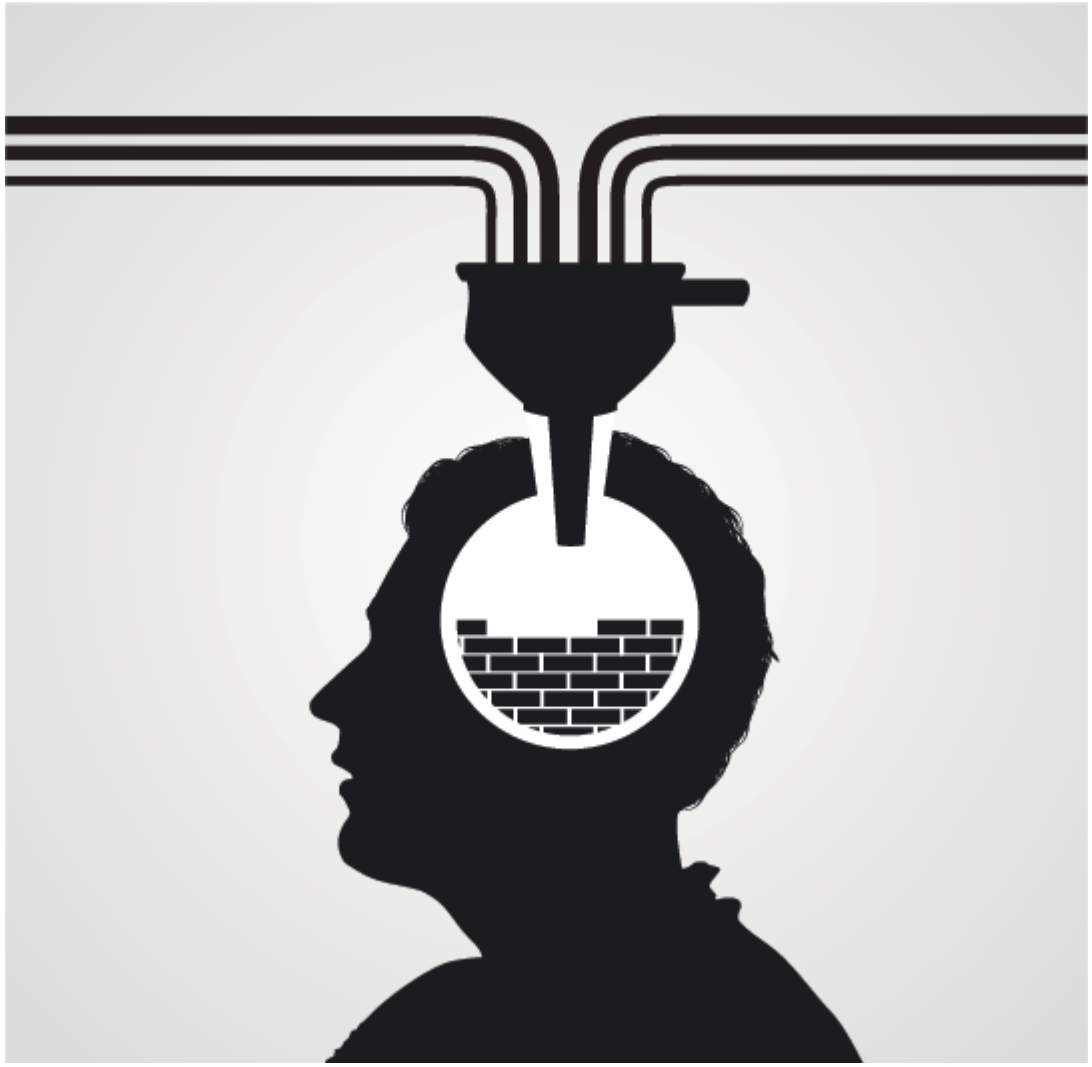
Main Goal

- Design a system for the histological characterization of the AZ in Fraser fir.

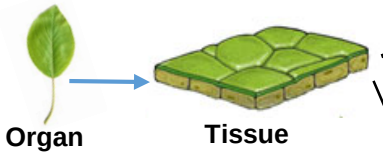
Short-term goals

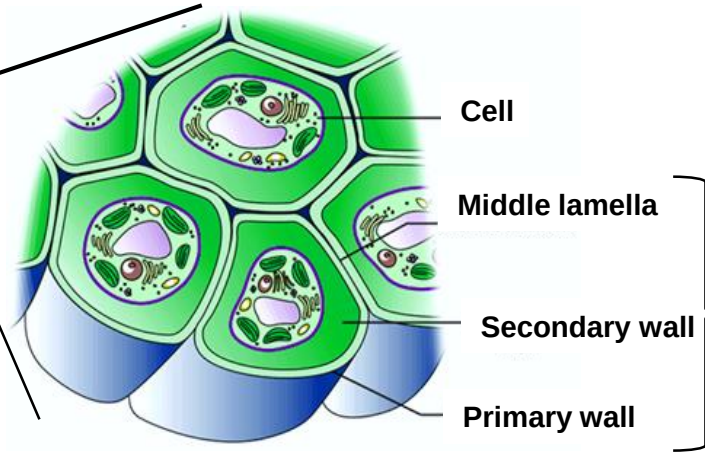
- Establish the **protocols for bright-field and confocal microscopy**.
- Identification and characterization of the AZ in firs.
- Identification of **potential anatomical differences** between trees that exhibit **good and poor needle retention**.

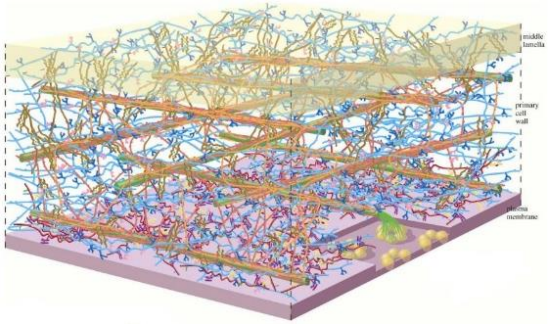
INTRODUCTION



Cellular anatomy related to abscission

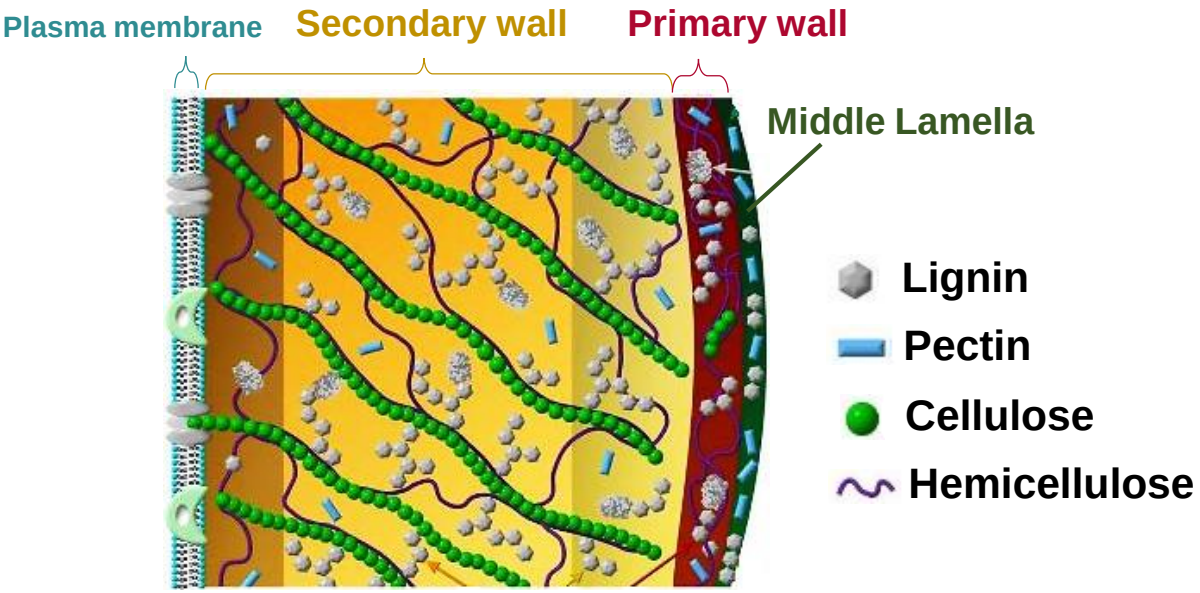






Cell wall

Heterogeneous natural matrix



Adapted from Animalia Life http://animalia-life.club/other/plant-cell-wall-lignin.html
https://www.slideshare.net/BenjaminMerritt/plant-cell-wall-mod

Plant abscission general model

1

Determination of abscission zone (AZ)

2

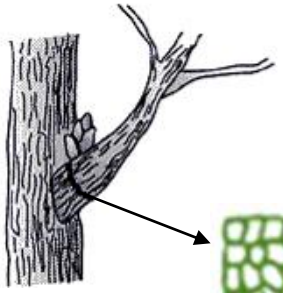
Competence to respond to abscission signals

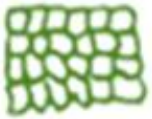
3

Activation of abscission

4

Post abscission transdifferentiation

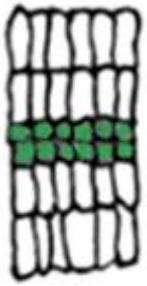


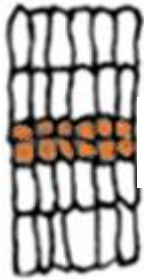


Development of the AZ

Pre-abscission program

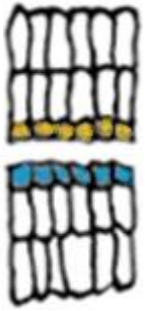
- **Phytohormones**
- **Environmental signals**

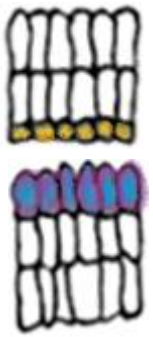




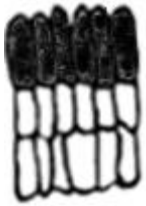
Dissolution of middle lamella

Loosening cell wall





Are elongation or expansion of cells essential?



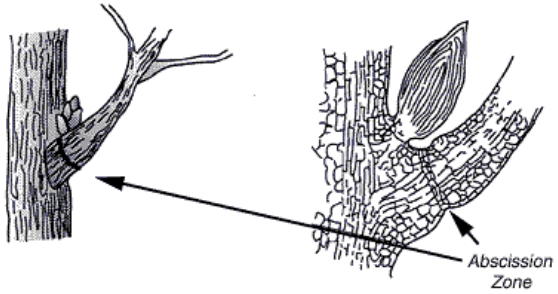
Differentiation of a protective layer

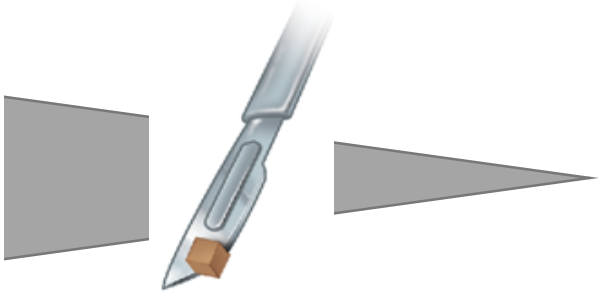
Cell division?

Adapted from Patterson (2001)

Histological methods

Isolation



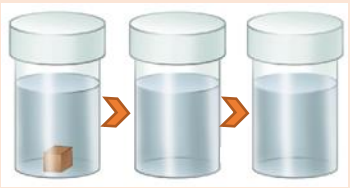


Fix tissue

Step of killing and preserving tissue

- Conserve the highest level of fidelity between living cells and stabilized (“fixed”) condition.
- Tissue may be stored for an indefinite period of time.

Dehydration



- Most embedding media are hydrophobic, thus water needs to be removed.
- Two methods:

1. *Graded dehydration series*
2. *Rapid dehydration system*

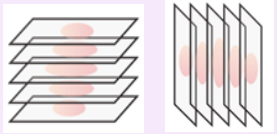
Embed

Paraffin wax

White resin

- Tissue must be encased in a solid matrix to add structural before sectioning.
- Infiltration and embedding.

Sectioning



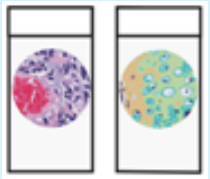
- Tissue are thinly sliced (sectioned).

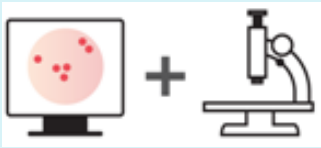
Microtome: use steel, glass, or diamond blades depending upon the specimen being sliced and the desired thickness of the sections being cut.

Staining & Mounting

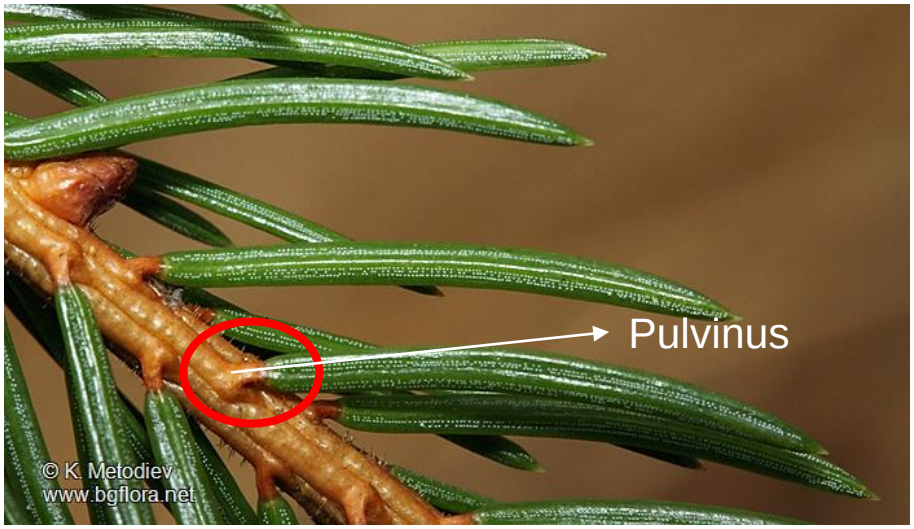
Light-Bright field

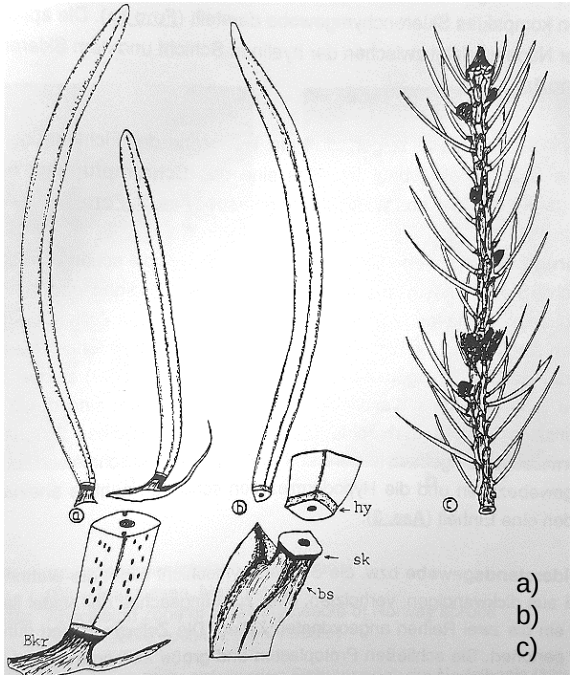
Confocal





Needle abscission in conifers





Types of needle loss:
- a) Mechanically detached needle loss
- b) Needle fall via abscission zone
- c) Needle loss causes by twig loss

Needle abscission in conifers

Environmental and Hormonal Physiology of Postharvest Needle Abscission in Christmas Trees

Article *in* Critical Reviews in Plant Sciences · January 2016

DOI: 10.1080/07352689.2015.1133965

CITATION 1

READS 45

1 author:

Dr. Arumugam Thiagarajan

Agriculture and Agri-Food Canada

35 PUBLICATIONS **94** CITATIONS

ABSTRACT

Several of the conifer species are increasingly adopted as Christmas trees worldwide. These species have become integral parts of the horticultural economies of North American and European countries. Postharvest characteristics such as needle abscission/retention, color, fragrance and rehydration abilities vary with species and these complex physiological traits are strongly modulated by hormonal and environmental factors. A large body of research indicates that prevalence of low temperature before harvest evokes cold acclimation responses that involve an increase in complex sugar concentrations, alterations in membrane structures and enhancements in scavenging abilities promoting postharvest needle retention. Adverse postharvest environmental factors, for example, high temperature and vapor pressure deficit are found to increase water stress, cause dehydration and accelerate needle abscission and/or discoloration. Postharvest water stress/cellular dehydration is one of the fundamental biophysical signals that triggers a cascade of hormonal changes, leading to needle abscission. Abscissic acid levels increase during cold acclimation as well as prior to abscission indicating a complicated and paradoxical role in abscission. Ethylene levels increase before abscission and are well proven to instigate the needle fall. Concentrations of cytokinins, auxins and polyamines decline postharvest. However, their interactive roles with other phytohormones orchestrating the abscission process still remain elusive. This review presents and discusses our current knowledge of the physiological aspects of pre-and postharvest environmental factors on needle abscission.

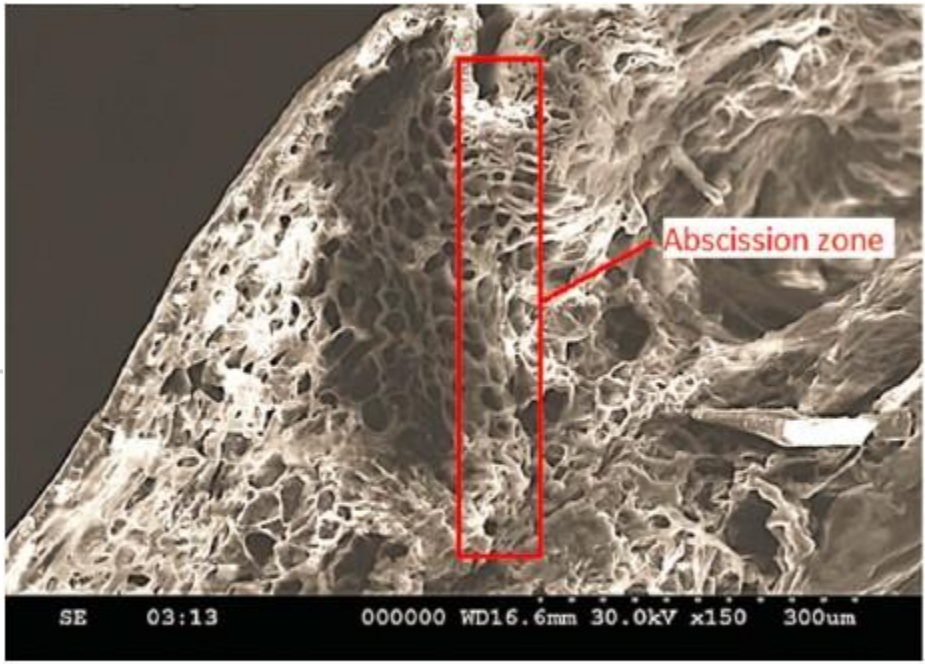
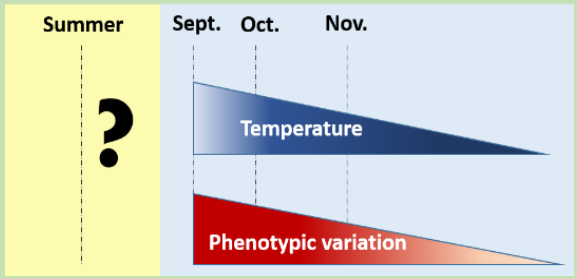


Figure 1. Scanning electron microscope image of the longitudinal section of the balsam fir needle illustrating the abscission zone (Thiagarajan, unpublished).

EXPERIMENTAL DESIGN

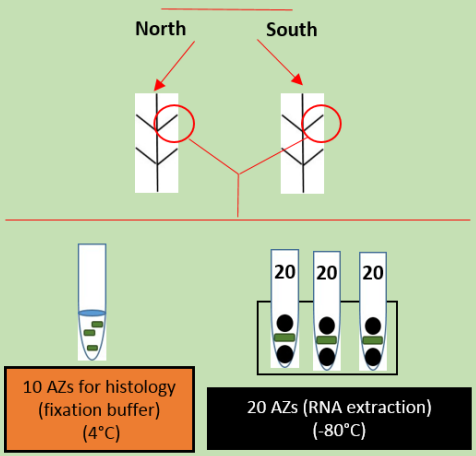
Experimental design – Collection of tissue

Field collection

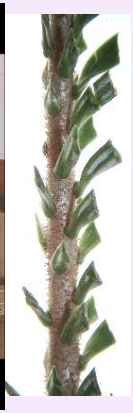


8x Good 8x Poor

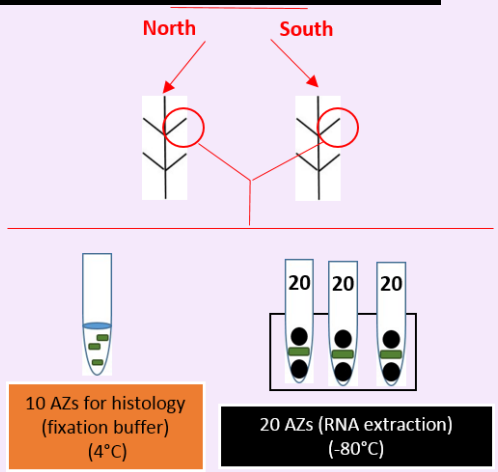
4 Field collections



Display-room collection



~3 x ~3 mm




Unexpected
Outcomes
Good
Outcomes

Outcome: Tissue collection and RNA-seq

- Collection of Fraser fir, Canaan fir and Balsam fir AZs for histological and transcriptome analyses.

Field_Coll_Num	Field_Coll_Date	Room_Coll_Date	DAC	Total samples	Total Clones	Good	Poor	Type
1	9/28/2016	9/28/2016	0	32	16	8	8	N+S
1	9/28/2016	10/10/2016	13	32	16	8	8	N+S
1	9/28/2016	10/17/2016	20	8	4	2	2	N+S
1	9/28/2016	10/21/2016	24	16	4	2	2	N+S
1	9/28/2016	10/24/2016	27	16	4	2	2	N+S
1	9/28/2016	11/14/2016	48	18	5	5	4	N+S
2	10/19/2016	10/19/2016	0	32	16	8	8	N+S
2	10/19/2016	10/31/2016	13	39	16	8	8	N+S
2	10/19/2016	11/7/2016	20	16	4	2	2	N+S
2	10/19/2016	11/18/2016	31	16	4	2	2	N+S
2	10/19/2016	11/22/2016	35	16	4	2	2	N+S
3	11/16/2016	11/16/2016	0	32	16	8	8	N+S
3	11/16/2016	11/28/2016	13	16	4	2	2	N+S
3	11/16/2016	12/2/2016	17	16	4	2	2	N+S
3	11/16/2016	12/5/2016	20	16	4	2	2	N+S
3	11/16/2016	12/9/2016	24	12	3	2	1	N+S
3	11/16/2016	12/12/2016	27	12	3	2	1	N+S
3	11/16/2016	12/16/2016	31	12	3	2	1	N+S
3	11/16/2016	12/20/2016	35	1	1	1	0	N+S

October

November

December

New Summer collection data!!

Outcome: Protocol standardization

- Improved methods for fixation, cutting, mounting, and staining AZ of Fraser fir for histological studies.

Fix tissue → **Dehydra…** → **Embed** → **…on** → **Staining & Mounting**

Fixatives:
- Formalin-based
- Alcohol-based
- Zenker's
- B5
- Bouin's fixatives
- Formaldehyde-based

?

…icrotome

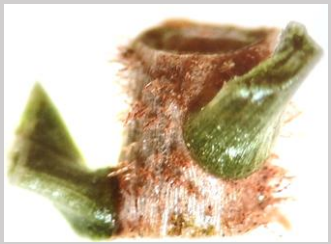
- Limited number of samples

- Protocol for studies of lignin and cellulose base on paraffin embedded samples

?

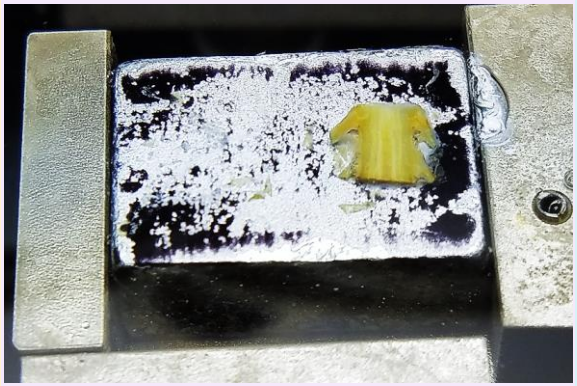
Outcome: Protocol standardization

Tissue isolation → **Fix tissue** → **Section** → **Staining & Mounting**

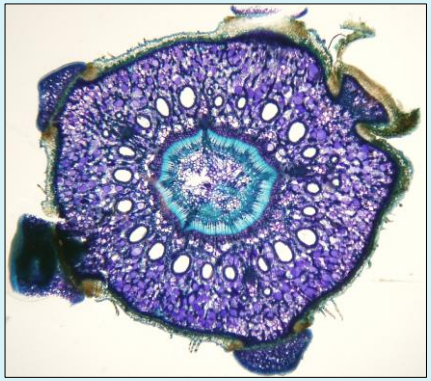


Commercial fixative (+10 Months)

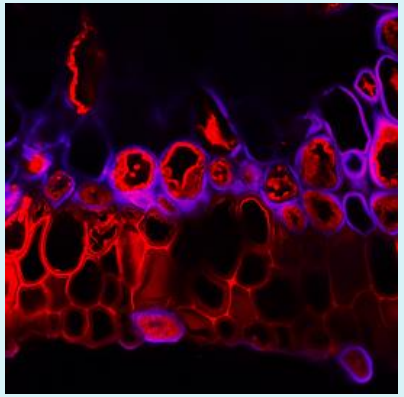
Vibratome



Staining protocol for bright field microscopy

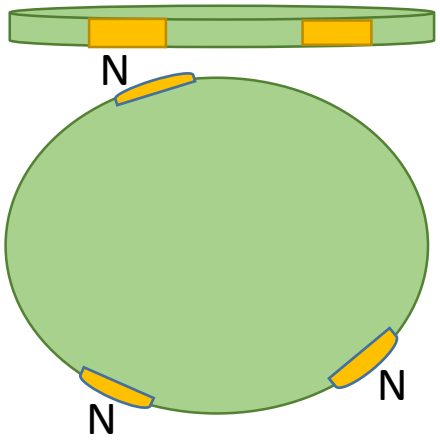


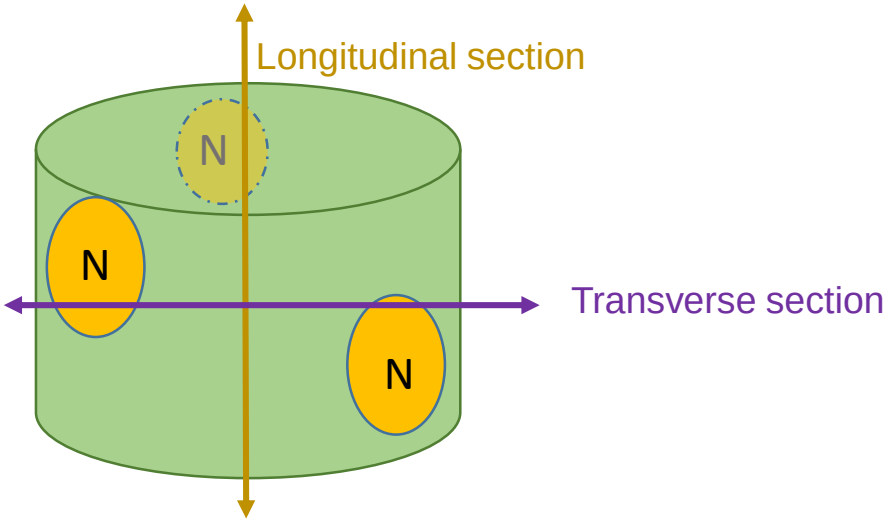
Staining protocol for confocal microscopy



Cutting & Staining Protocol

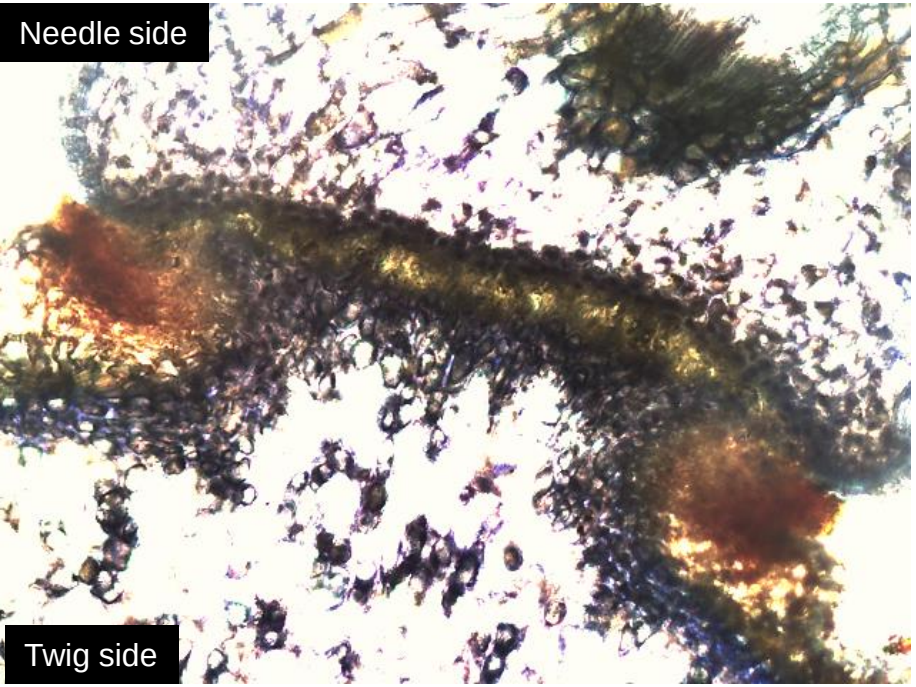
Outcome: Cutting protocol



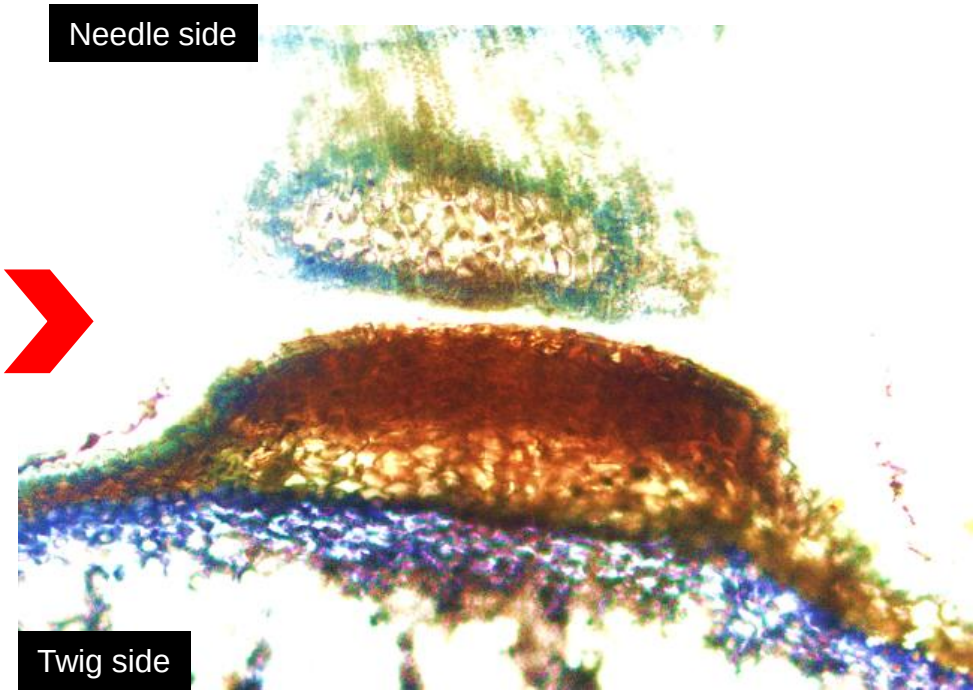


Outcome: Toluidine Blue staining

Before abscission



After abscission

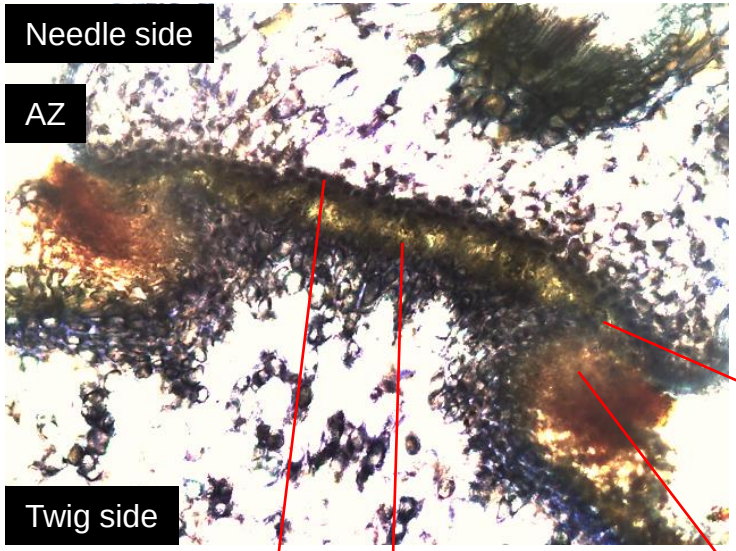


?

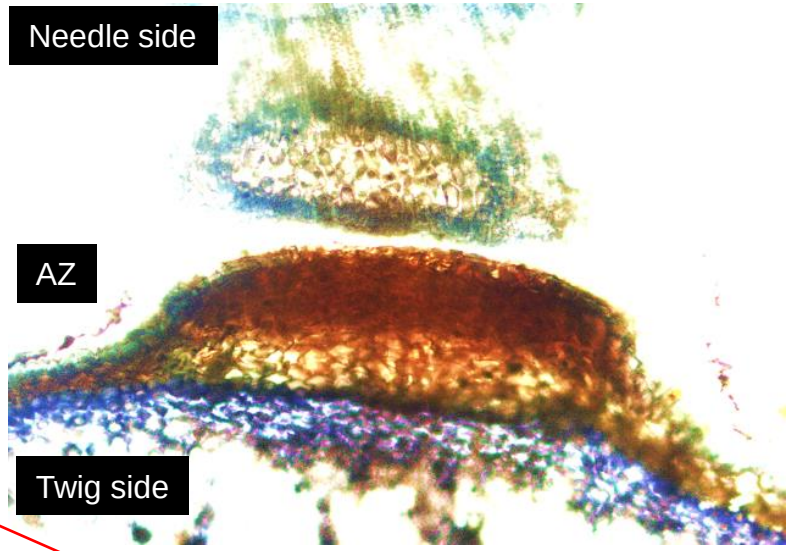
- When?
- What are those reddish-brown spots?
- How many structures could be recognized?
- Can we identify good and poor NR clones?

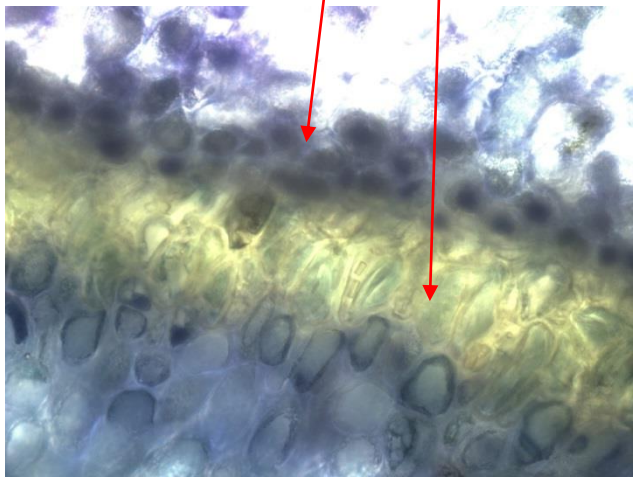
Outcome: Toluidine Blue staining

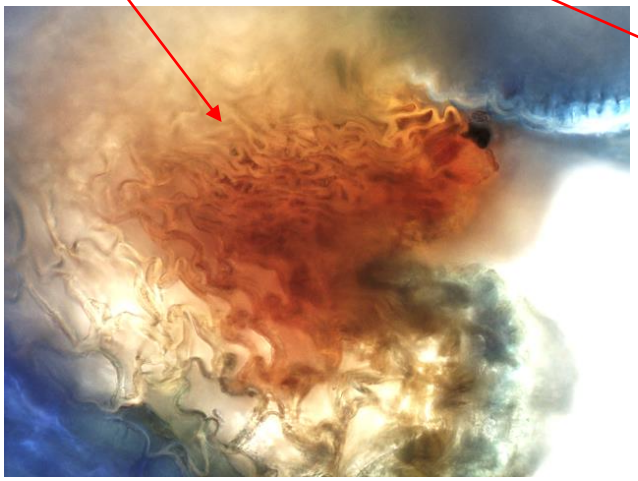
Before abscission

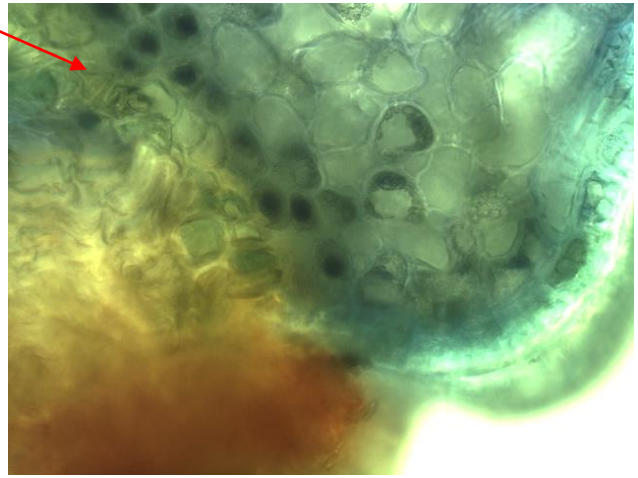


After abscission









Histological characterization of needle abscission in Fraser fir

Outcome: 3-D bright field microscopy analysis

- Designed a method to obtain a 3-D view of the AZ using bright field microscopy.

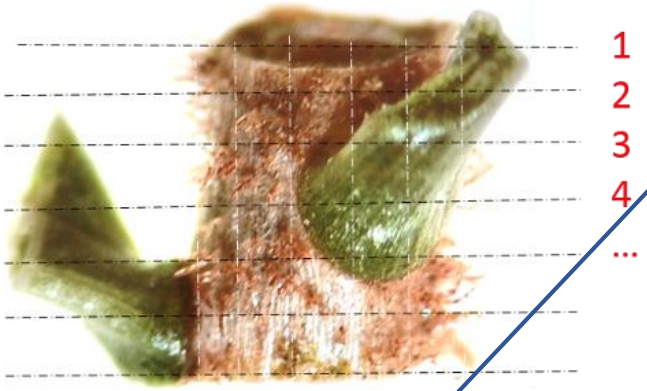


Plate 1

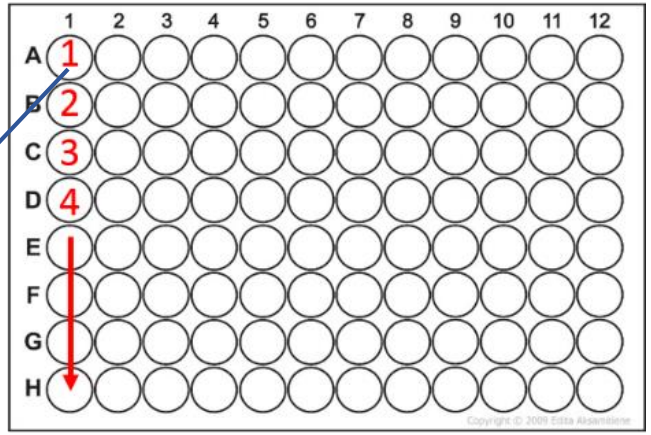
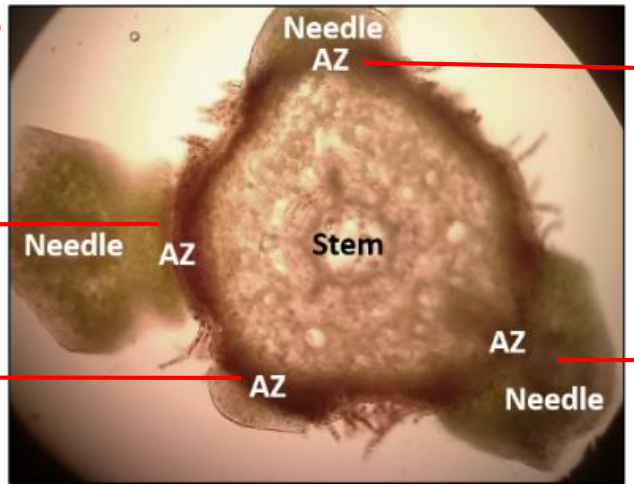


Plate 1 – A1



AZ-4

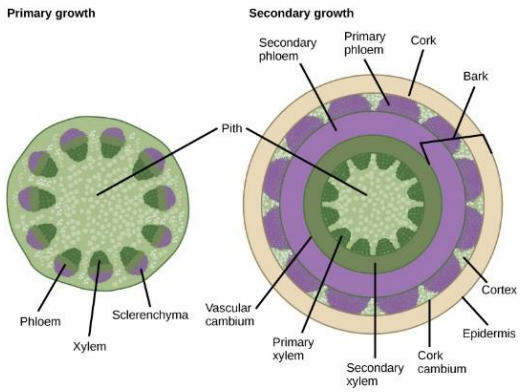
AZ-3

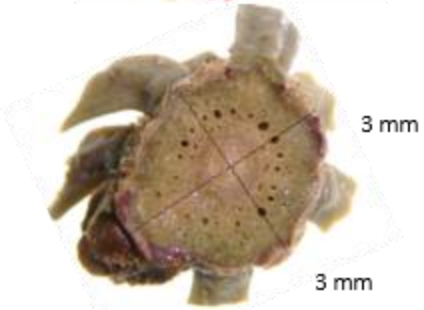
AZ-2

AZ-1

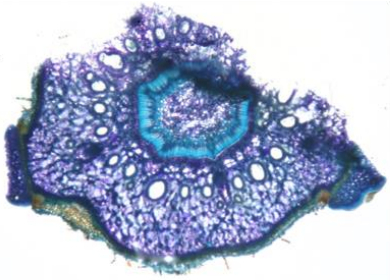
Outcome: 3-D bright field microscopy analysis

Clone 26 – Good
3rd FC
17 DAC – (5% NL)
TOB (Toluidine Blue)

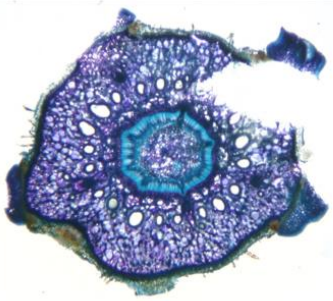




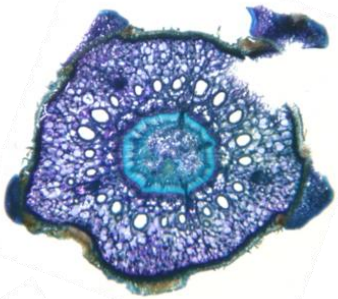
P6A1



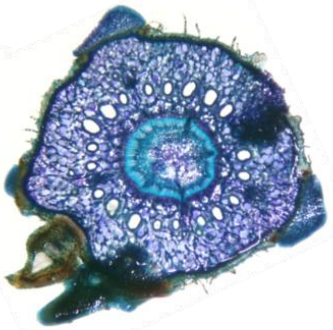
P6B1



P6C1



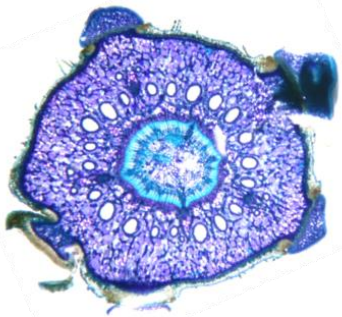
P6D1



P6E1

P6F1

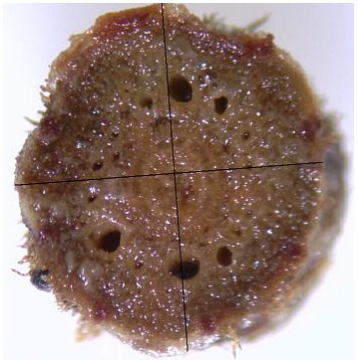
P6G1



Outcome: 3-D bright field microscopy analysis

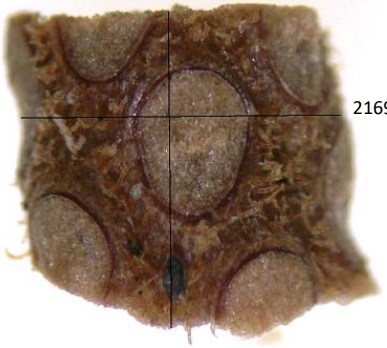
Clone 72 – Poor
3rd FC (November)
17 DAC – (50-80% NL)
TOB (Toluidine Blue)

2505 mm



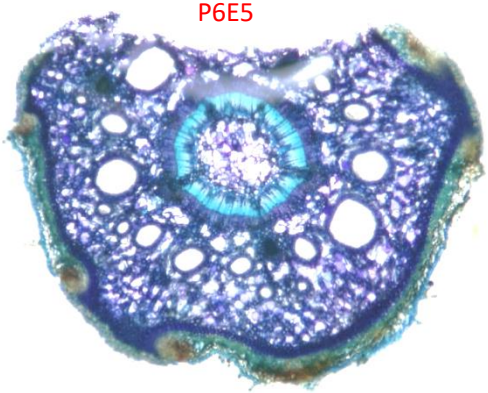
2198 mm

2169 mm

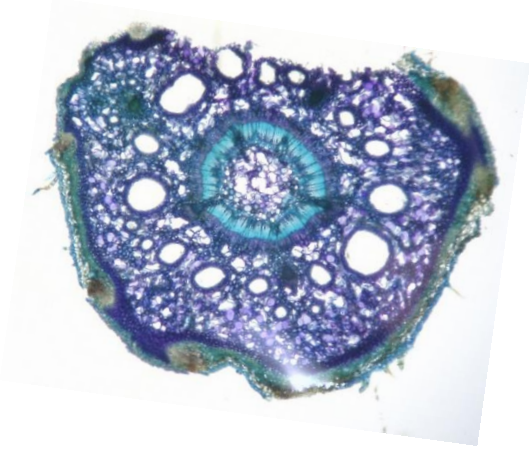


2140 mm

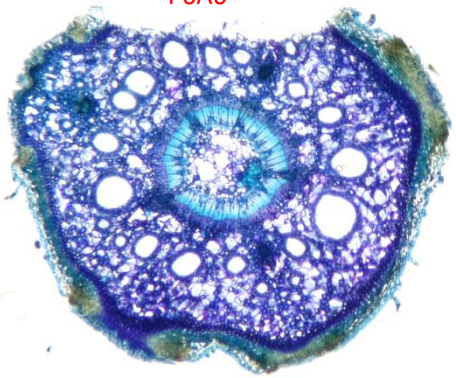
P6E5



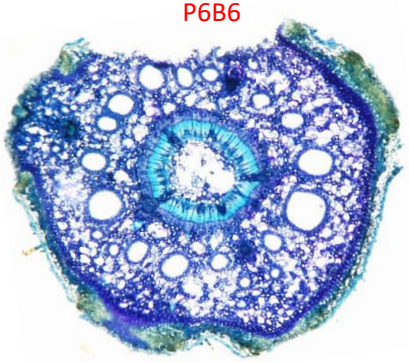
P6F5



P6A6

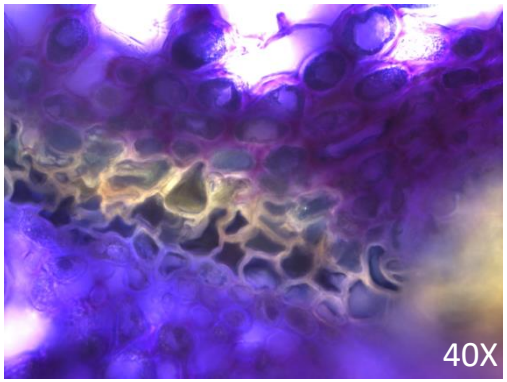


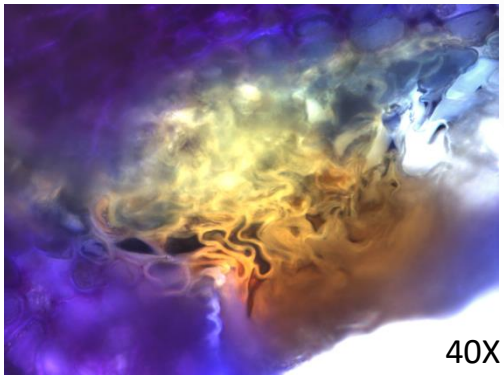
P6B6

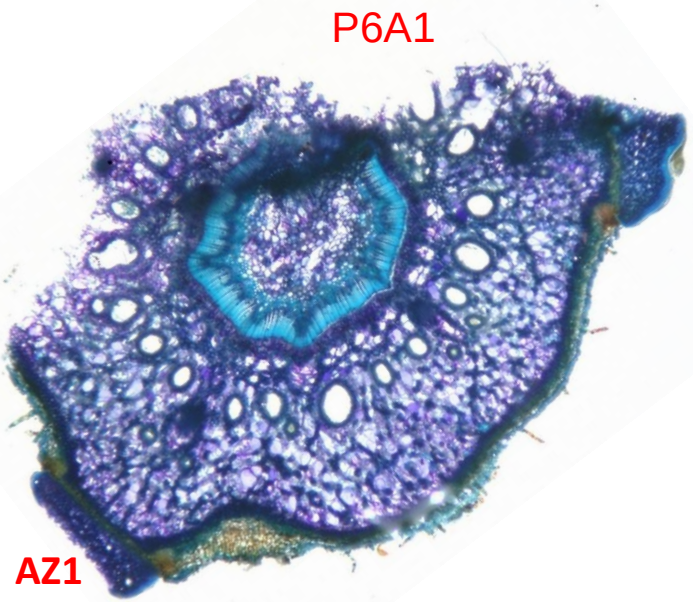


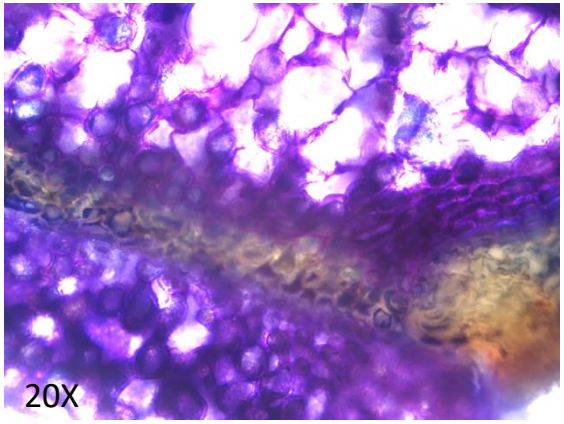
Outcome: Characterization of needle abscission in Fraser fir

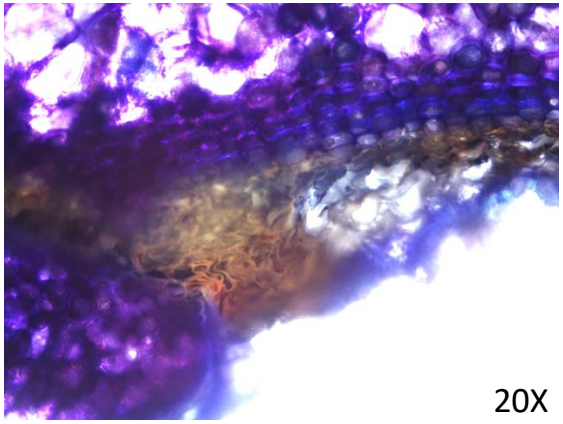
Clone 26 – Good
3rd FC (November)
17 DAC – (5% NL)
TOB (Toluidine Blue)





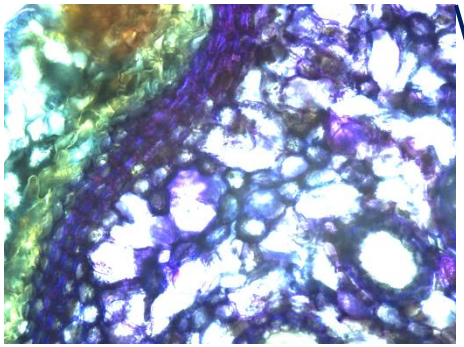


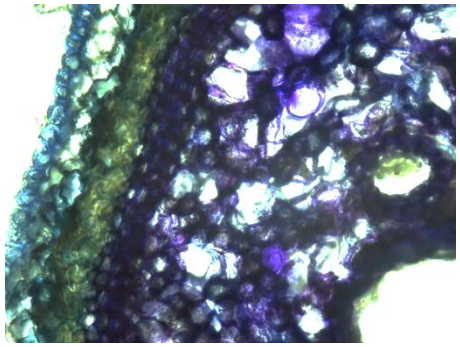


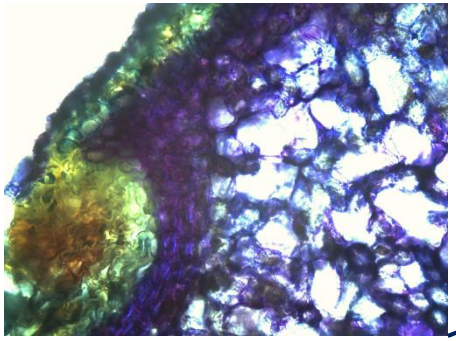


Outcome: Histological characterization of needle abscission in Fraser fir

Clone 72 – Poor
3rd FC (November)
17 DAC – (~80% NL)
TOB – 20X

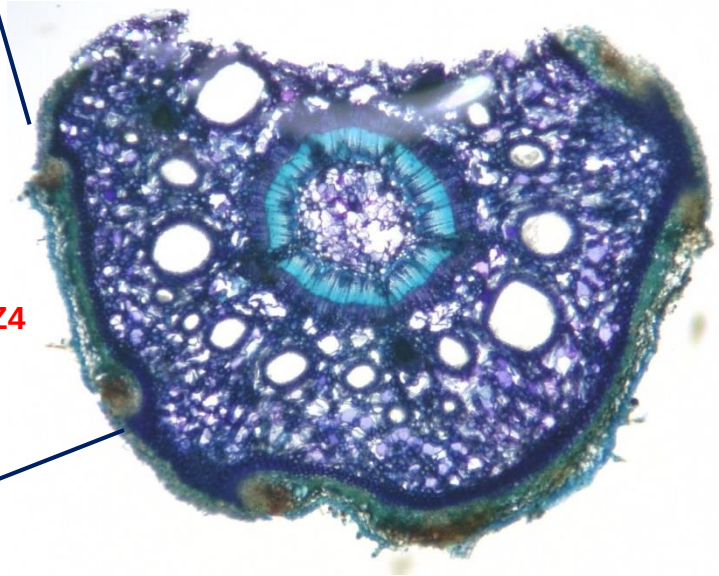






P6E5

AZ4



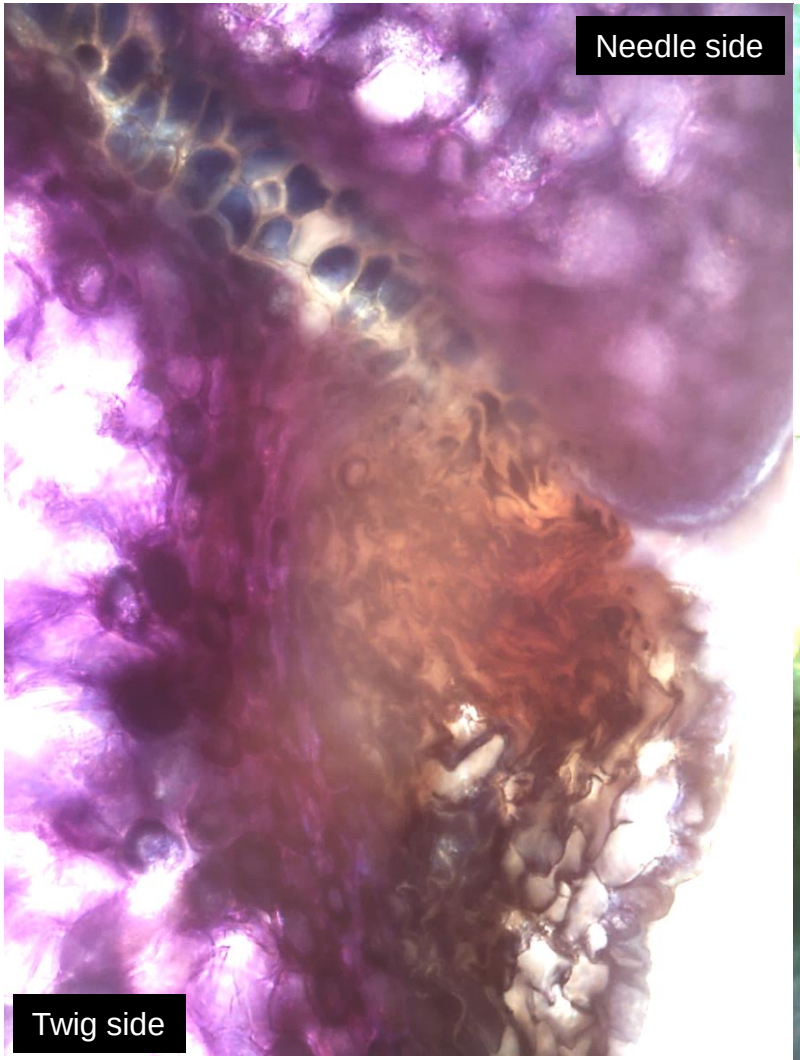
Histological differences between phenotypes

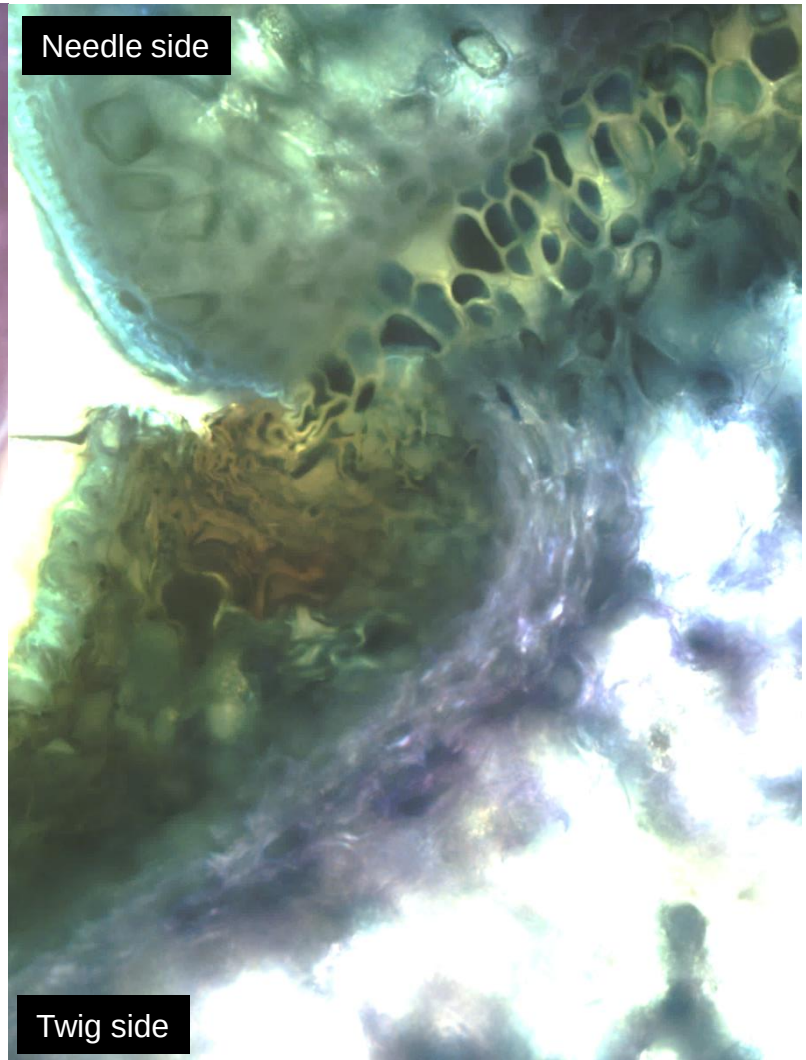
Outcome: Histological differences between phenotypes

03.07.2017
Clone 26 – Good NRP
0 % NL
2nd FC (October)
0 DAC
TOB

0% Needle retention October

03.07.2017
Clone 72 – Poor NRP
0 % NL
2nd FC (October)
0 DAC
TOB



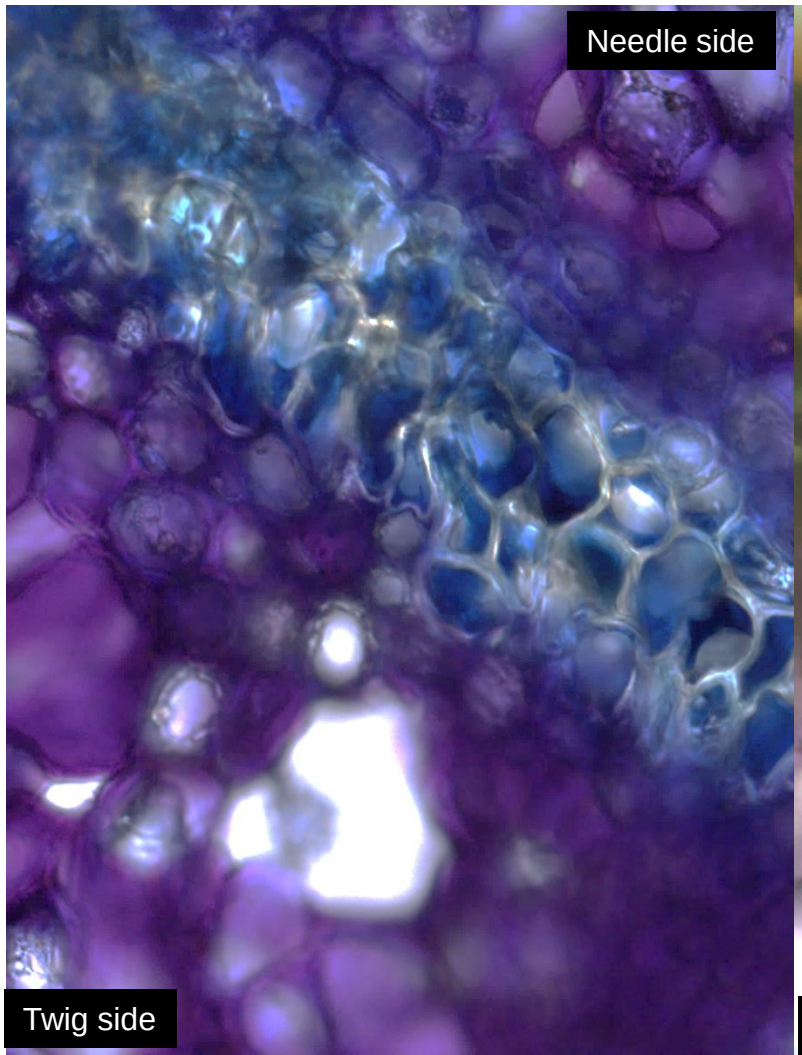


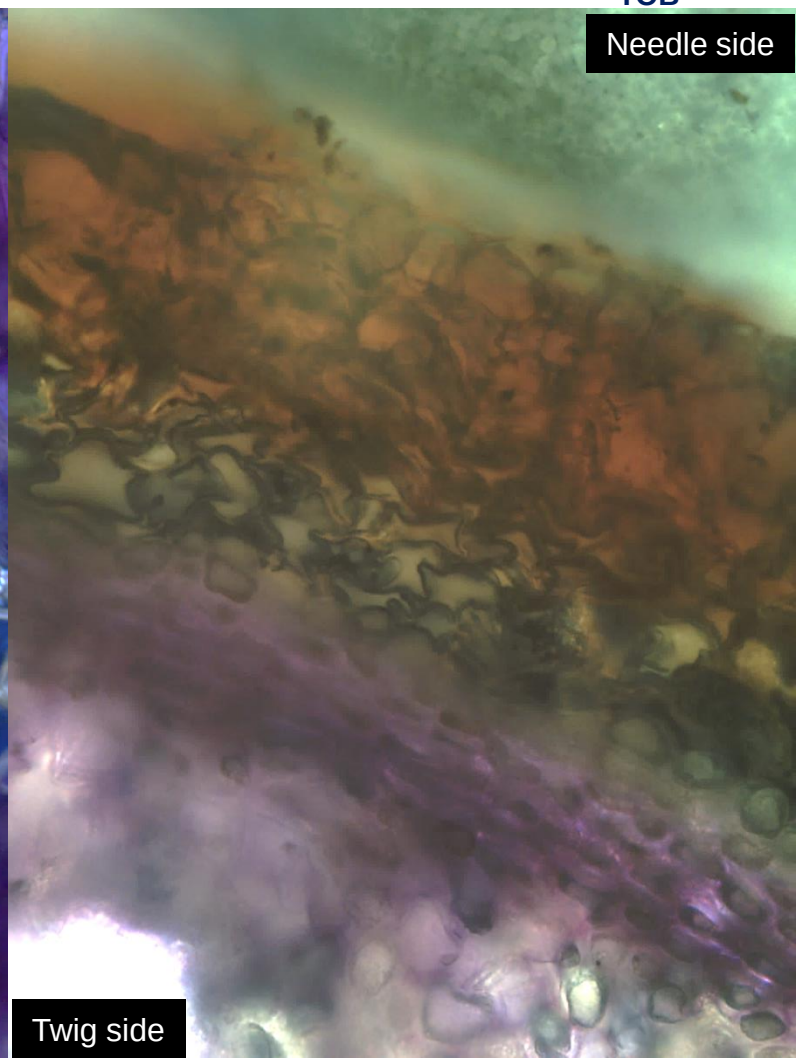
Outcome: Histological differences between phenotypes

04.06.2017
Clone 26 – Good NRP
15 % NR
2nd FC (October)
35 DAC
TOB

15% - 75% Needle retention October

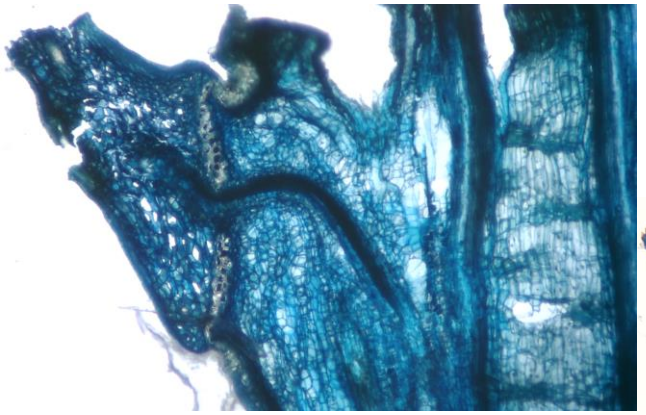
04.06.2017
Clone 72 – Bad NRP
75 % NR
2nd FC (October)
35 DAC
TOB

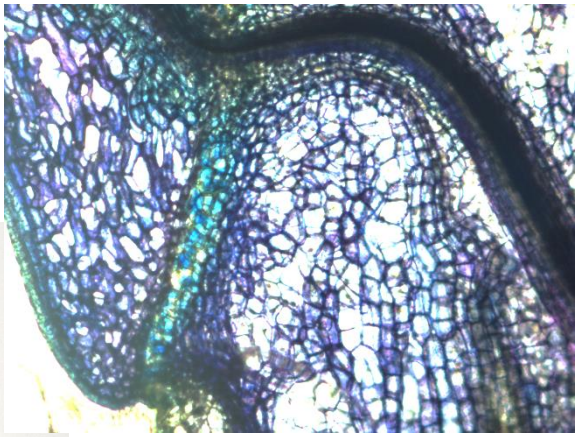


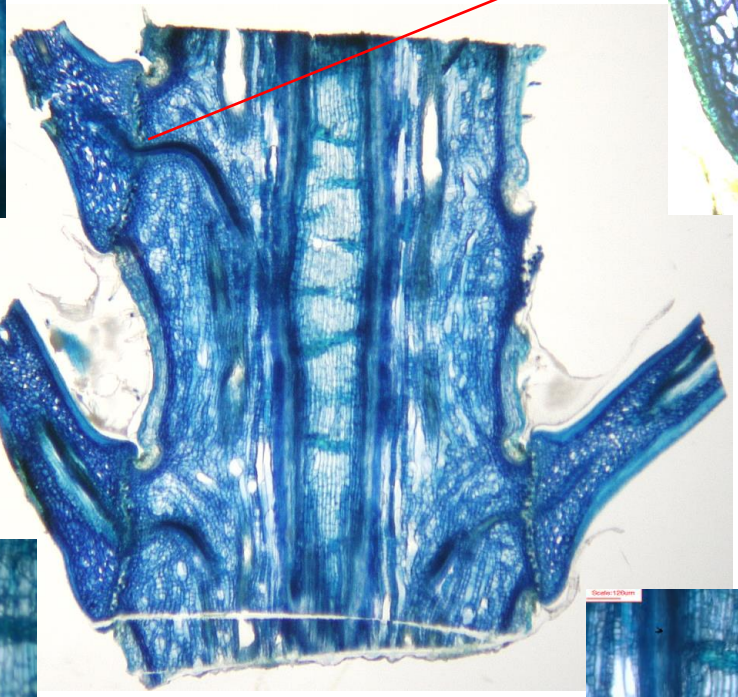


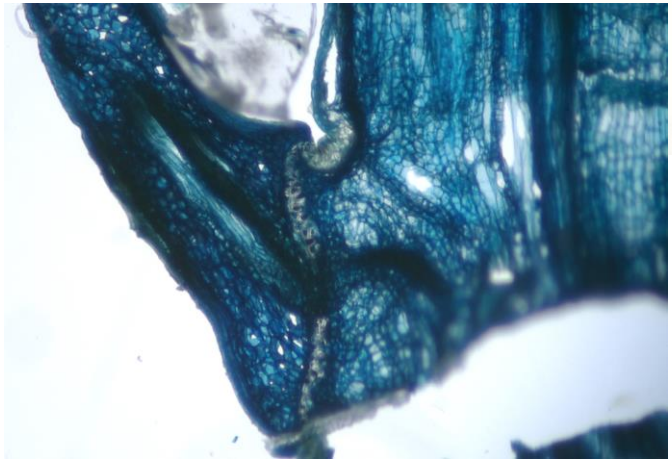
AZ in Fraser fir was already formed in Summer

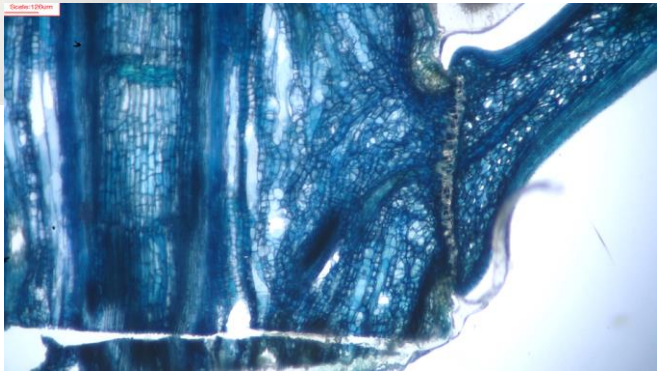
Outcome: Transverse section – Summer Collection



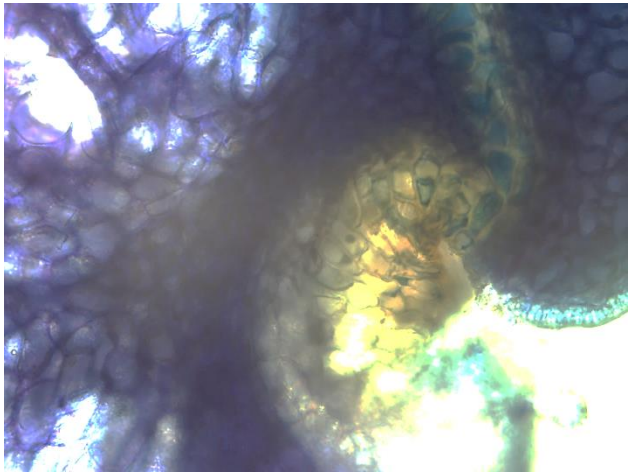
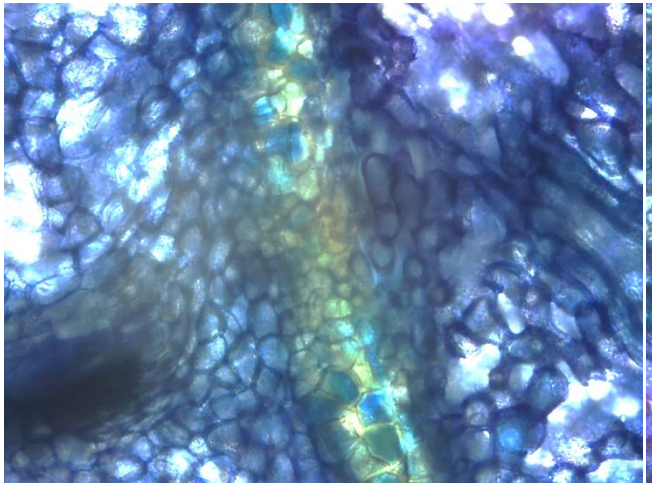
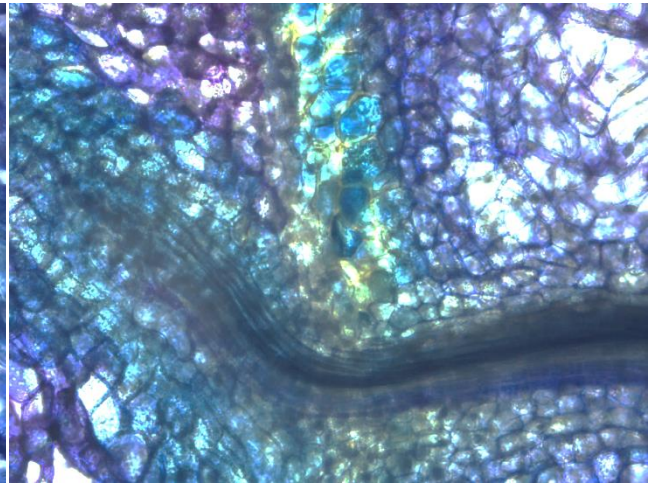
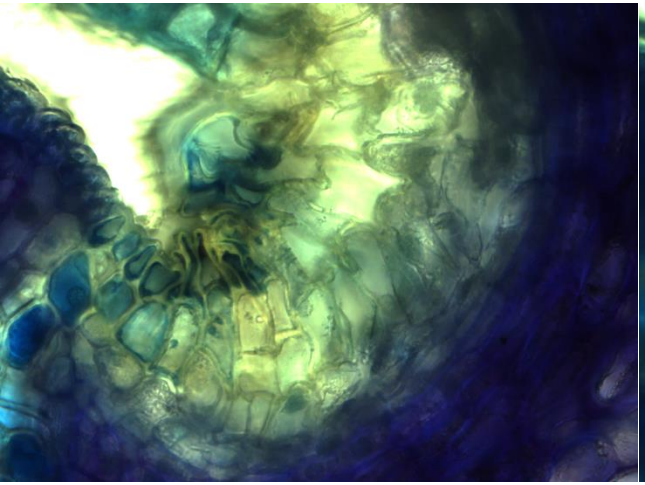
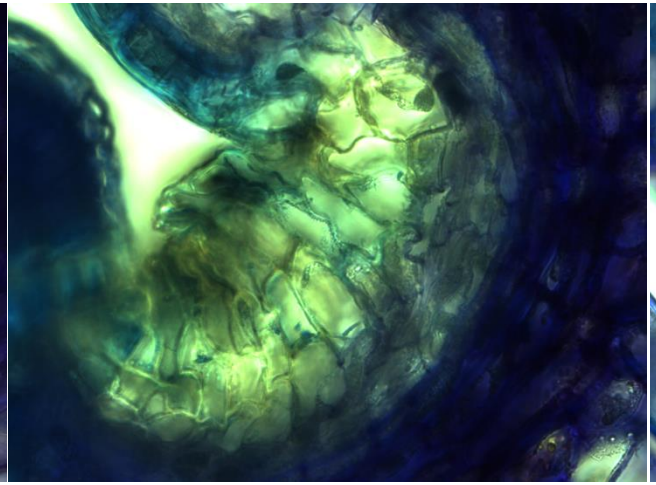
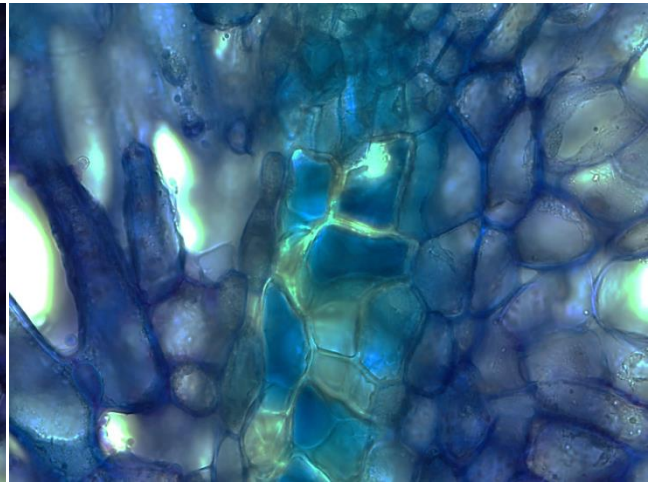








Outcome: Transverse section – Summer Collection

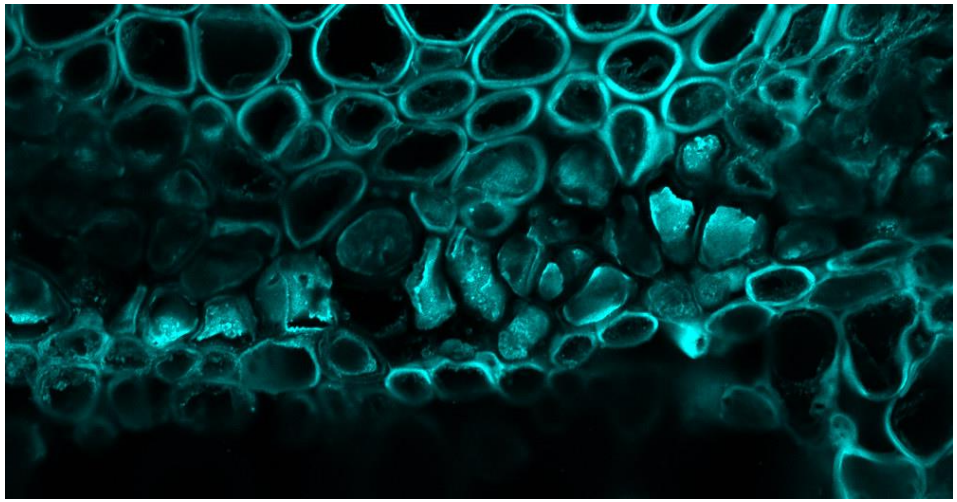







Confocal microscopy protocol to study Fraser fir AZ

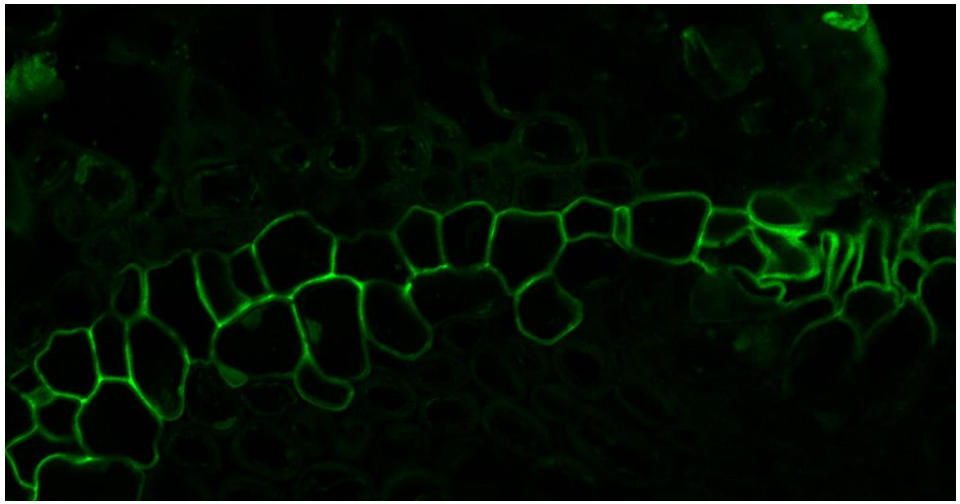
Outcome: Confocal microscopy

07-10-2017
Clone 72 – Poor clone
1st – Summer collection

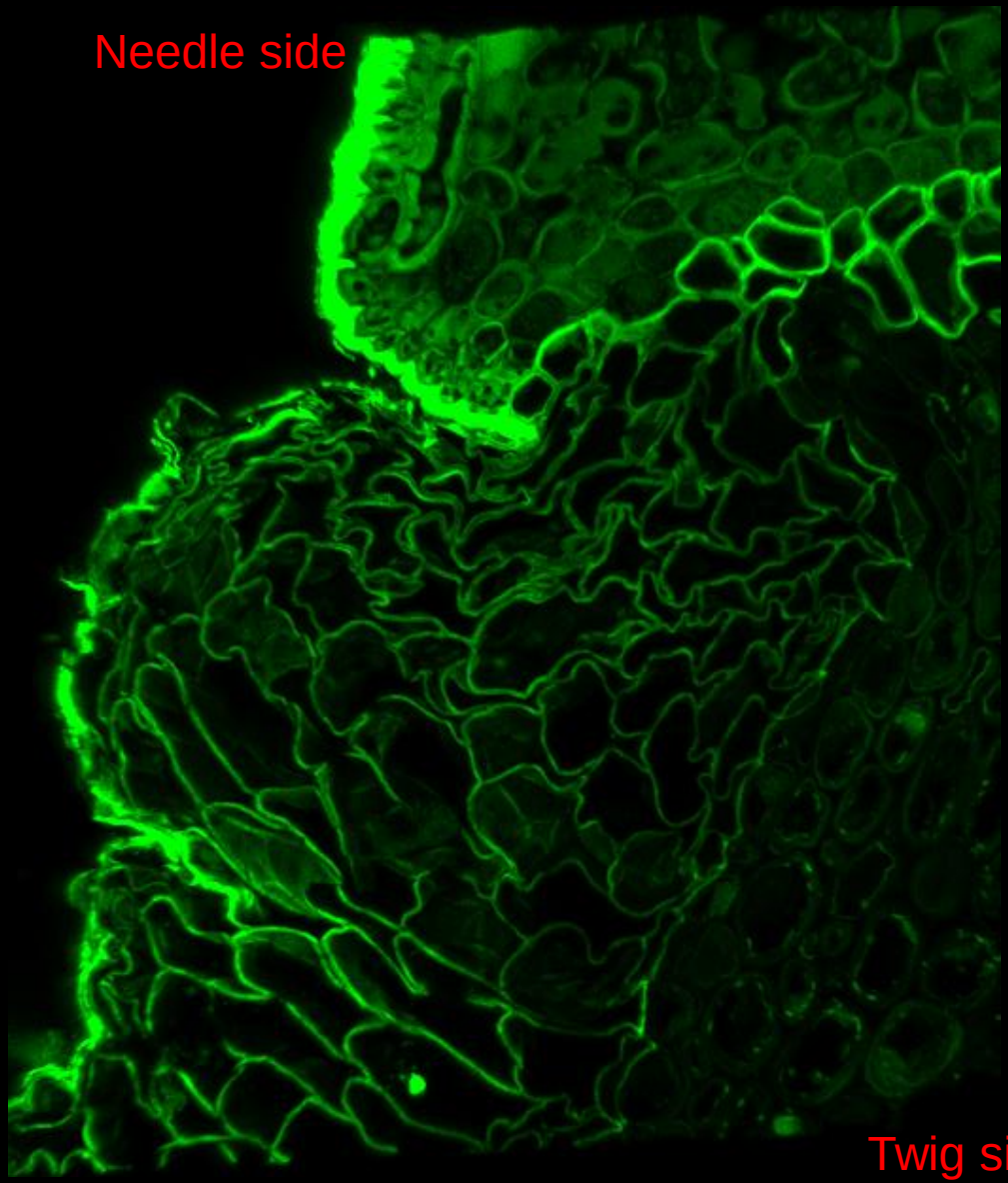
07-10-2017_AZ1(72) - Calcofluor – “tooth cells”



07-10-2017_AZ1(72) - Safranine- “hyaline cells”

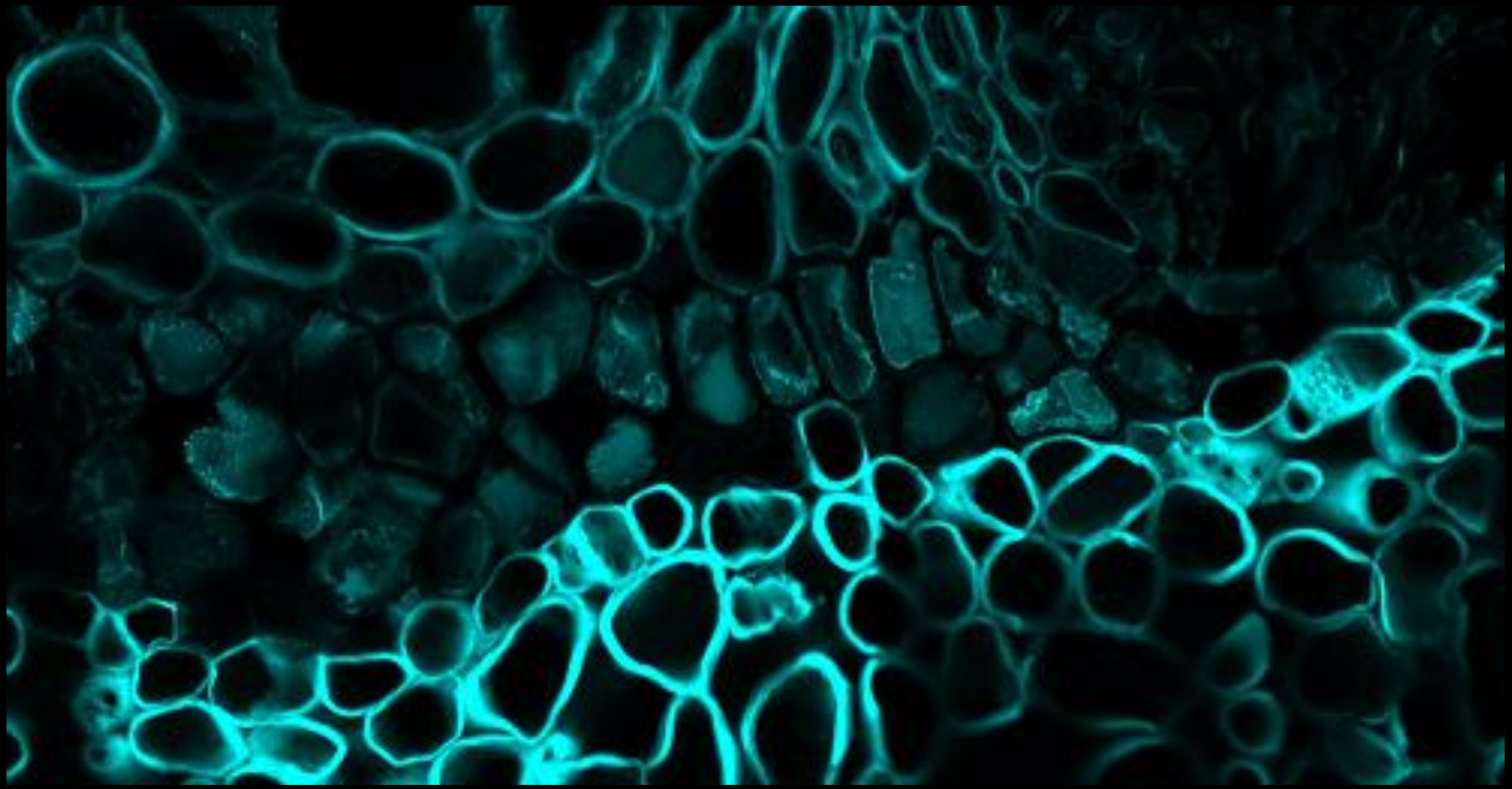


Outcome: Confocal microscopy



Outcome: Confocal microscopy

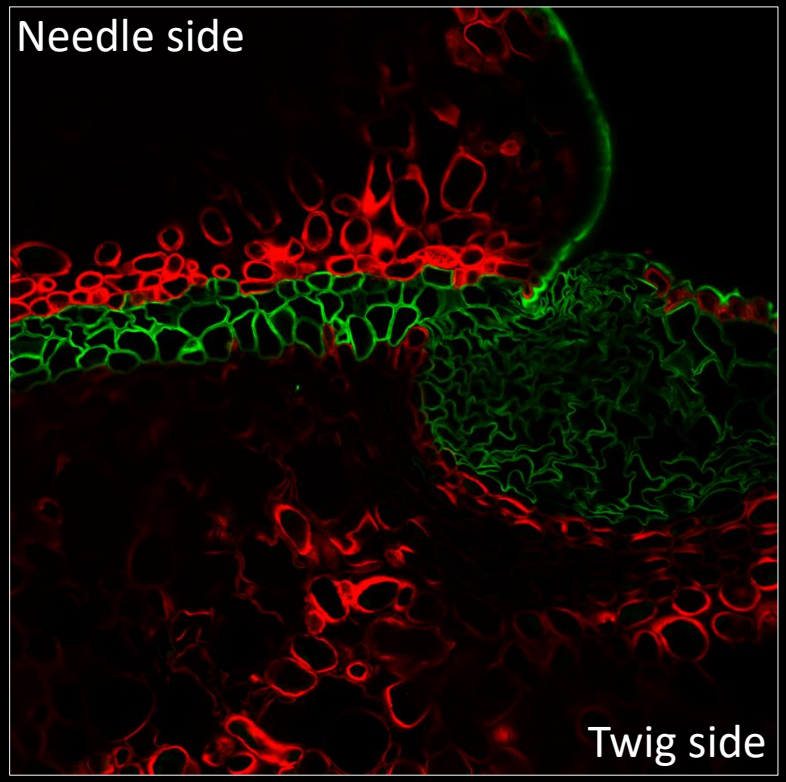
Needle side



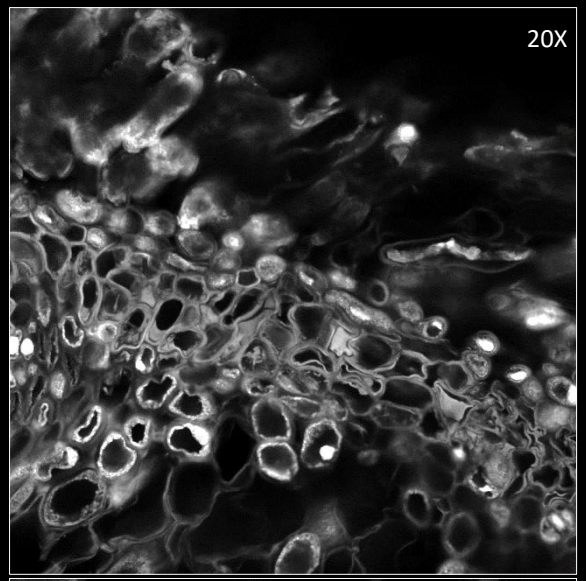
Twig side

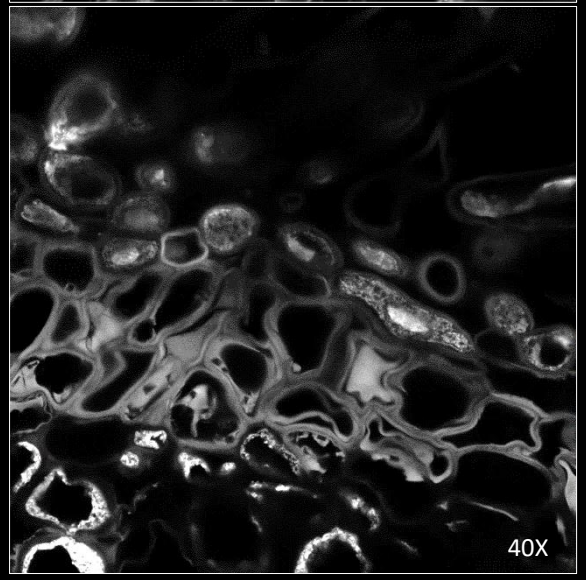
Outcome: Confocal microscopy

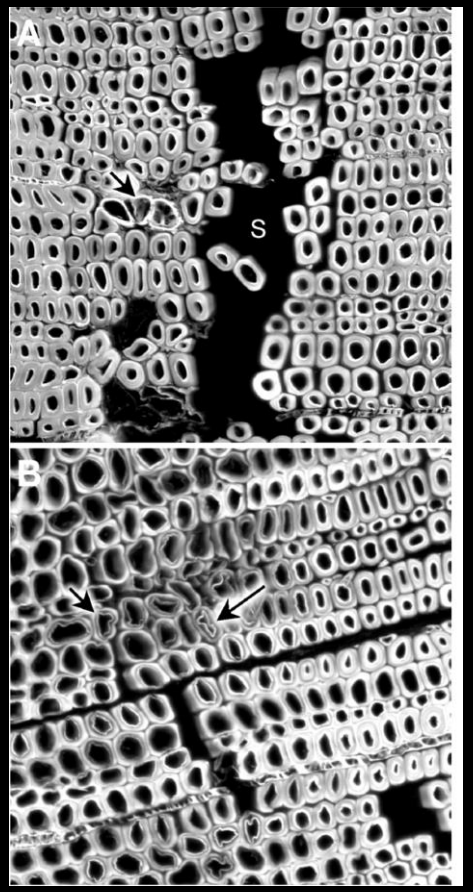
Safranin
Calcofluor



Possible evidence of drought stress





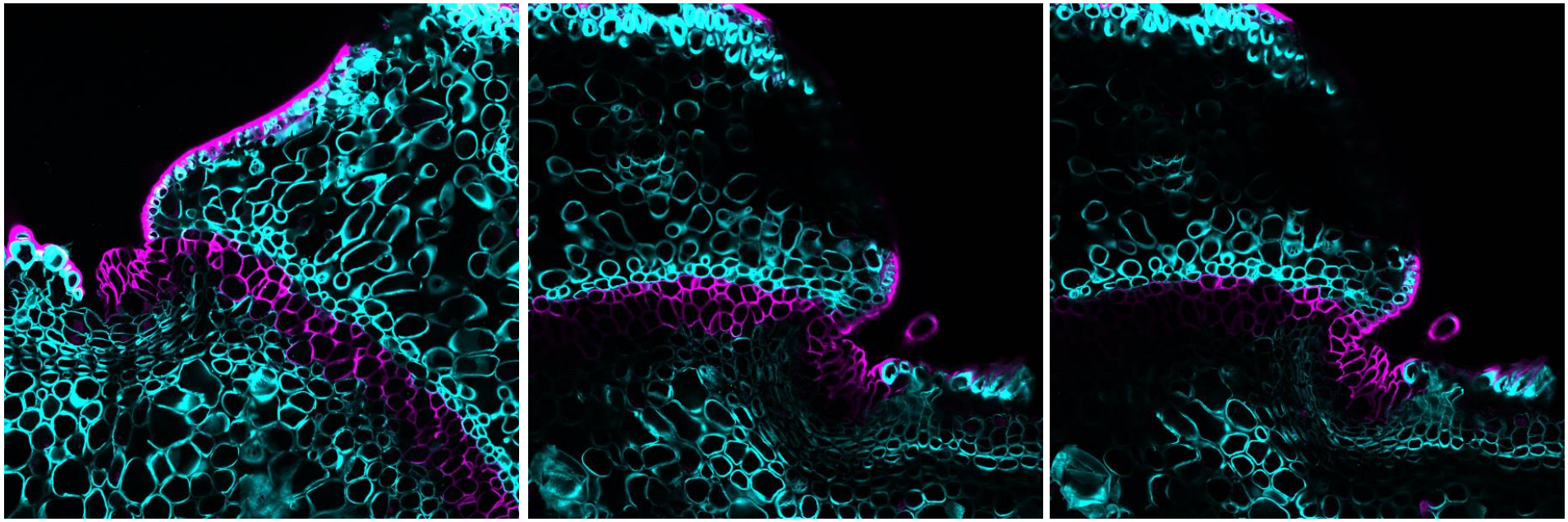


Abnormal lignin distribution in wood from severely drought stressed Pinus radiata trees. (PDF Download Available).

Outcome: Confocal microscopy

Safranin
Calcofluor

1st Summer collection



Conclusions/Discussion

- We have standardized protocols for bright field and confocal microscopy for the identification of the AZ in Fraser, Balsam and Canaan firs.
- Our tissue collection will facilitate the molecular characterization of the AZ in three different firs.
- We will complete the characterization of needle abscission in Fraser fir using more samples from different time points.
- Examine differences between poor and good needle retention phenotypes.

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Dr. Eva Johannes
Cellular and molecular imaging - NCSU

Dr. Gary Chastagner
Plant pathology and Extension
WSU

Katie Coats
Scientific assistant
WSU

NC STATE UNIVERSITY College of Natural Resources

Molecular Tree Breeding Lab

USDA


Thank you for your attention!

Questions?