Deer Browse of Coastal WRC

Nimpkish Study Data overview

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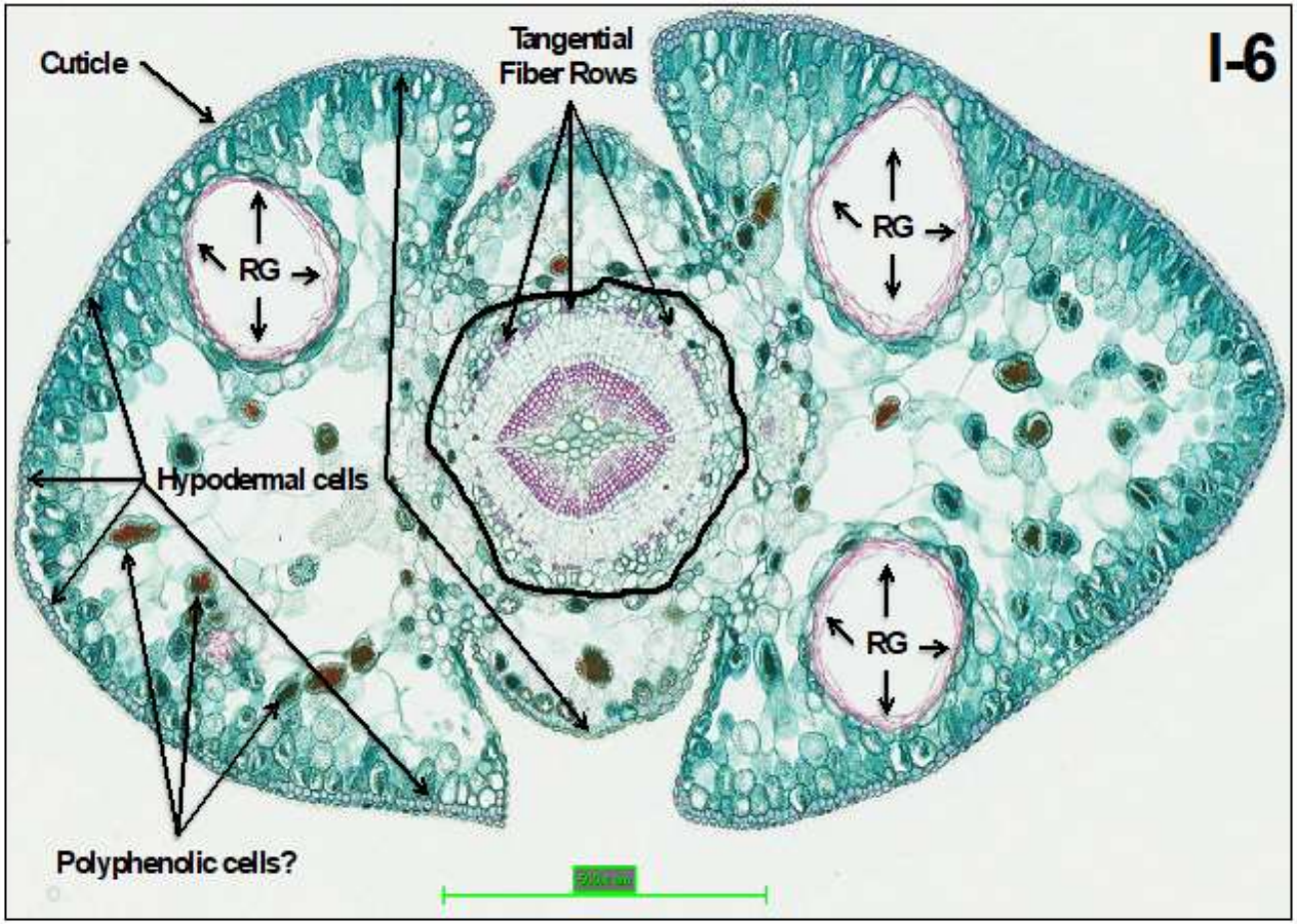
November 1st, 2021

Dr. John Russell

- Dr. Russell approached me in Nov. 2017
- Had a feeling that WRC preferred by deer 'felt different'
- Foliar waxes?
- Other traits?
  - Terpenes
  - Anatomy

Preliminary Study



- The goal for WRC histology is to evaluate phenotypic differences between families. Specifically, we want to quantify:
  - Resin gland area
  - Tangential fiber rows
  - Amount of lignified tissues

Figure 4. Anatomy of young foliage at internode 6. Foliar structures are still present and resin glands are intact. As foliage ages, the leaves shrink and stem expands.

WRC and Deer Browse

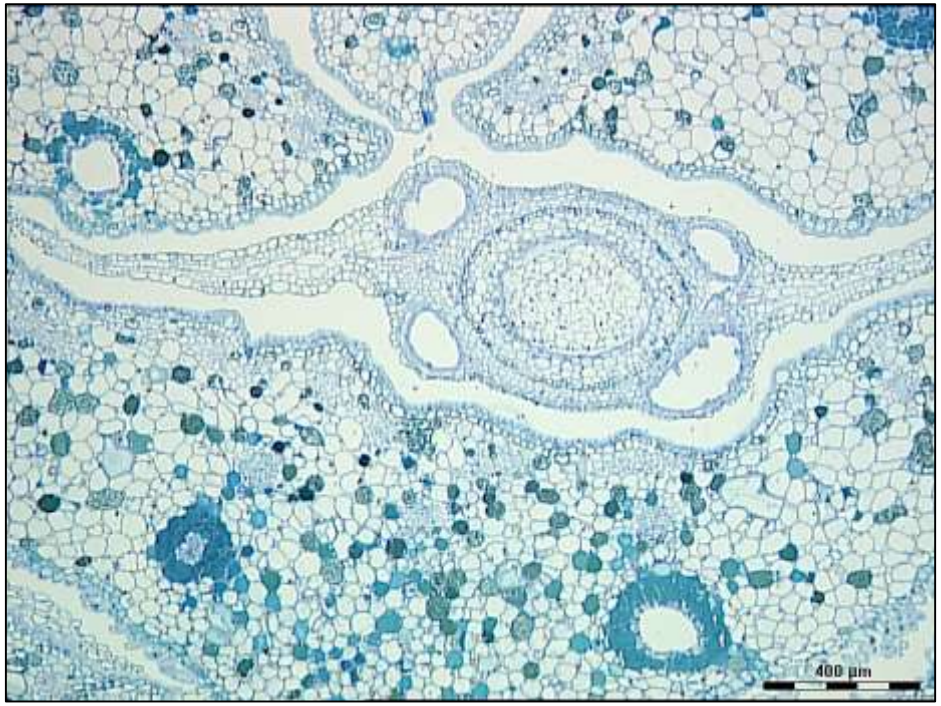
- Deer browse causes significant damage to seedlings
  - ~$20 - $25 mil a year to protect (2008)
- Genetic variation in WRC genotypes based on foliar chemistry
- Terpene chemistry associated with deer browse resistance
- Terpenes stored in resin glands/vesicles

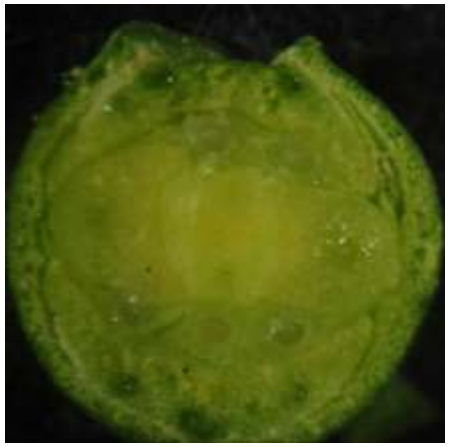
WRC feeding choice trial

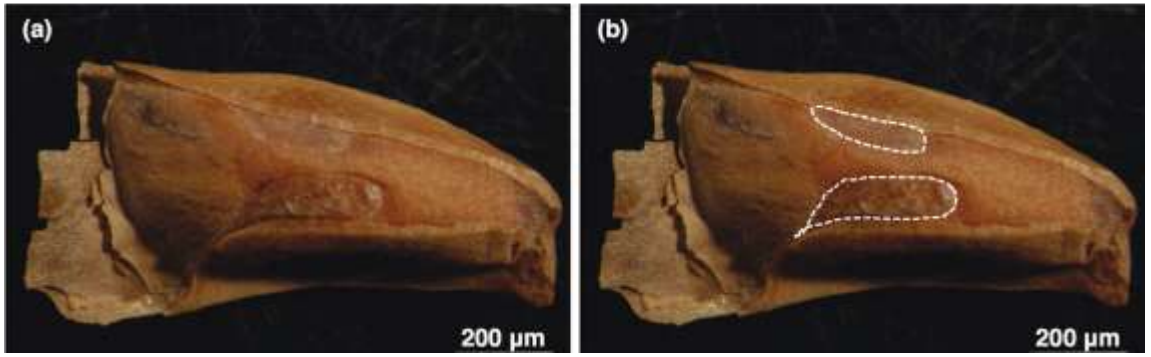
Russell. 2008. *USDA-FS Proceedings*.

WRC Terpenes

- **WRC terpenes are located in resin glands/vesicles**
  - **Seeds (vesicles)**



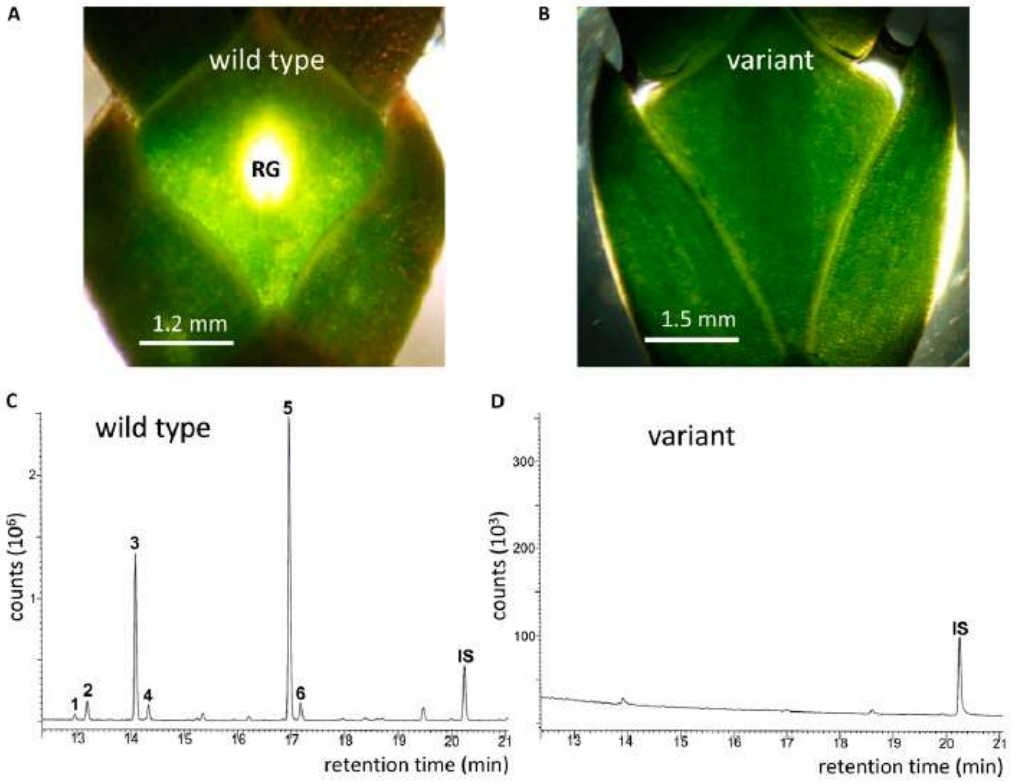




Kshatriya et al. 2018. *CJFR*.

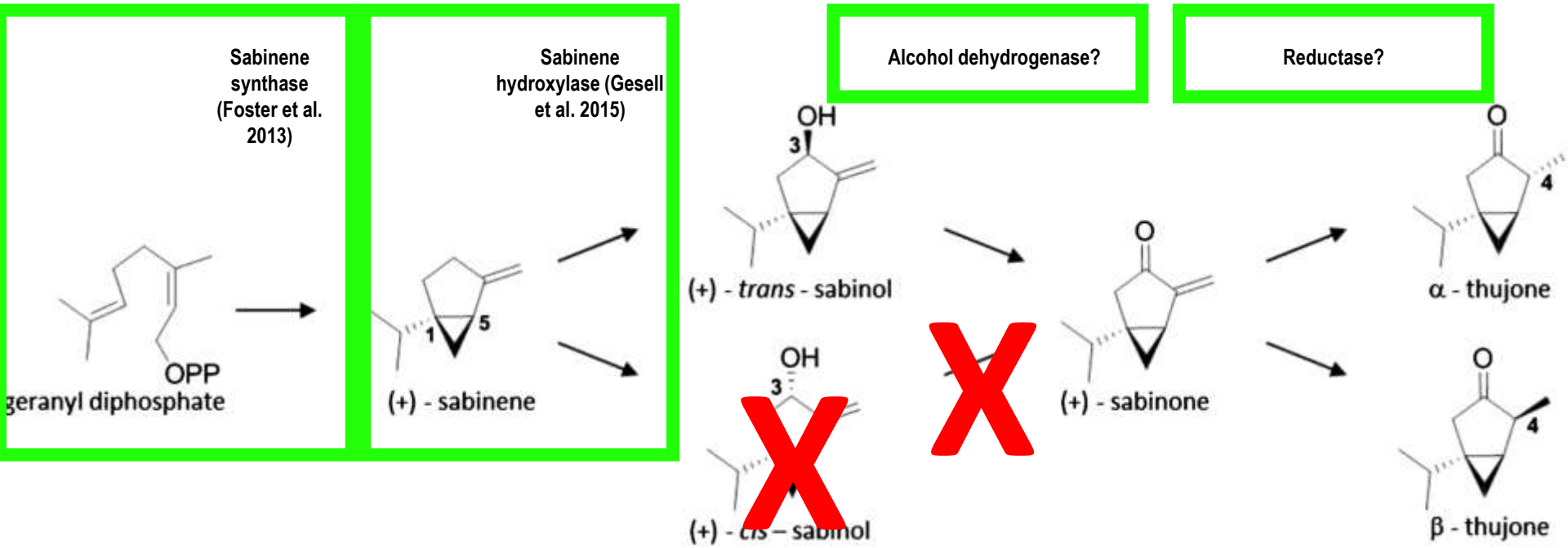
WRC Terpenes

- WRC terpenes are located in resin glands/vesicles
  - Seeds (vesicles)
  - Foliage (glands)
- (+)-Sabinene, α- & β-thujone dominate the terpenoid profile
  - ~80% of monoterpenes
- Sabinene associated with fungal resistance
- Thujones are cytotoxic at high levels with potential neurological impacts
  - Absinthe



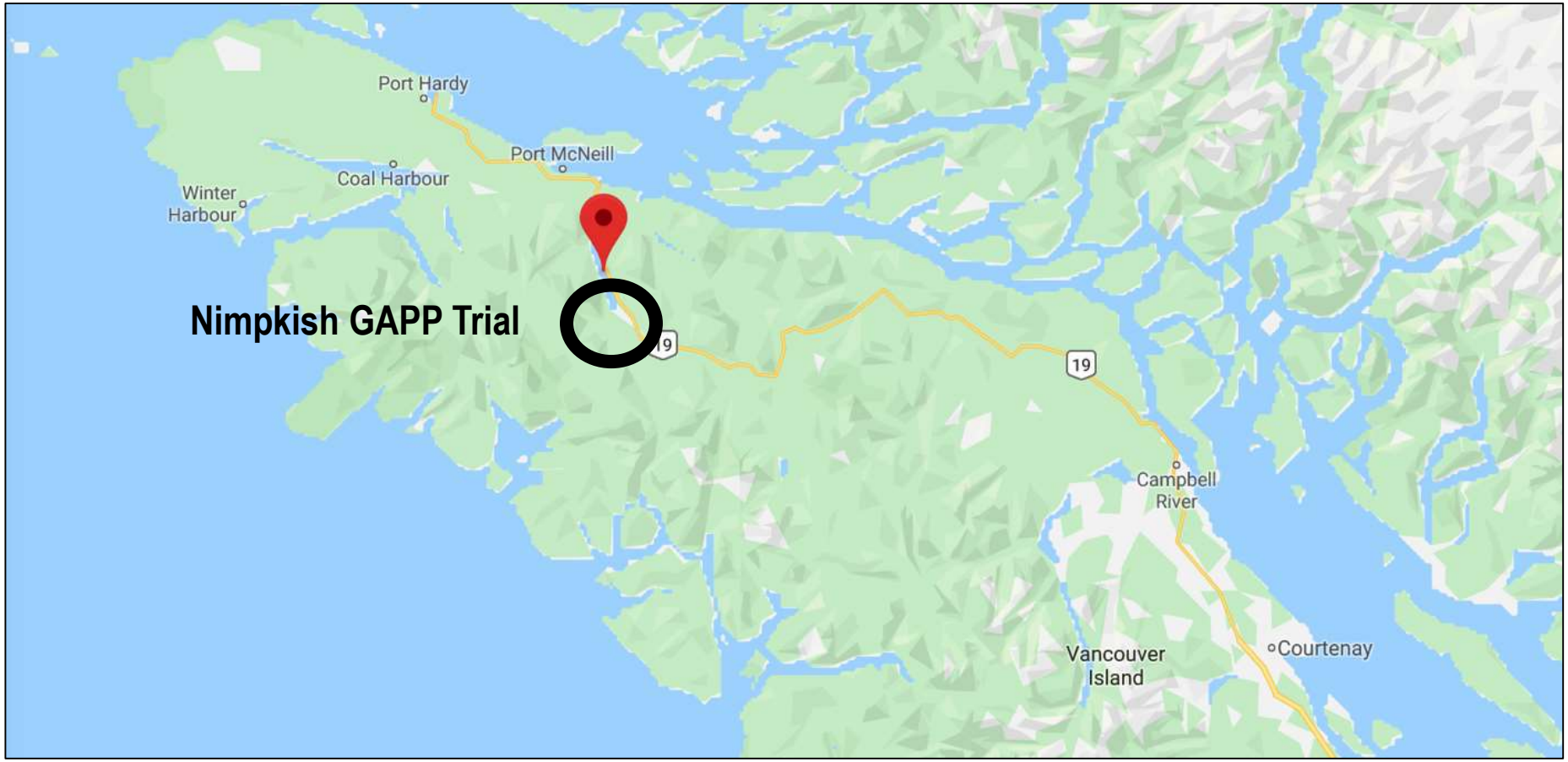
Foster et al. 2013. *Plant Phys.*

WRC Terpenes



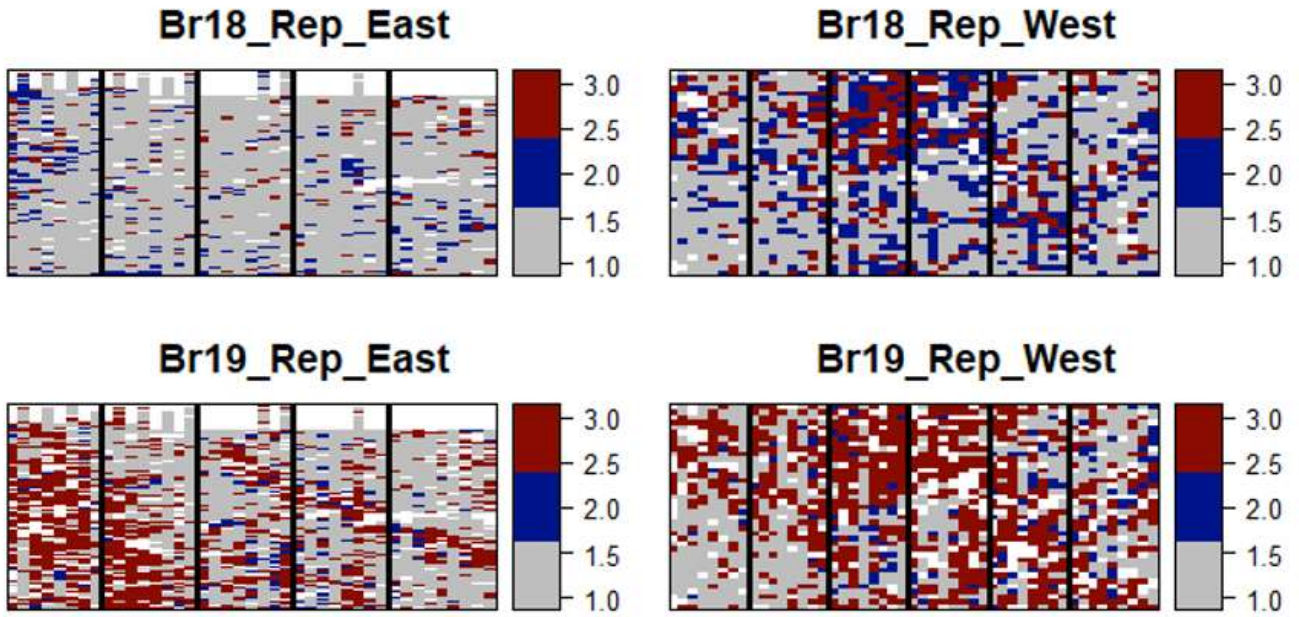
Gessell et al. 2015. *Plant Phys.*

GAPP Plantings



Deer Browse at Nimpkish

- Grey is little to no browse
- Blue is moderately browsed
- Red is heavily browsed
- White is dead/missing
- Browse got worse from 2018 to 2019



Family BV Rank	Family #	BV Mean	Designation
1	493	-0.33	Best
2	525	-0.29	Best
4	412	-0.25	Best
7	522	-0.17	Best
8	335	-0.14	Best
52	519	0.14	Worst
53	554	0.22	Worst
55	436	0.23	Worst
56	502	0.27	Worst
57	526	0.34	Worst

- Breeding values were calculated from pedigree information and deer browse scores
- Families were ranked; 5 best and 5 worst were selected

Preliminary Site Observations

- Nimpkish had heavy and even browse
- Trees rather small

Nimpkish West Block

Preliminary Site Observations

- Nimpkish had heavy and even browse
- Trees rather small
- Sampled in early-March 2020 to coincide with deer browse peak

Healthy unbrowsed WRC

Preliminary Site Observations

- Nimpkish had heavy and even browse
- Trees rather small
- Sampled in early-March 2020 to coincide with deer browse peak
- Deer tend to feed only on young shoots

Browsed WRC

Preliminary Site Observations

- Nimpkish had heavy and even browse
- Trees rather small
- Sampled in early-March 2020 to coincide with deer browse peak
- Deer tend to feed only on young shoots
- Some trees are heavily browsed (lollipop trees)

Lollipop WRC

Driving Questions and Assumptions

Driving Question:

What are the traits driving differences among families?

Key observations:

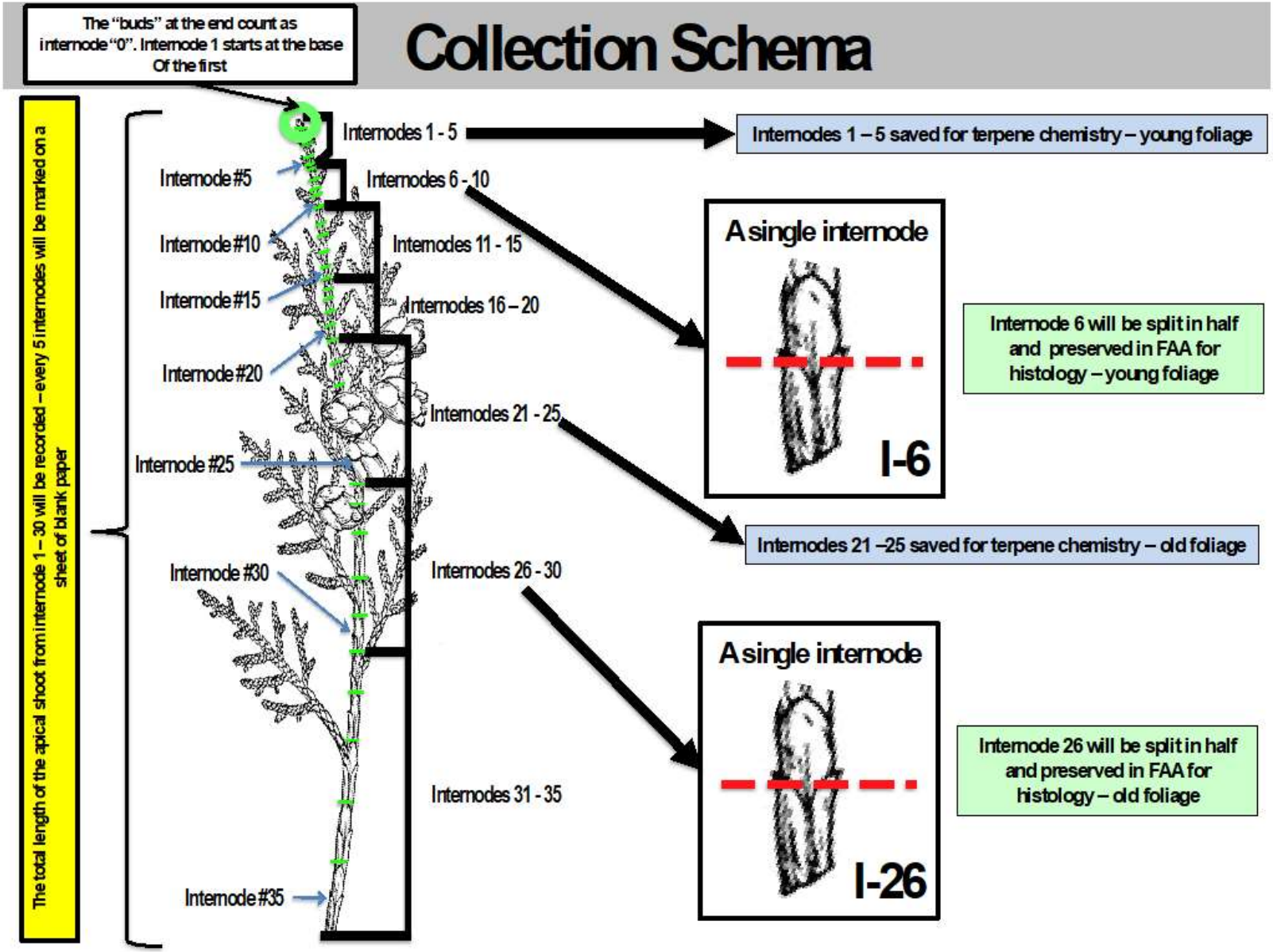
Deer tend to feed on young tissues

Traits evaluated:

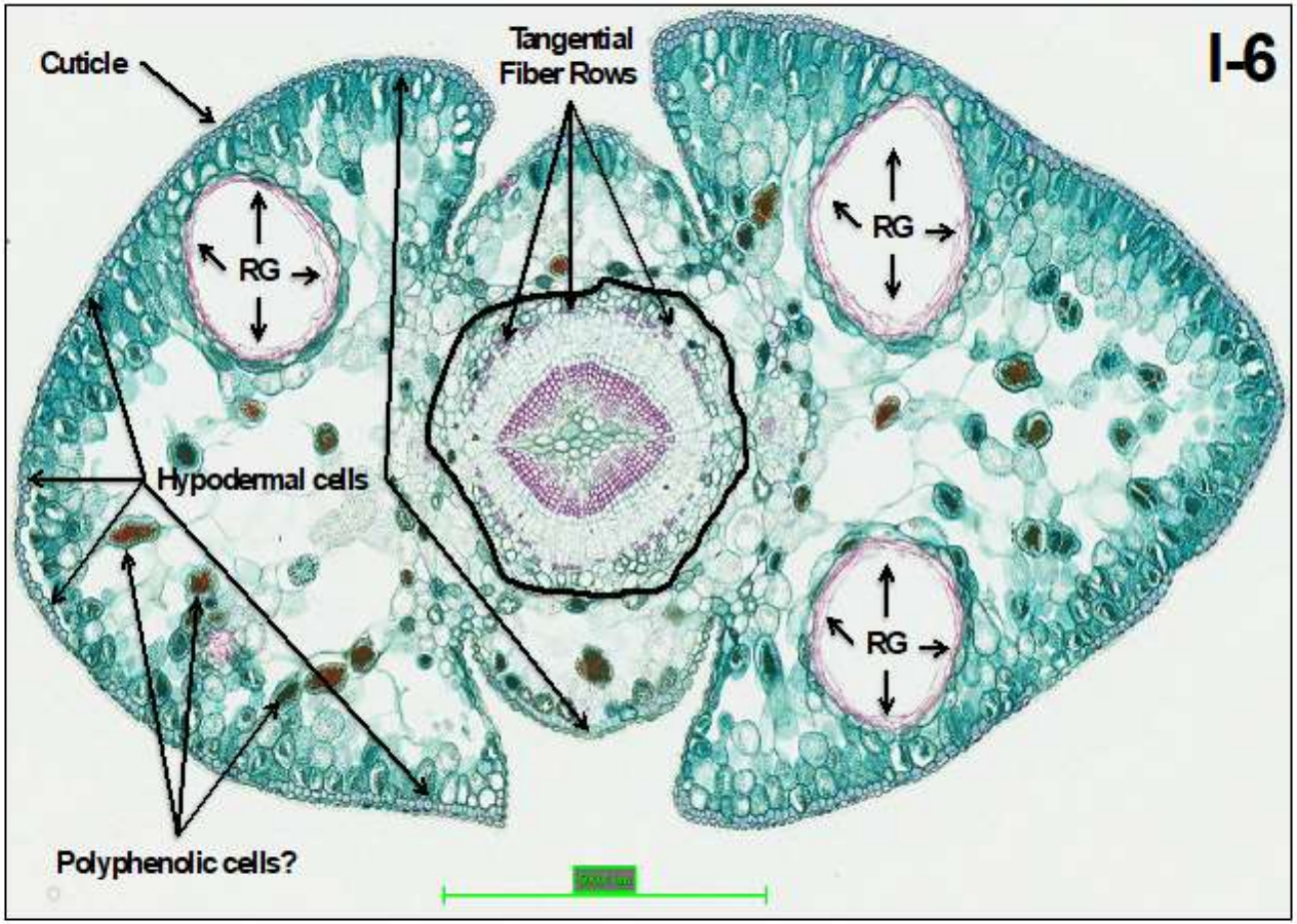
Anatomy (resin glands & tangential fiber rows);
Chemistry (mono-, sesqui-, & di-terpenes)

Deer Browse at Nimpkish

- I-6 = young tissue
- I-26 = old tissue
- I-6 & I-26 used for histology analyses
- I-1 to I-5 = young tissue chemistry
- I-21 to I-25 = old tissue chemistry



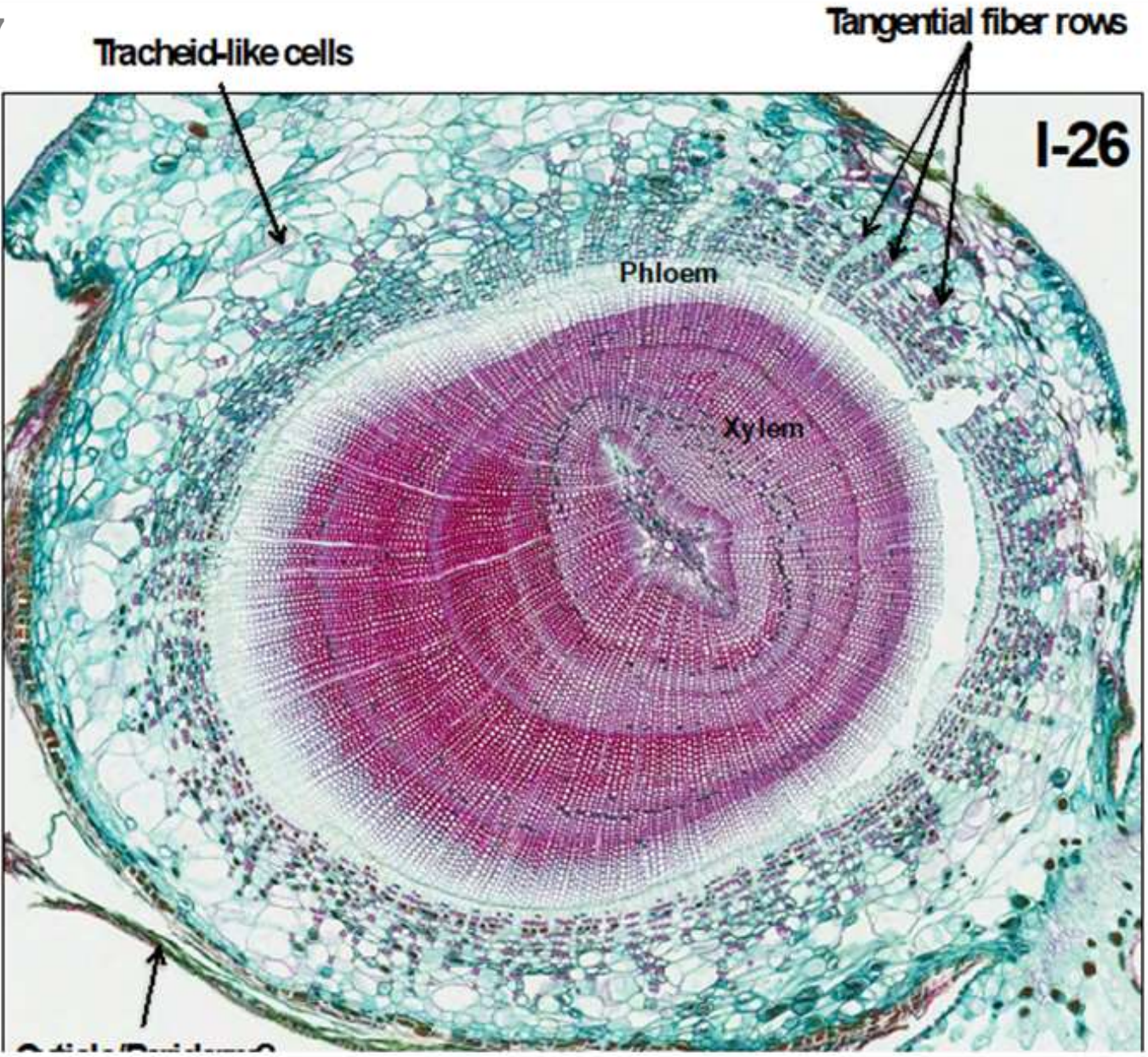
Anatomy of Internode 6



- The goal for WRC histology is to evaluate phenotypic differences between families. Specifically, we want to quantify:
  - Resin gland area
  - Tangential fiber rows
  - Amount of lignified tissues

Figure 4. Anatomy of young foliage at internode 6. Foliar structures are still present and resin glands are intact. As foliage ages, the leaves shrink and stem expands.

Anatomy of Internode 26



- The goal for WRC histology is to evaluate phenotypic differences between families. Specifically, we want to quantify:
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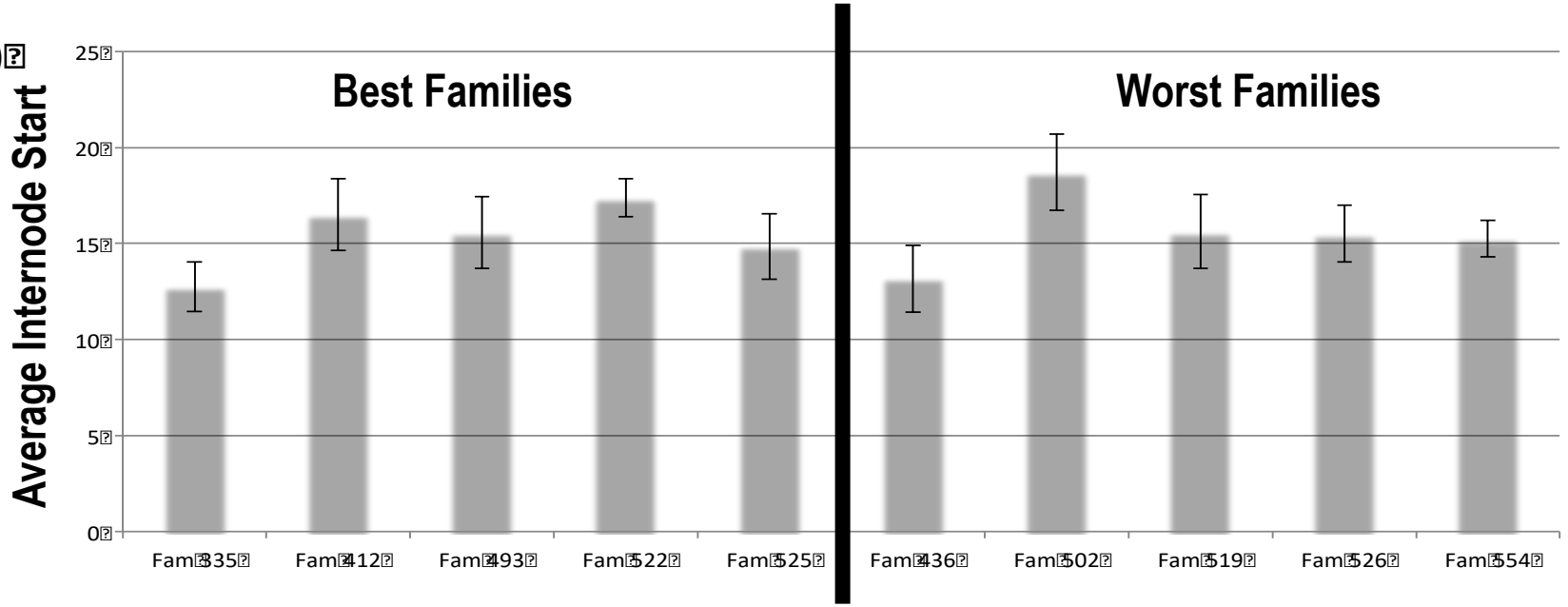
Initial Observations

Start of Lignification

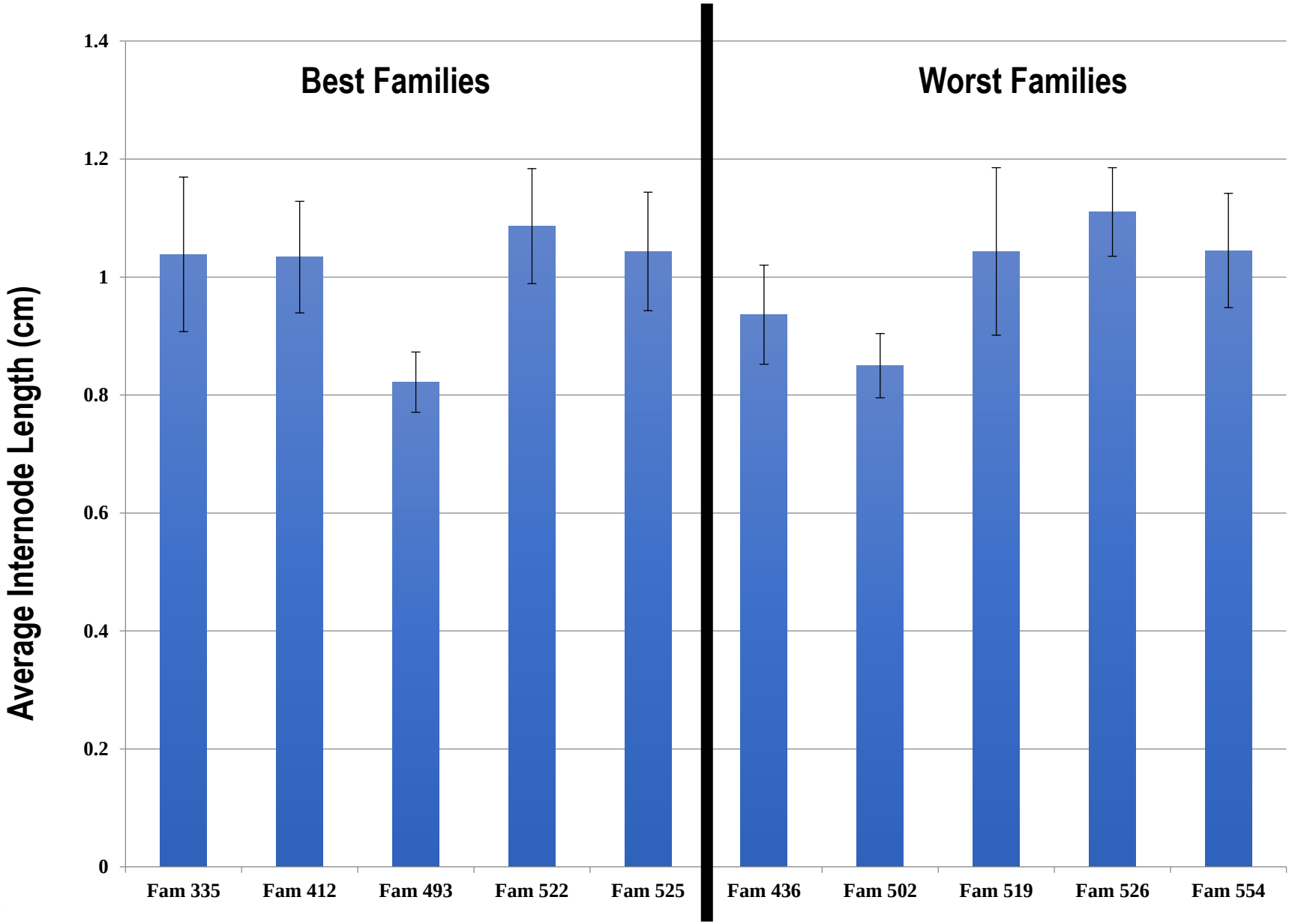
a)



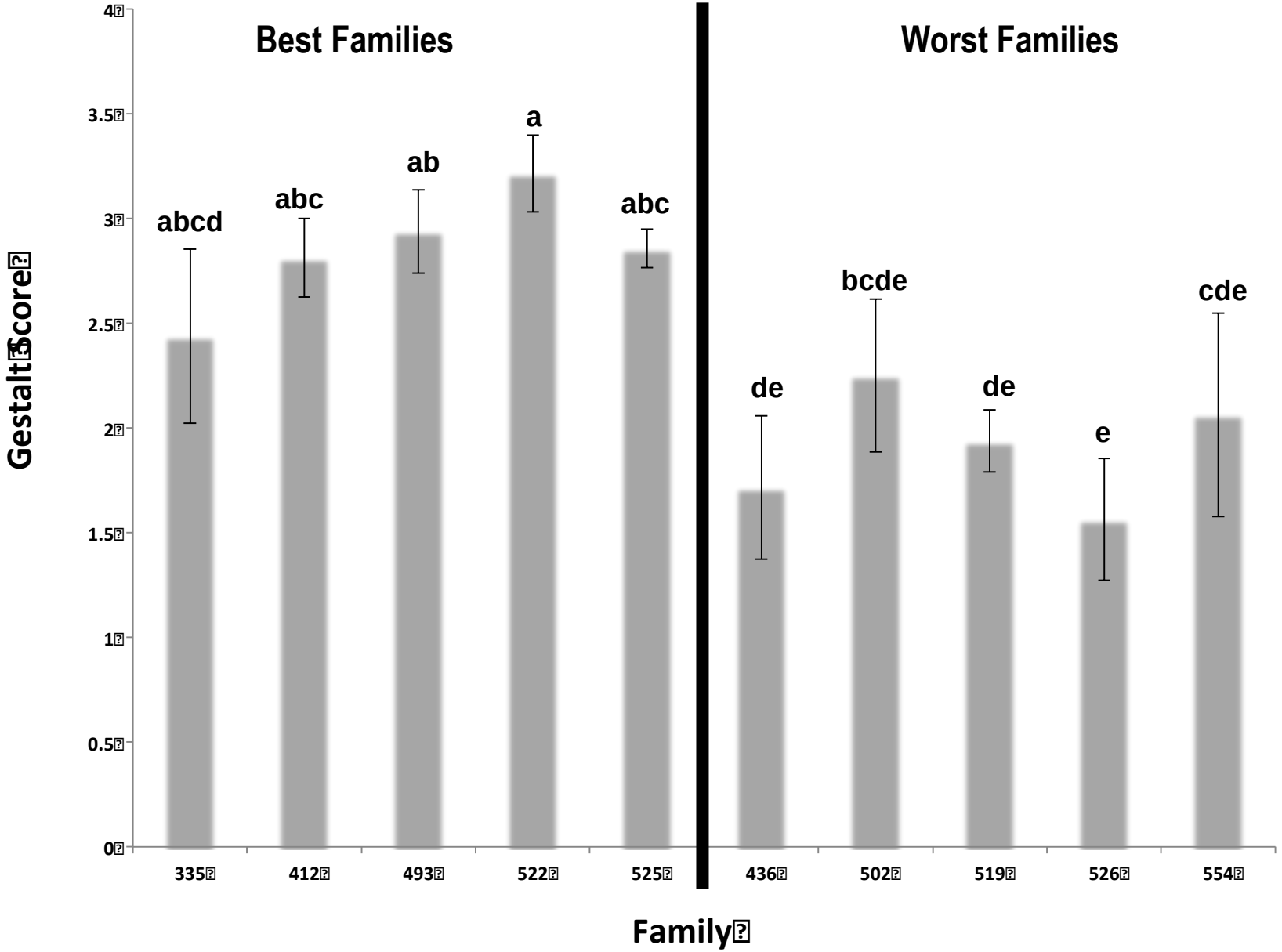
b)

c)

Average Internode Length



Gestalt


Best Families
Worst Families
Gestalt Score
4
3.5
3
2.5
2
1.5
1
0.5
0
abcd
abc
ab
a
abc
de
bcde
de
e
cde
335
412
493
522
525
436
502
519
526
554
Family

Gestalt

Figure 9. Examples of WRC gestalt. These images are representative of samples with a gestalt score of: a) 4; b) 3; c) 2; d) 1, and e) 0.

Gestalt Criteria:

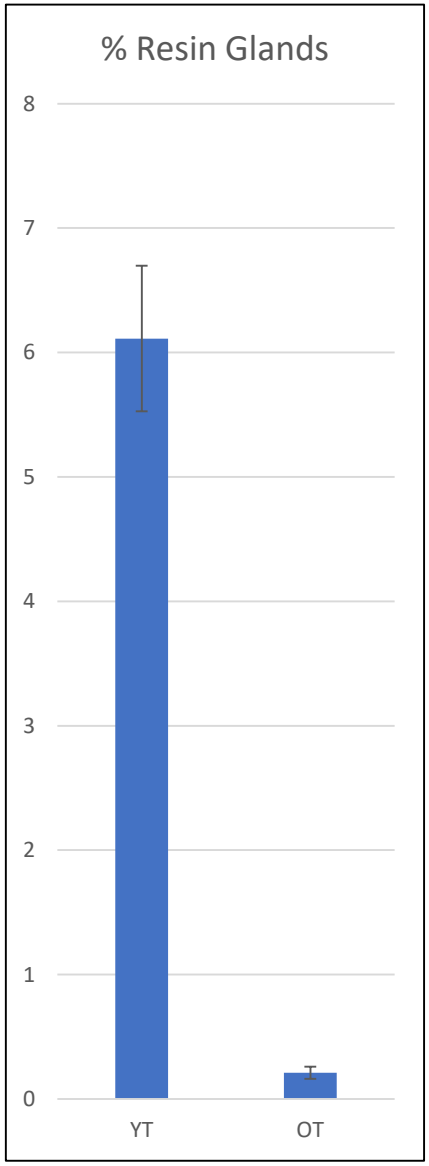
1) Is the growth more or less linear/symmetrical including apical growth and lateral branches. If so, +1 point.

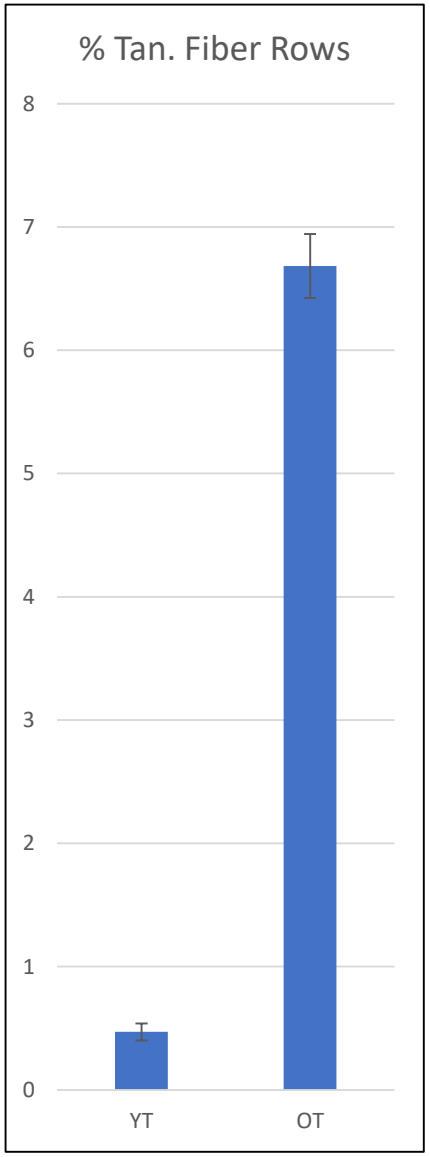
2) Is the foliage sleak/symmetrical, thinly spaced without bushy growth? If branches and foliage has no/minor overlap then the sample receives a score of +1. 0.5 if they are spaced with some overlap (see panel b).

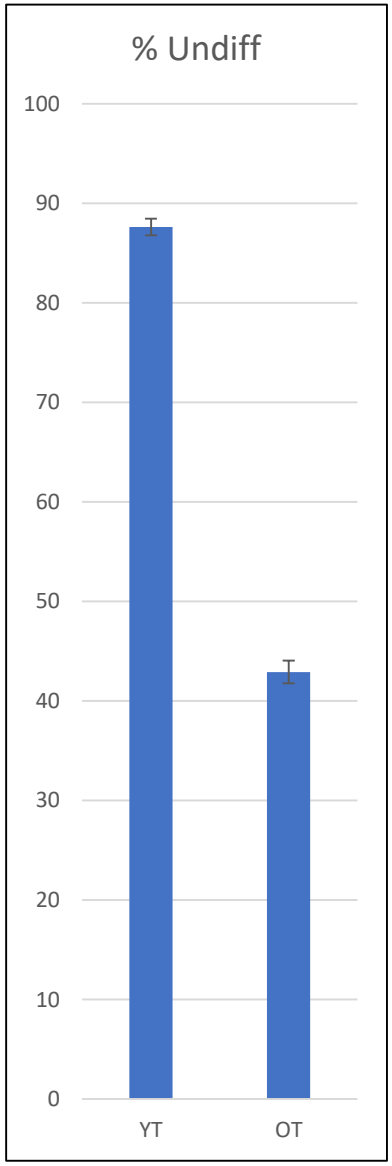
3) Are the internodes compact with a woody appearance? If so, +1 point.

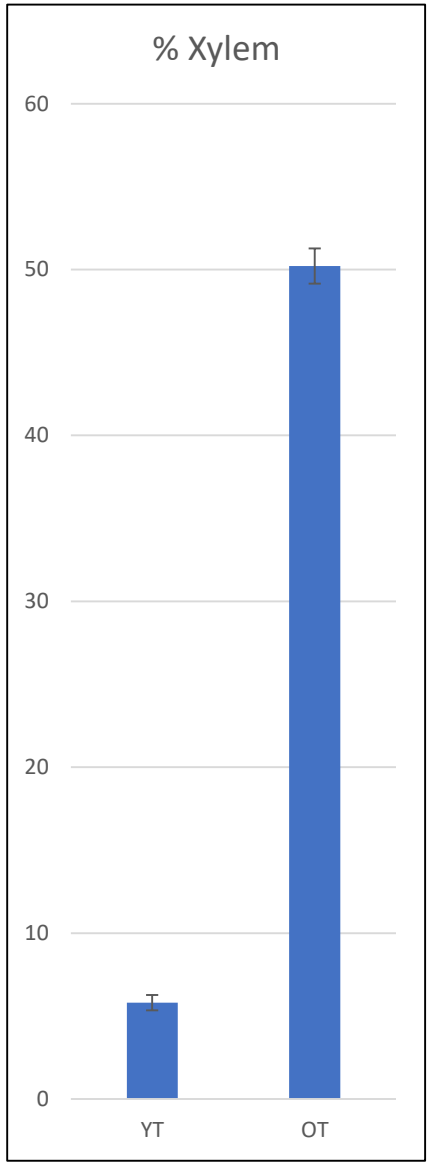
4) Has the tree been browsed at all? If so, -1 point.

Histology - Overview

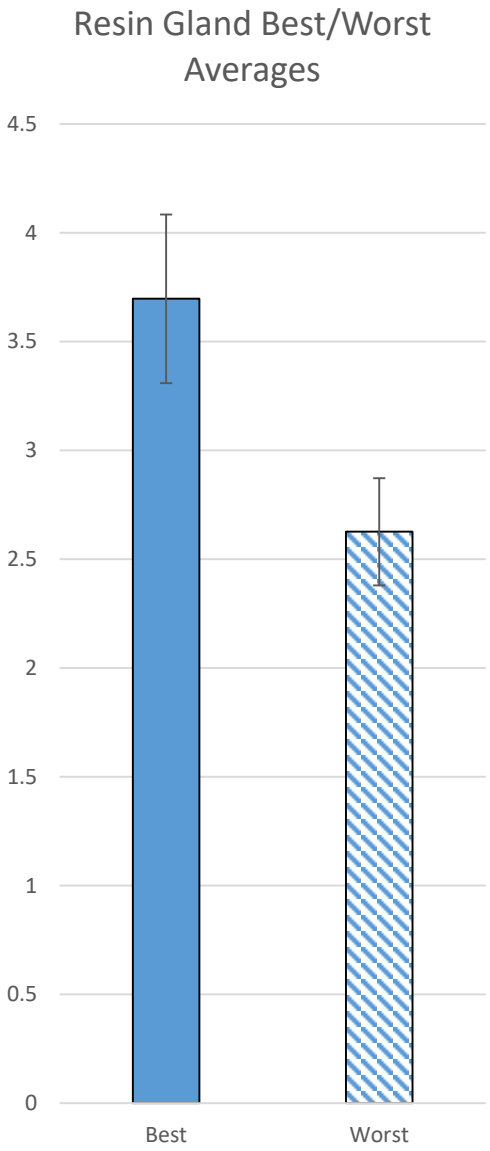


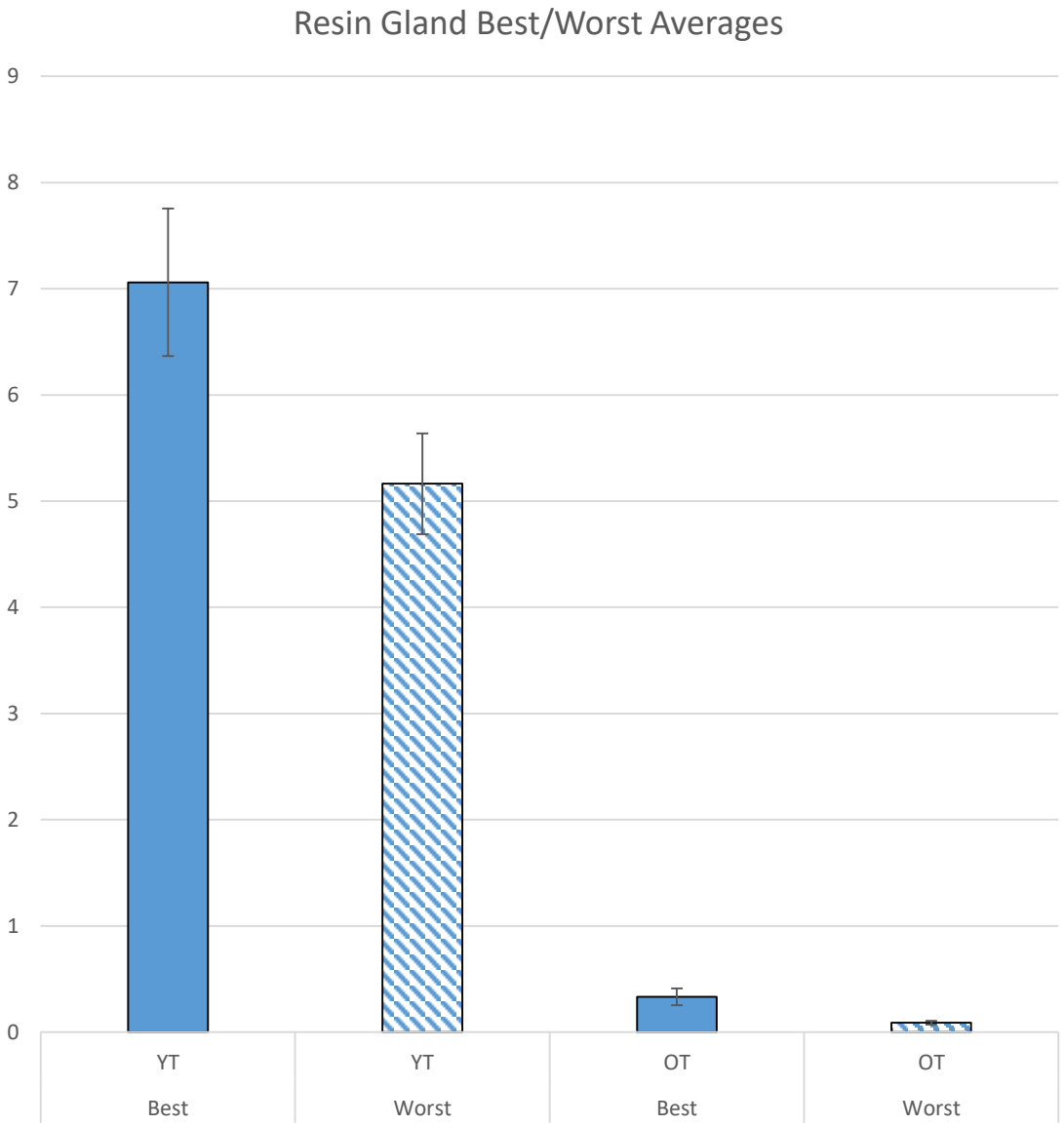




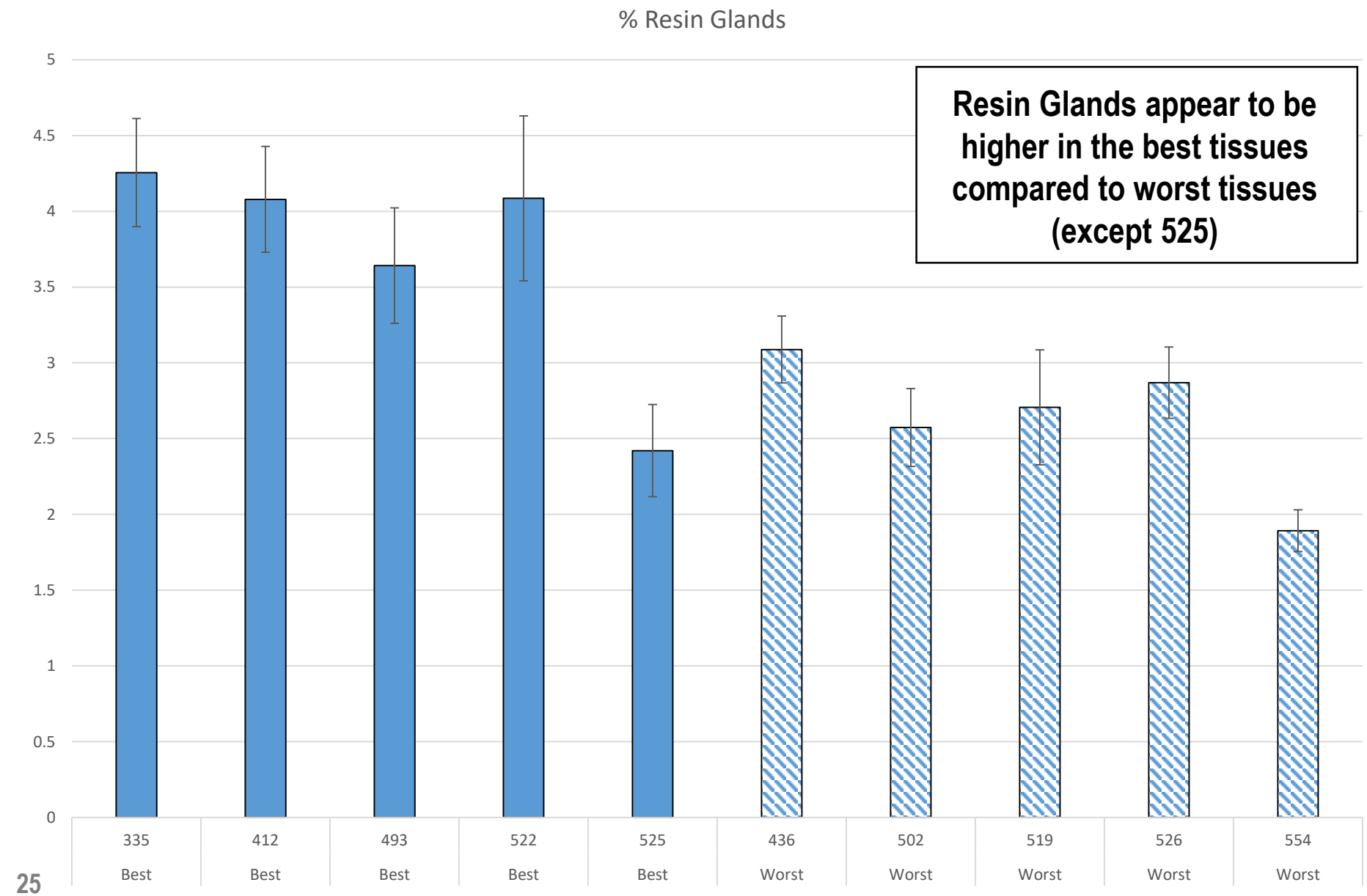


Histology – Resin Glands

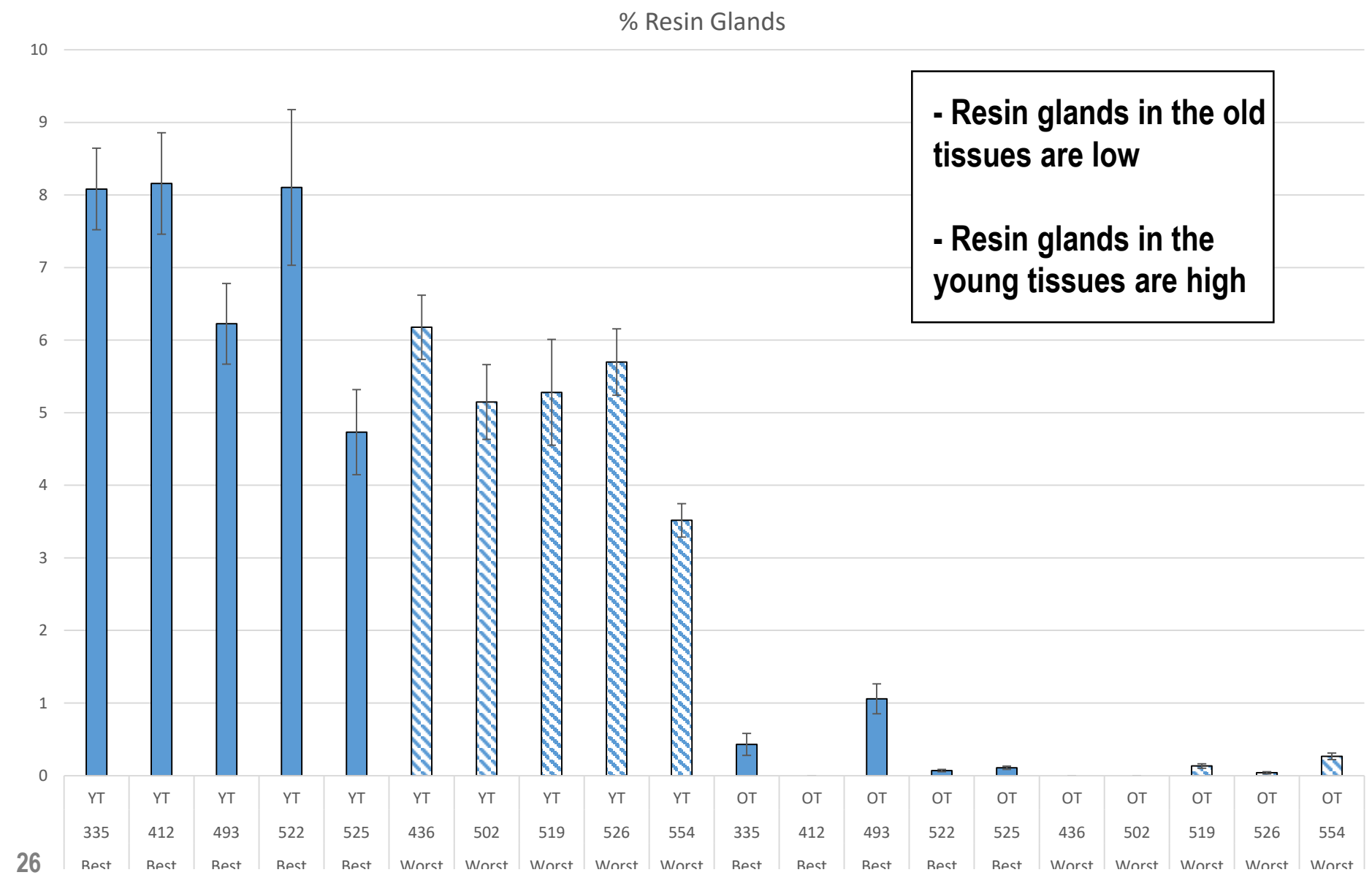




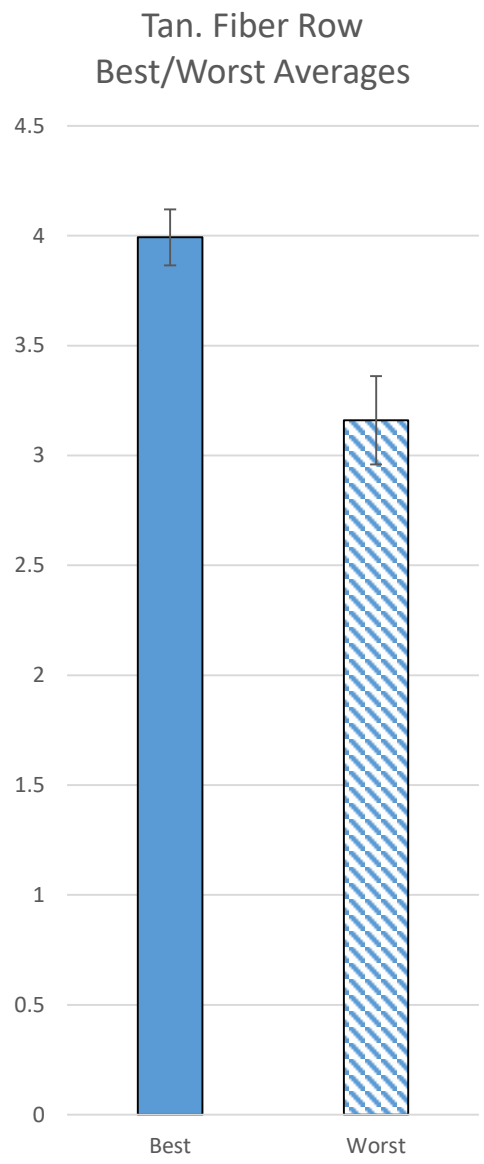
Histology – Resin Glands (Family)

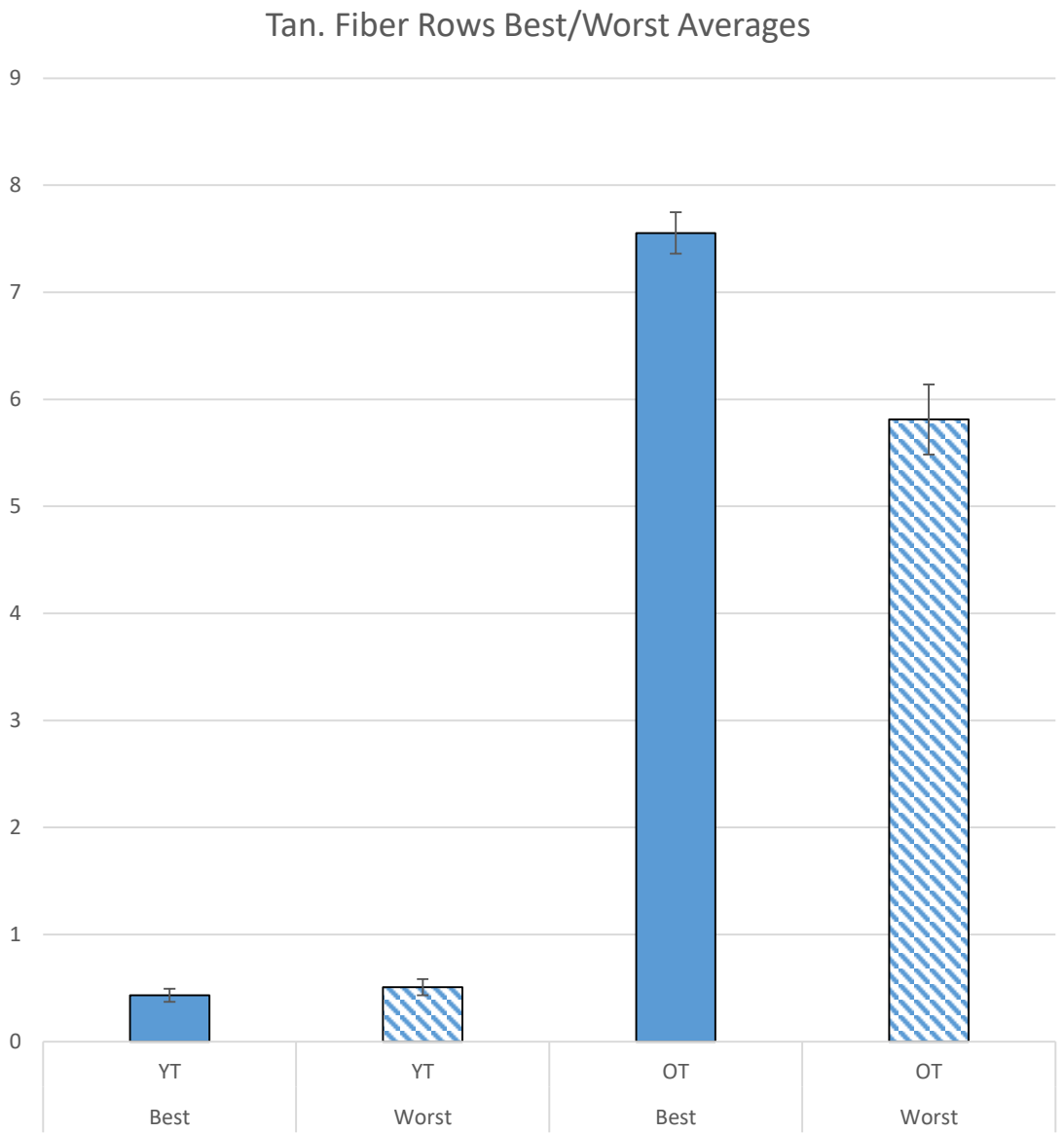


Histology – Resin Glands (Family x Tissue)

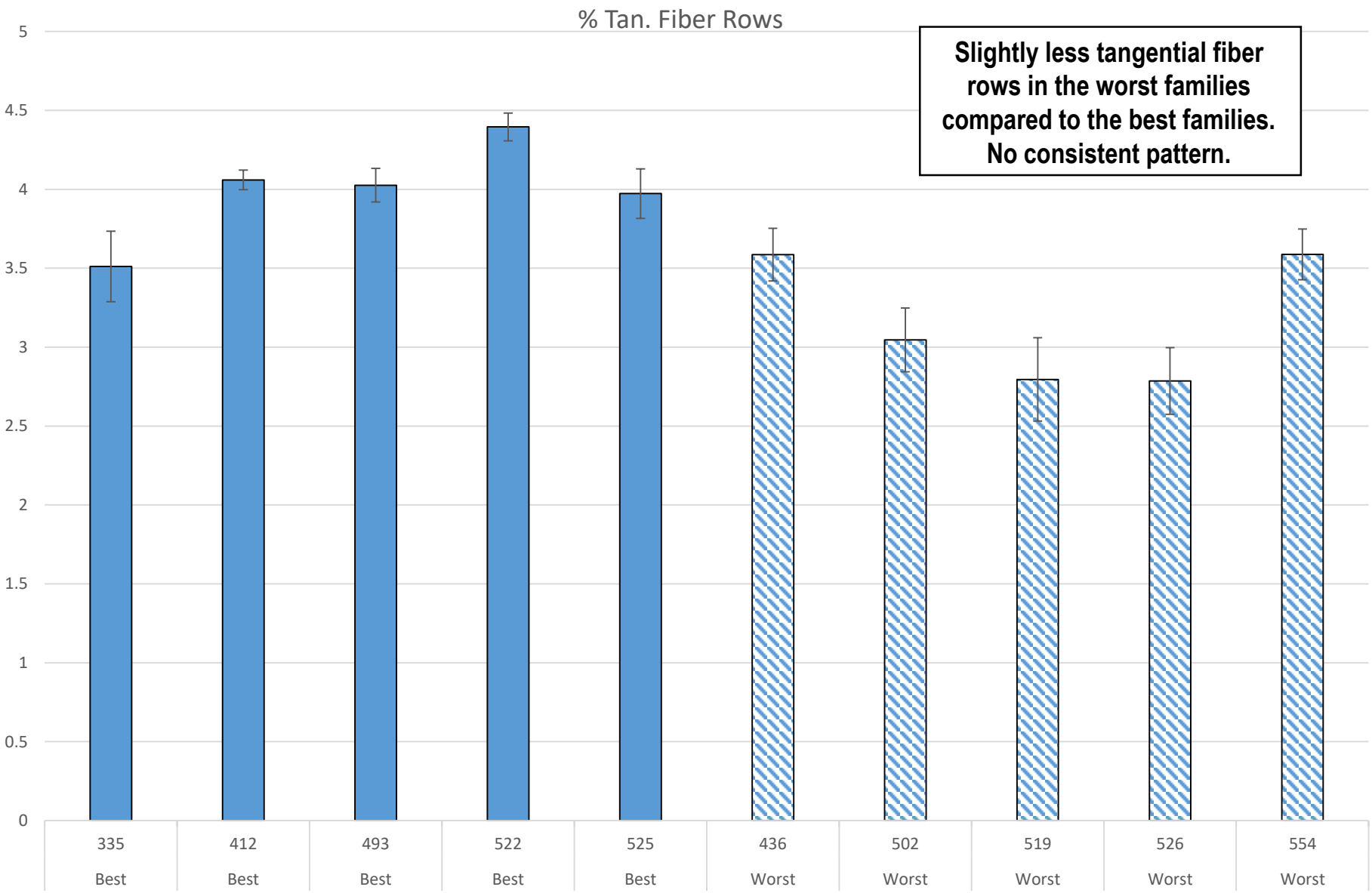


Histology – Tang. Fiber Rows

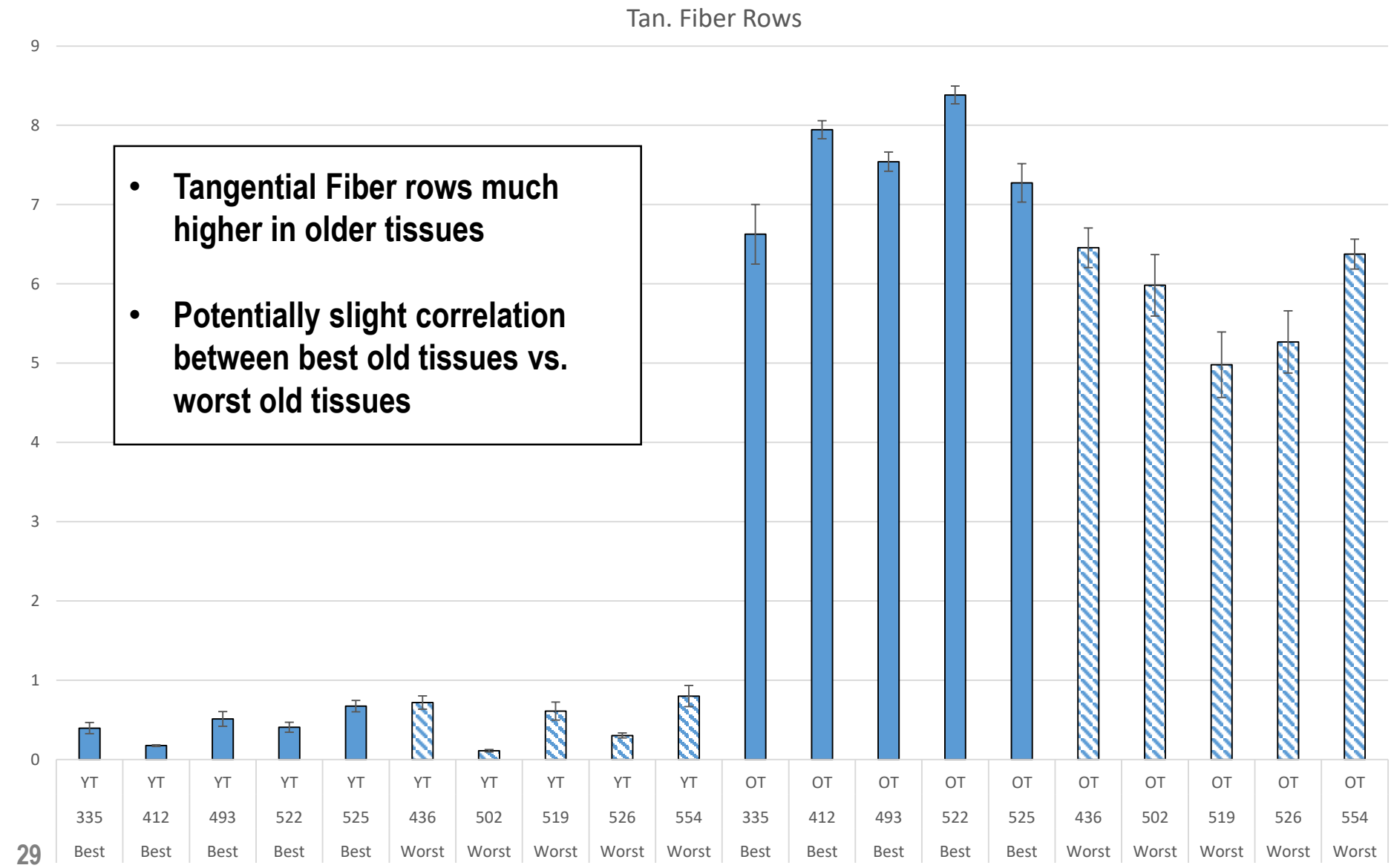




Histology – Tang. Fibers (Family)

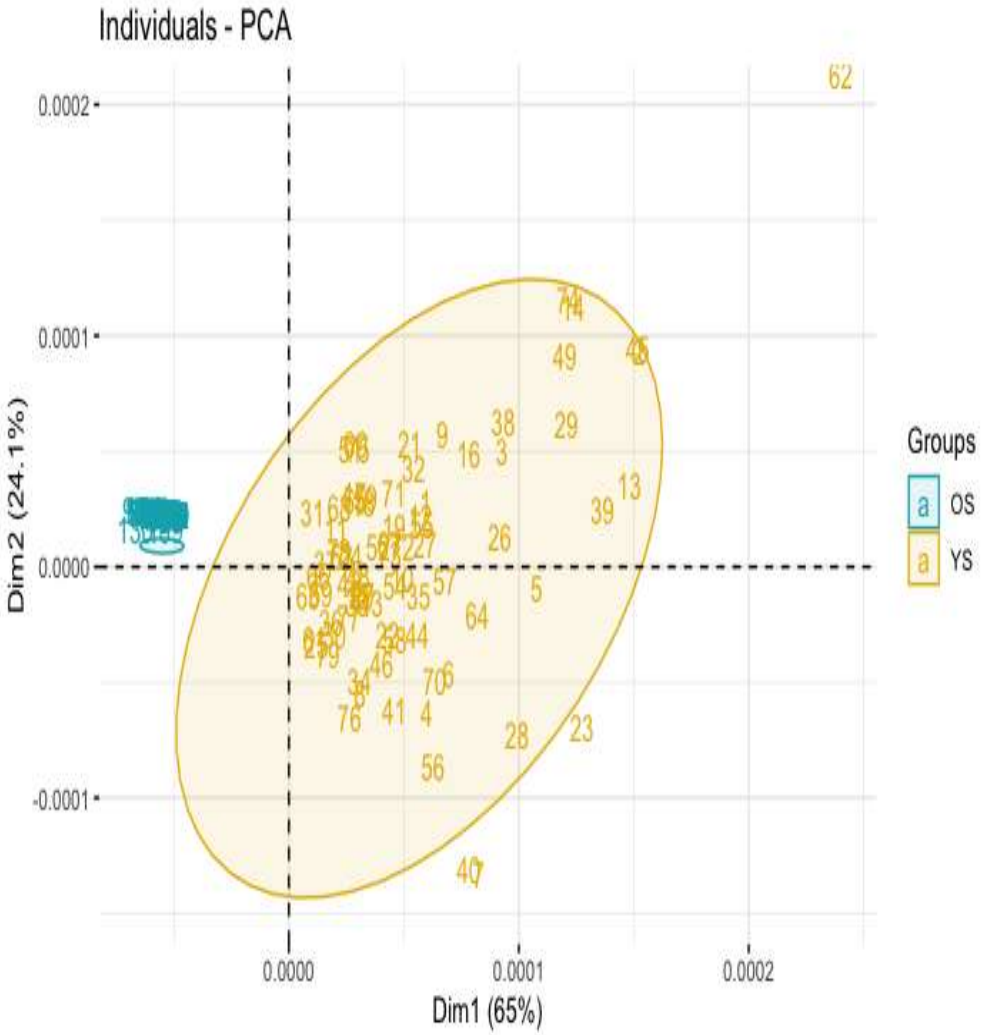


Histology – Tang. Fibers (Family x Tissue)



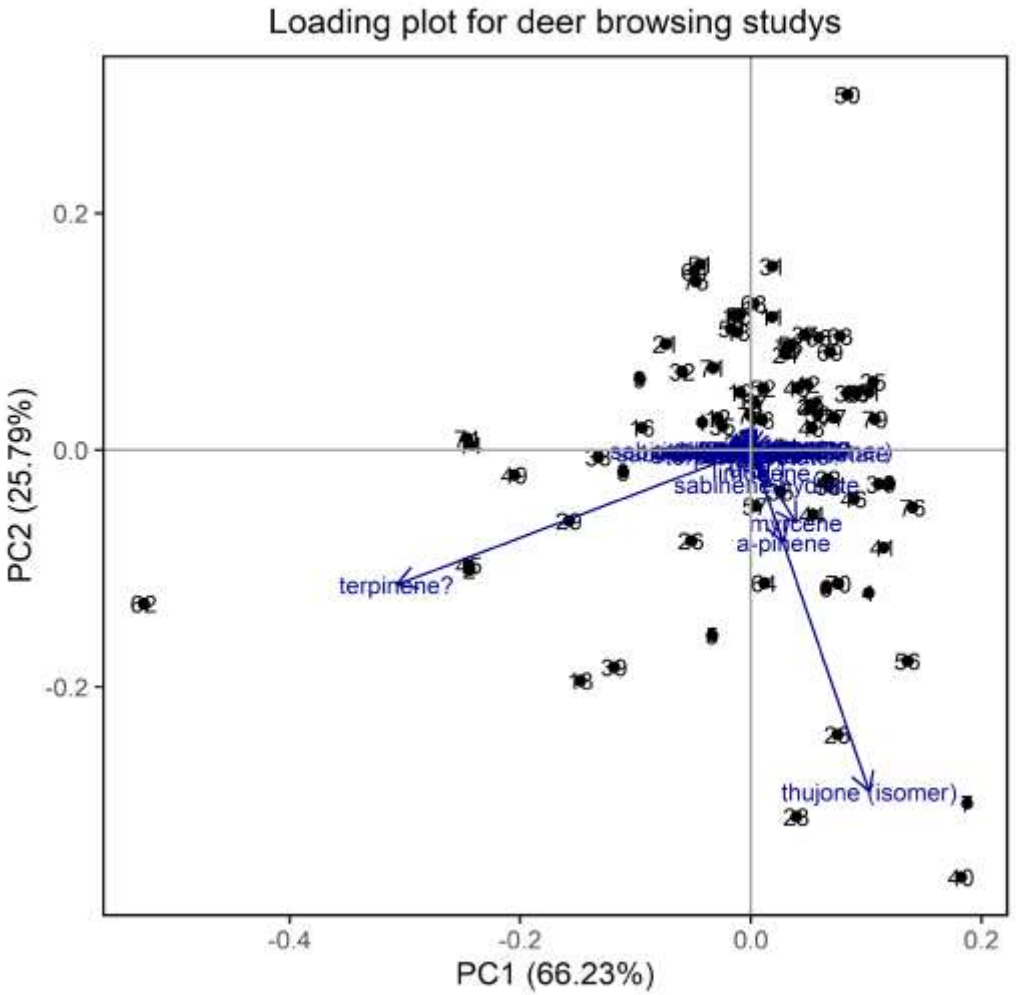
Chemistry – Monoterpenes (Summary)

- 72 mono- & sesquiterpenes were identified in young and old tissues
- Worked with a stats class to explore dataset
  - Yingqiao Wang
  - Ifeoma Ojialor
- Used the following:
  - PCA
  - Biplots
  - Pairwise comparisons
  - Spearman correlations



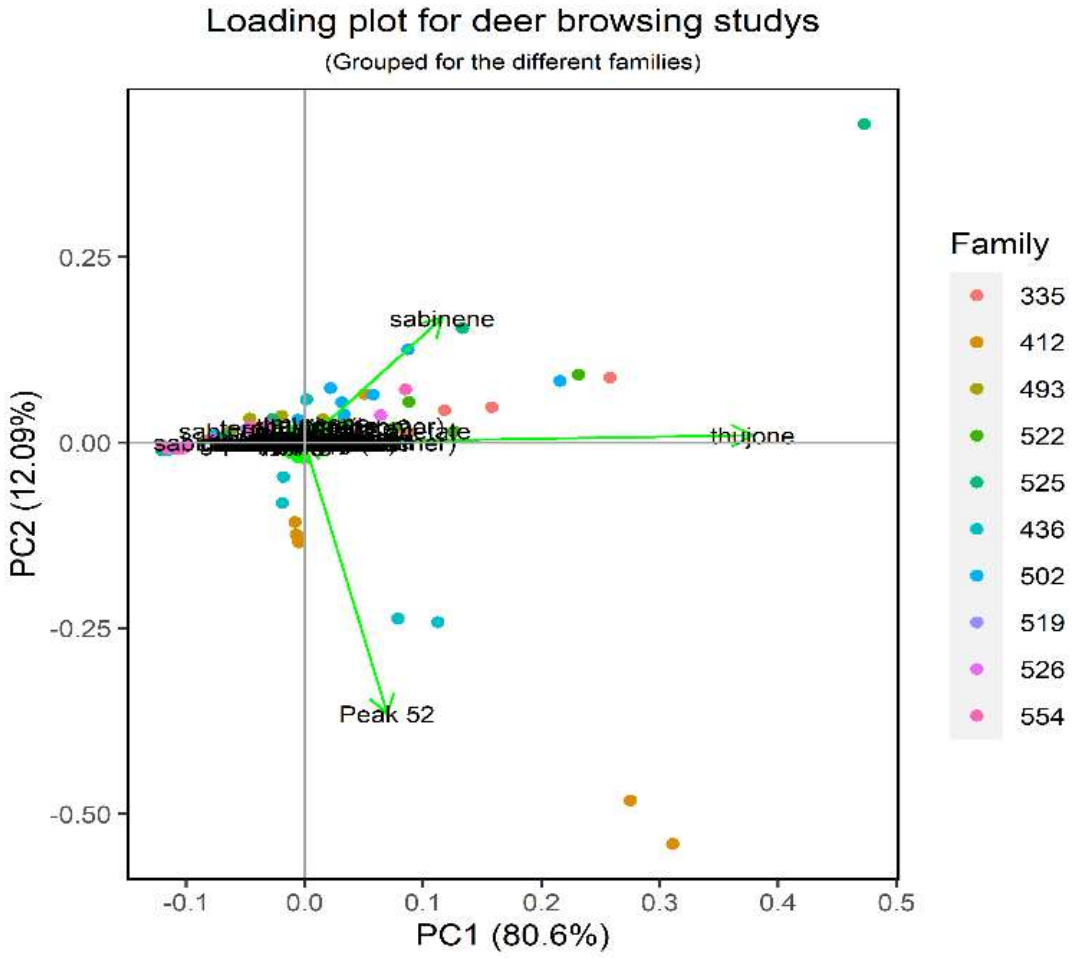
Chemistry – Monoterpenes (Young Stems)

- **Non-parametric MANOVA found 34 (of 72) compounds differed significantly among the 10 families (not shown)**
- **What is driving differences between YS & OS?**
- **Differences between YS and OS driven mainly by differences/presence of:**
  - **Terpinene, thujone (isomer), a-pinene, myrcene, sabinene hydrate**



Chemistry – Monoterpenes (Old Stems)

- Graph needs improved
- Old stem differences driven mainly by presence of sabinene, thujone, & peak 52
- Peak 52 higher in family 412 & 519
- Sabinene and thujone historically associated with deer browse preference



Chemistry – Monoterpenes (Spearman correlation)

- 12 compounds showed association with deer browse score
- 2 compounds (peak 4 and 26) shared a positive association with DB (i.e. more browse and more of these compounds)
- 10 compounds have a negative correlation (i.e. more compound and less browse)
- 0.2 correlation coefficient is considered moderate in biology
- Height and DB strongly correlated (data not shown)
  - Taller = less browse

Table 4: Spearman Correlation Test of the Deer Browse Score and the Terpene Compounds [Significant Results]

Terpene Compounds	P-Value	Rho
Peak 4	0.03	0.17
Sabinene hydrate acetate	0.03	-0.18
Peak 26	0.01	0.2
Unk 5	0.02	-0.18
Fenchene	0.002	-0.22
Peak 52	0.003	-0.24
Peak 56	0.004	-0.23
Peak 60	0.002	-0.24
P-cymen-8-ol	0.04	-0.16
Peak 64	0.01	-0.2
Unk 11	0.001	-0.26
Peak 67	0.01	-0.2

Summary

- Family differences for lig. start, internode length, & gestalt
- Major differences between YS & OS for RG and TFR
- Slight relationship between RG and TFR for best and worst (needs further analysis)
- Histological variation is tight within family
- Major difference in terpene profiles of YS vs. OS
  - What is important for biology of deer and feeding preference?
- Focus on compounds in YS for better families
  - OS compounds might be less important
- Deer browse preference is complex and likely involves a multitude of different traits (family specific)

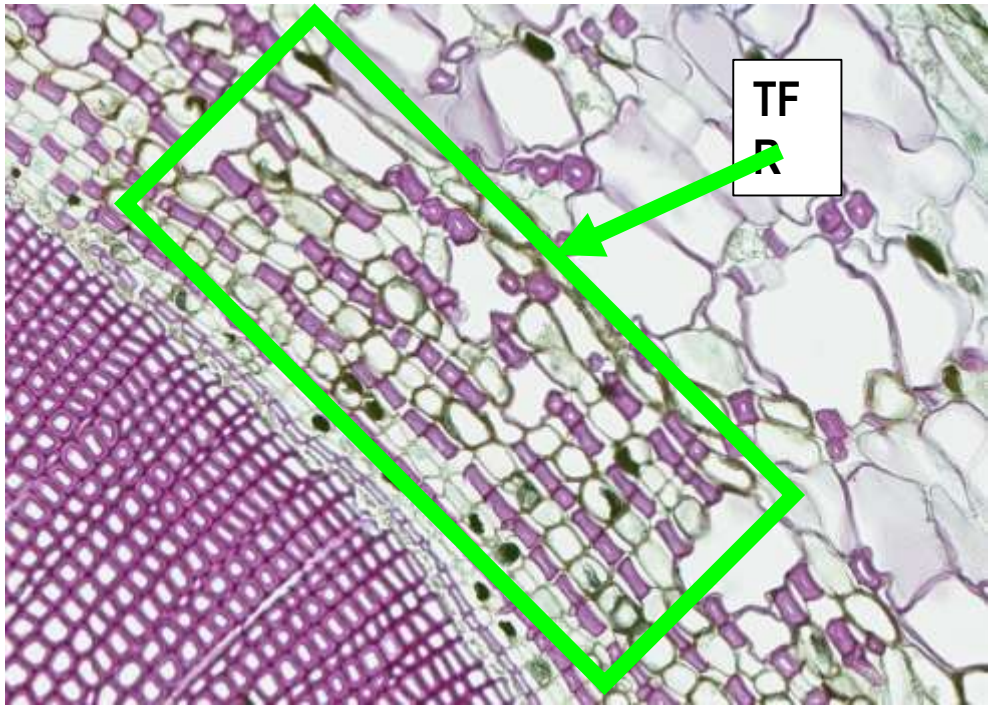
Deer skull at Nimpkish

Conclusions

- Focus on best families
- Dig into traits
- Develop BV based on clonal analysis of best families/individuals within a family
- Integrate all traits of interest with DB scores
- Identify targets
- Faster growth could offset browse?
- Different families have different mechanisms for DB preference.
  - Tolerance (i.e., compensatory growth)
  - “Resistance” (physical/chemical?)
  - Provenance effects?

Next Steps

- More thorough analysis of ALL variables
  - Multivariate
  - PCA
  - Correlations
- Look into specific associations between individual traits and DB scores
- Incorporate diterpenes
  - All integrated and data ready, not yet analyzed
  - Willie Adams prepared a master data sheet
- Identify terpenes of interest
  - Get solid compound IDs
- Write & publish paper!
  - January 2023?
- Suggest phenotyping strategy/tool for DB preference in WRC



Tangential fiber rows in old stems

Thanks!

Willie Adams