Performance and Value of CAD-deficient Pine

Principal Investigators

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Collaborators

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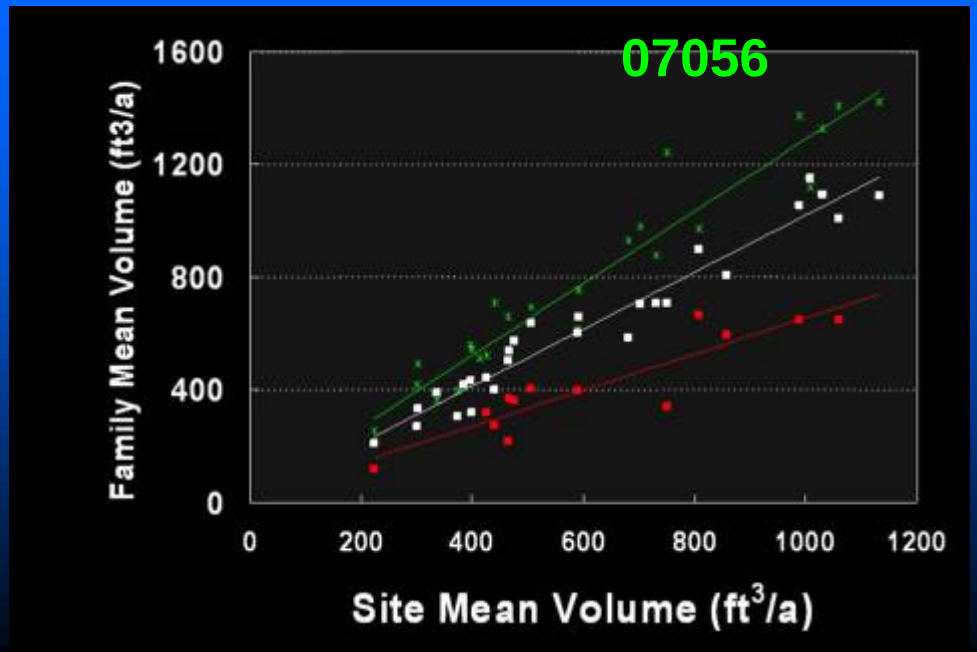
- John Kadla, UBC, Canada
- John MacKay, Université Laval, Québec

Background

- Tree 7-56 was an outstanding selection from NCSU Tree Improvement Program

  Producing on average 30% more volume than commercial check lots

  – Most widely planted progenies in the south



CAD Genotypes

+/+ “wild-type” or normal

+/- *cad*-n1 heterozygotes, “partially CAD deficient”

-/- *cad*-n1 homozygotes, “totally CAD deficient”

+ / +

+ / -

- / -

Previous Studies for Cad-nl Heterozygotes

- Wu et al. (1999) reported heterozgotes trees produced 14% more debarked volume than wild-type trees at age 4 years.
- Dimmel et al. (2001) found heterozygous trees resulted in significant lower kappa (lignin content %) numbers than wild type.

This Study is to Characterize the Difference with

- Large sampling size
- Use all *cad*-n1 heterozygotes selections
- Trials in different locations
- Different treatments
- A range of ages

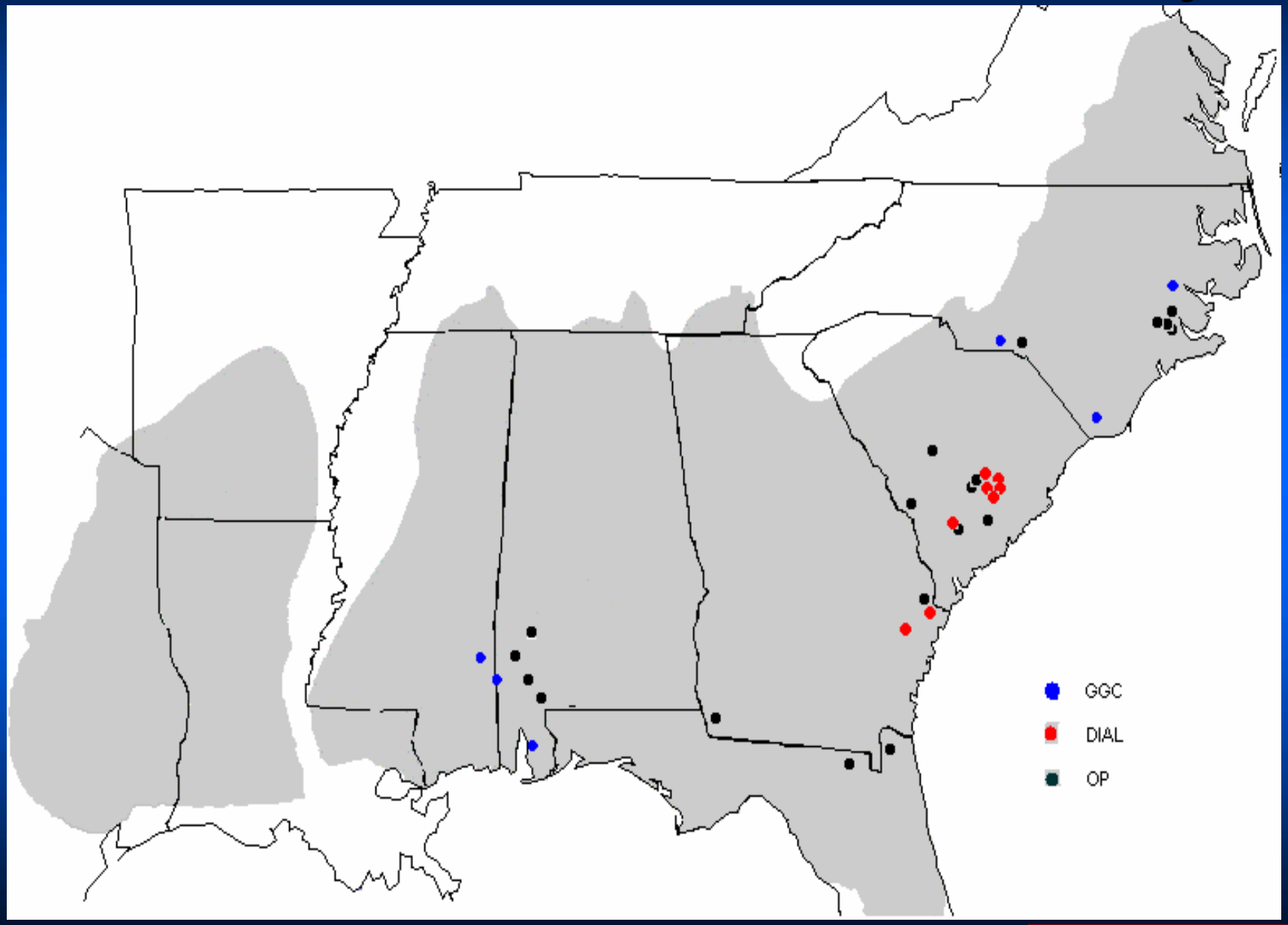
Objectives

- To identify associations between CAD genotype and growth performance from age 6 to rotation age in multiple genetic and environmental backgrounds
- To compare lignin formation in partially CAD-deficient and “normal” trees,, in both juvenile and mature wood
- To conduct laboratory studies to confirm the value of partially CAD-deficient wood for energy savings in pulp production

Experimental Approach

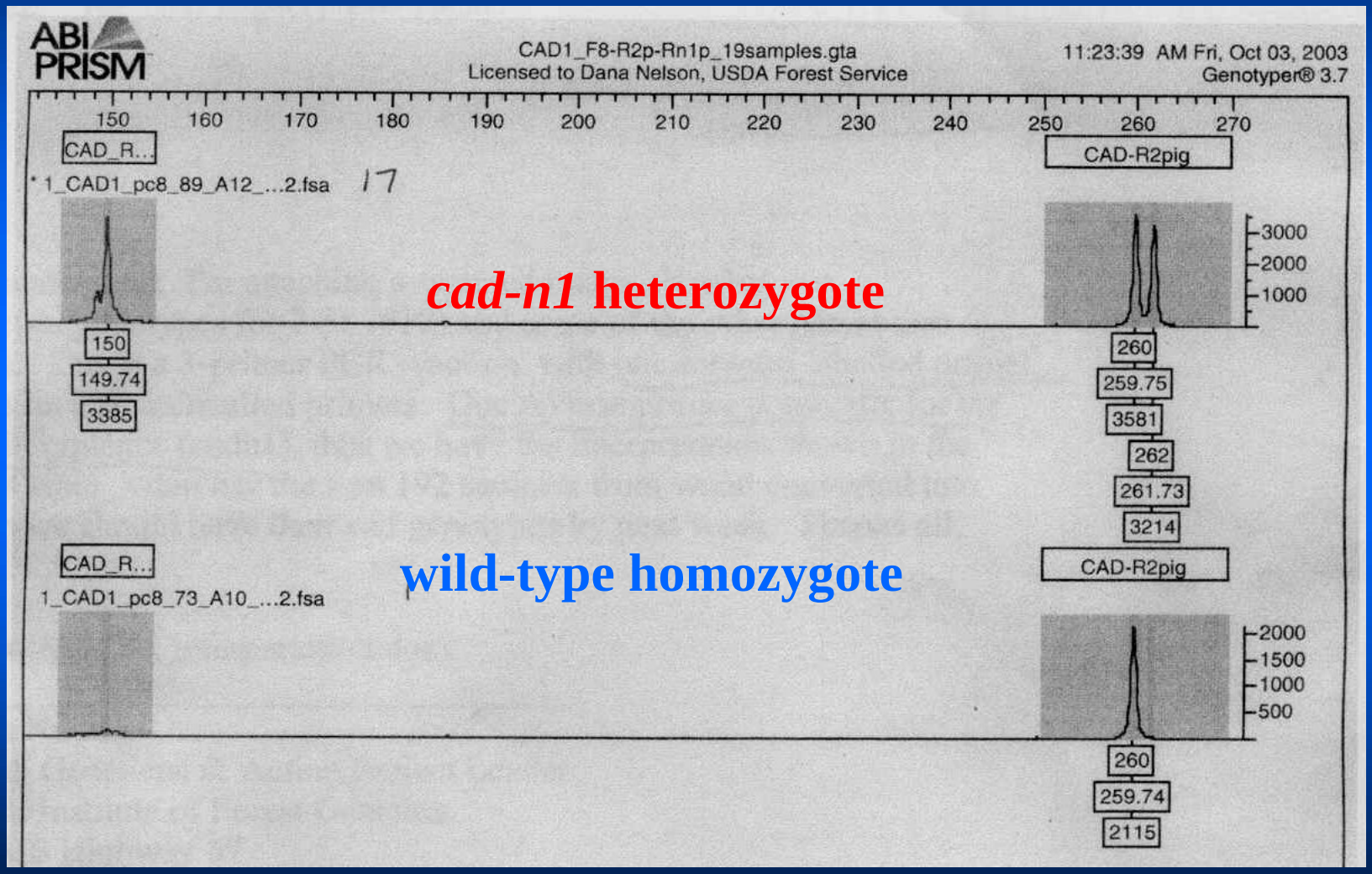
- Field sampling: 1st, 2nd, and 3rd generation descendants of 7-56
- Genotyping
- Growth assessment
- Evaluation of wood physical, chemical and pulping properties
- Quantitative genetic analysis of differences

Distribution of Field Trials in CAD Project



DNA Collection from Cambium

Genotyping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Licensed to Dana Nelson, USDA Forest Service
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Genotyper® 3.7
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CAD_R...
CAD-R2pig
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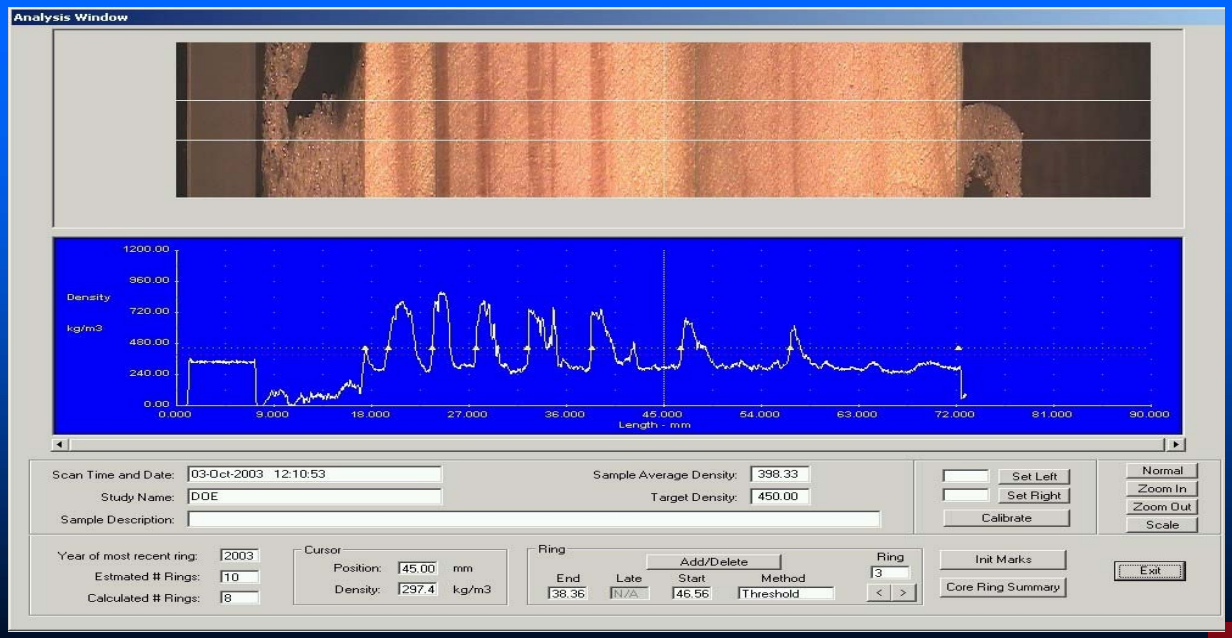
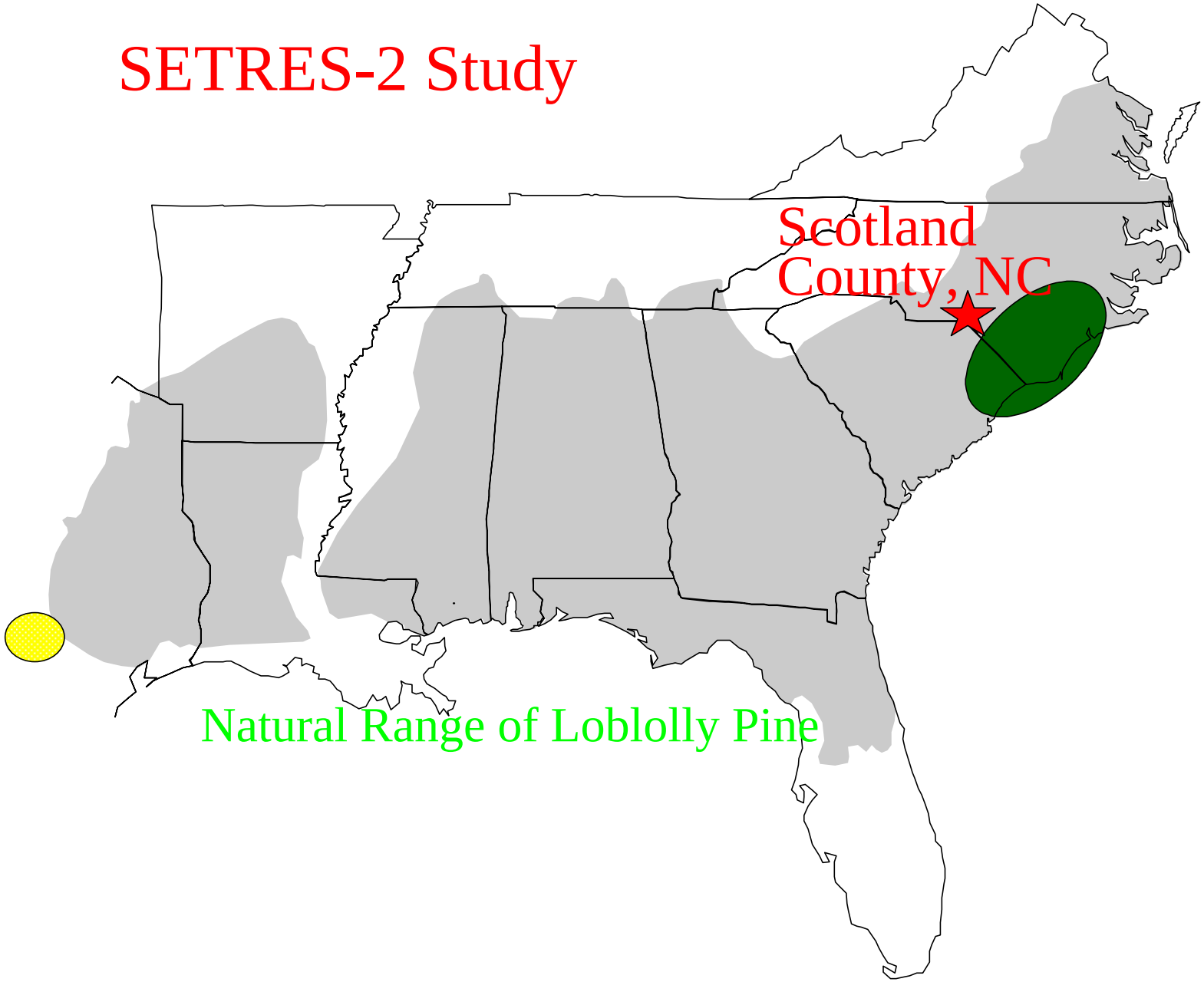
Wood Core Collections

X-ray Densitometry

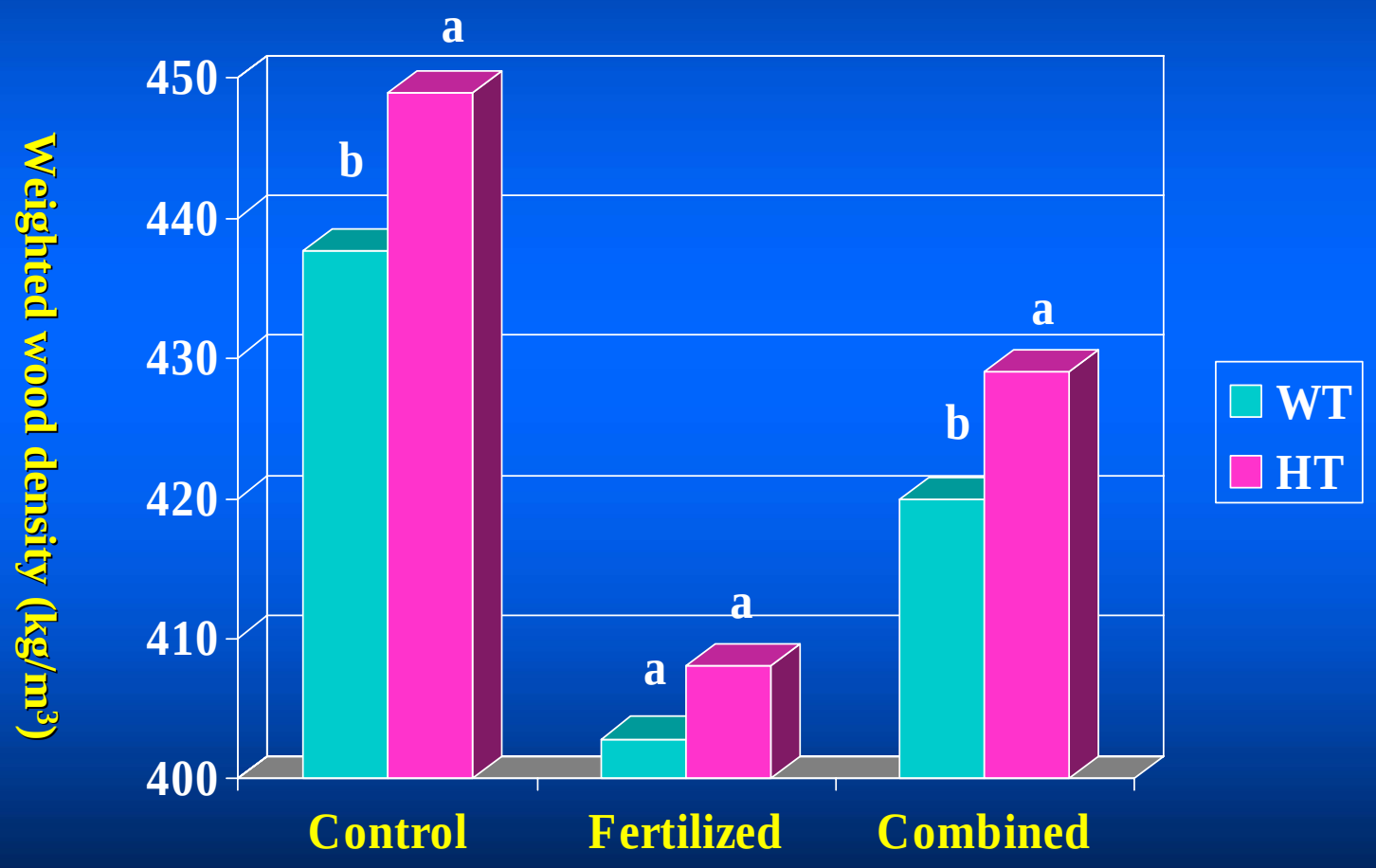


SETRES-2 Study

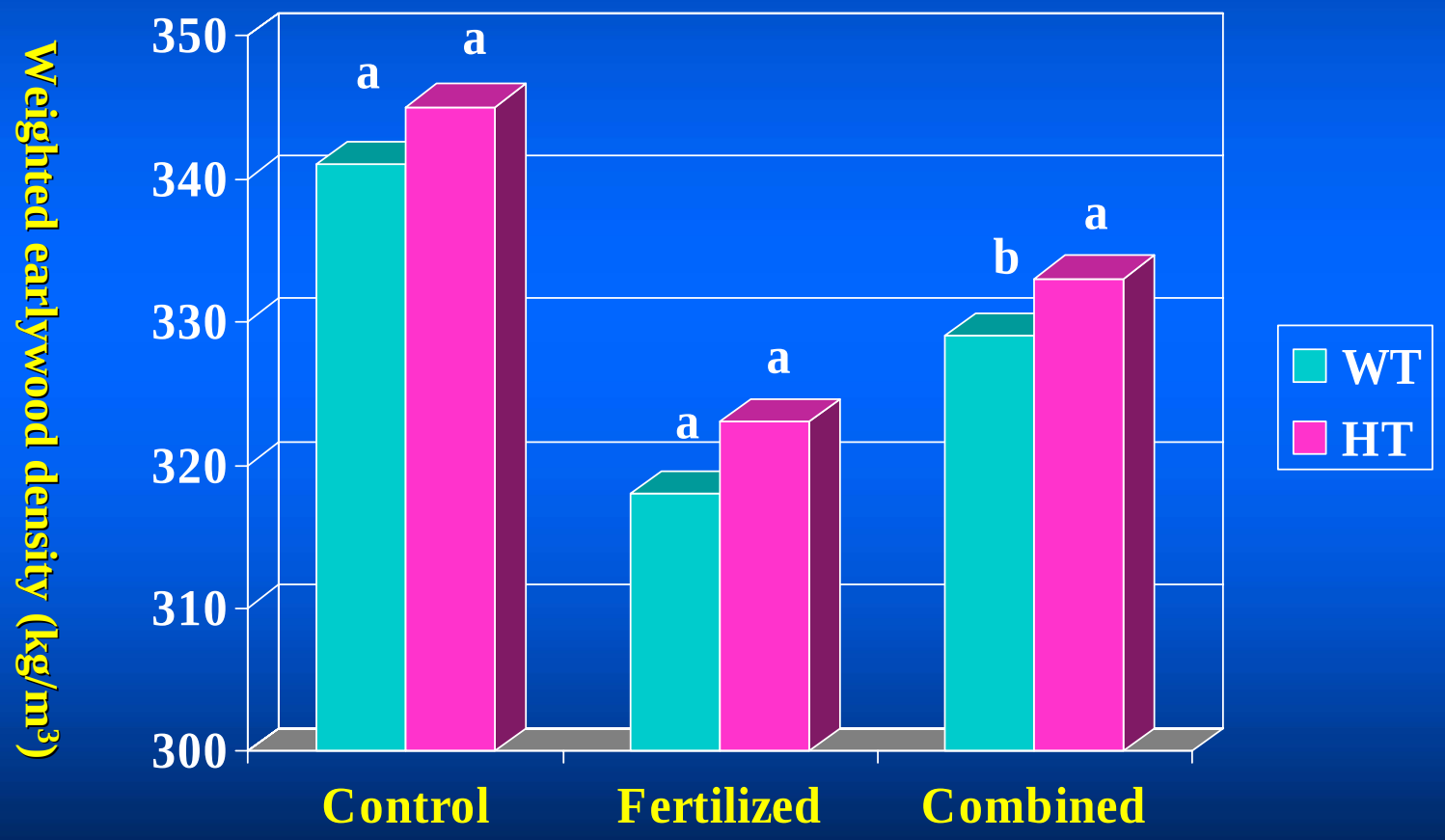


Control
Fertilized

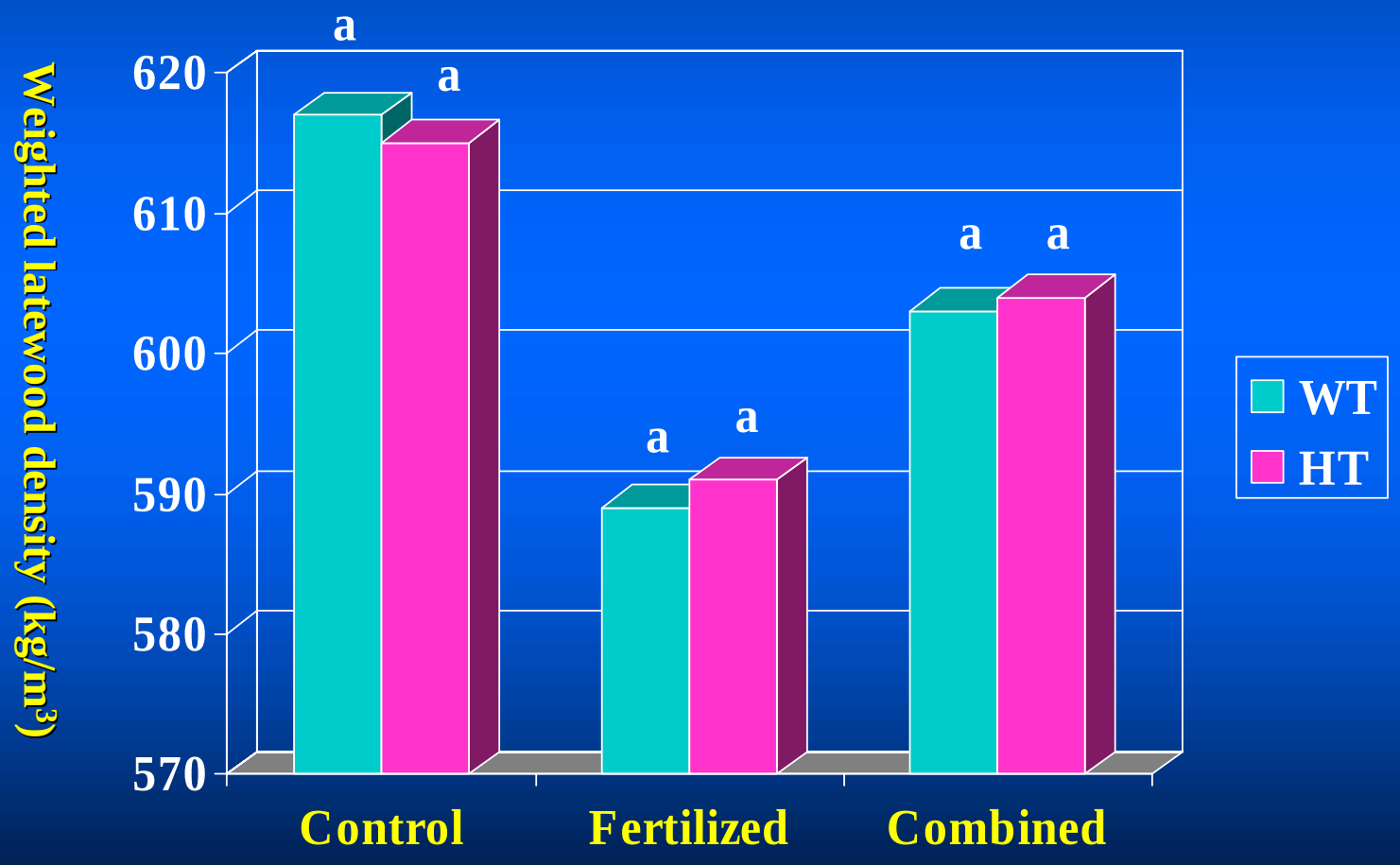
Wood Density for Cad-nl Heterozygotes (HT) and Wild-type (WT) in 9 Years



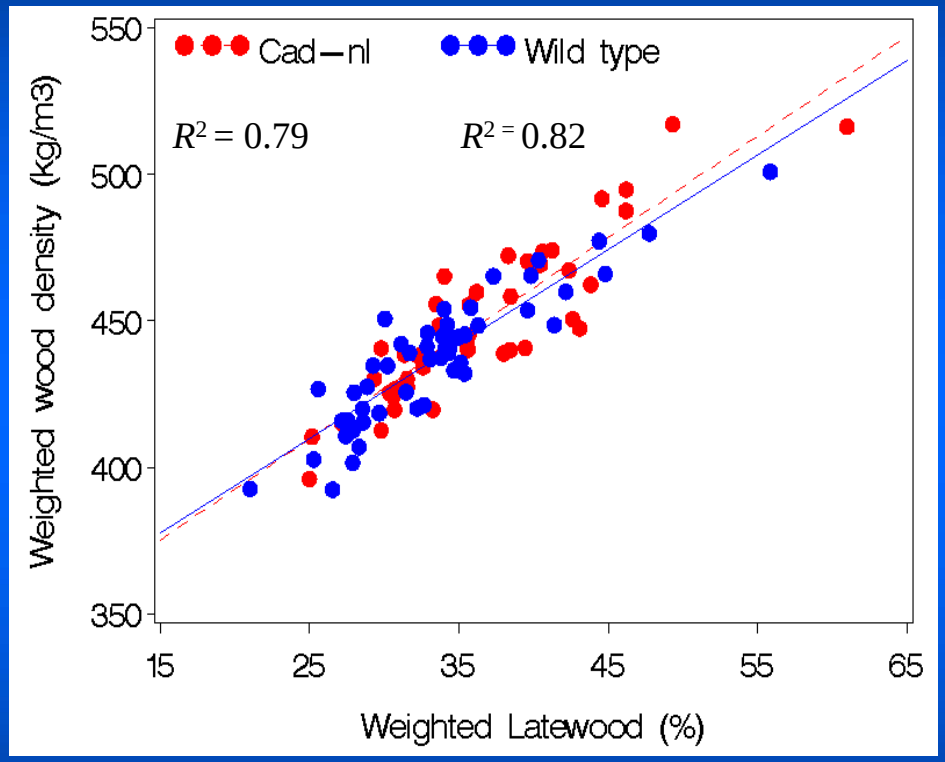
Earlywood Density for Cad-nl Heterozygotes (HT) and Wild-type (WT) in 9th Year



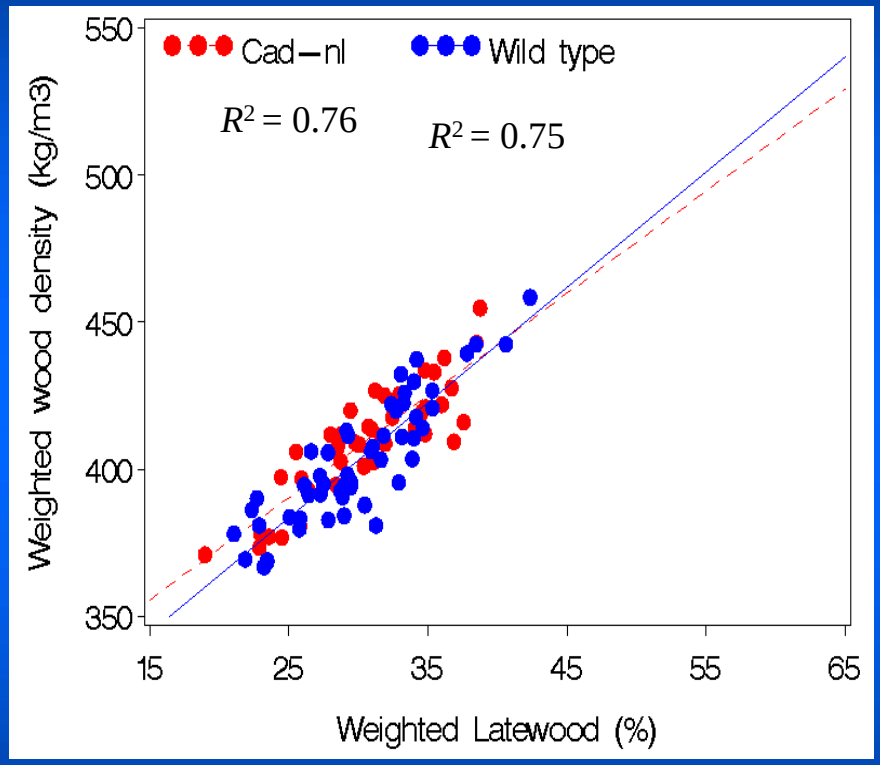
Latewood Density for Cad-nl Heterozygotes (HT) and Wild-type (WT) in 9th Year



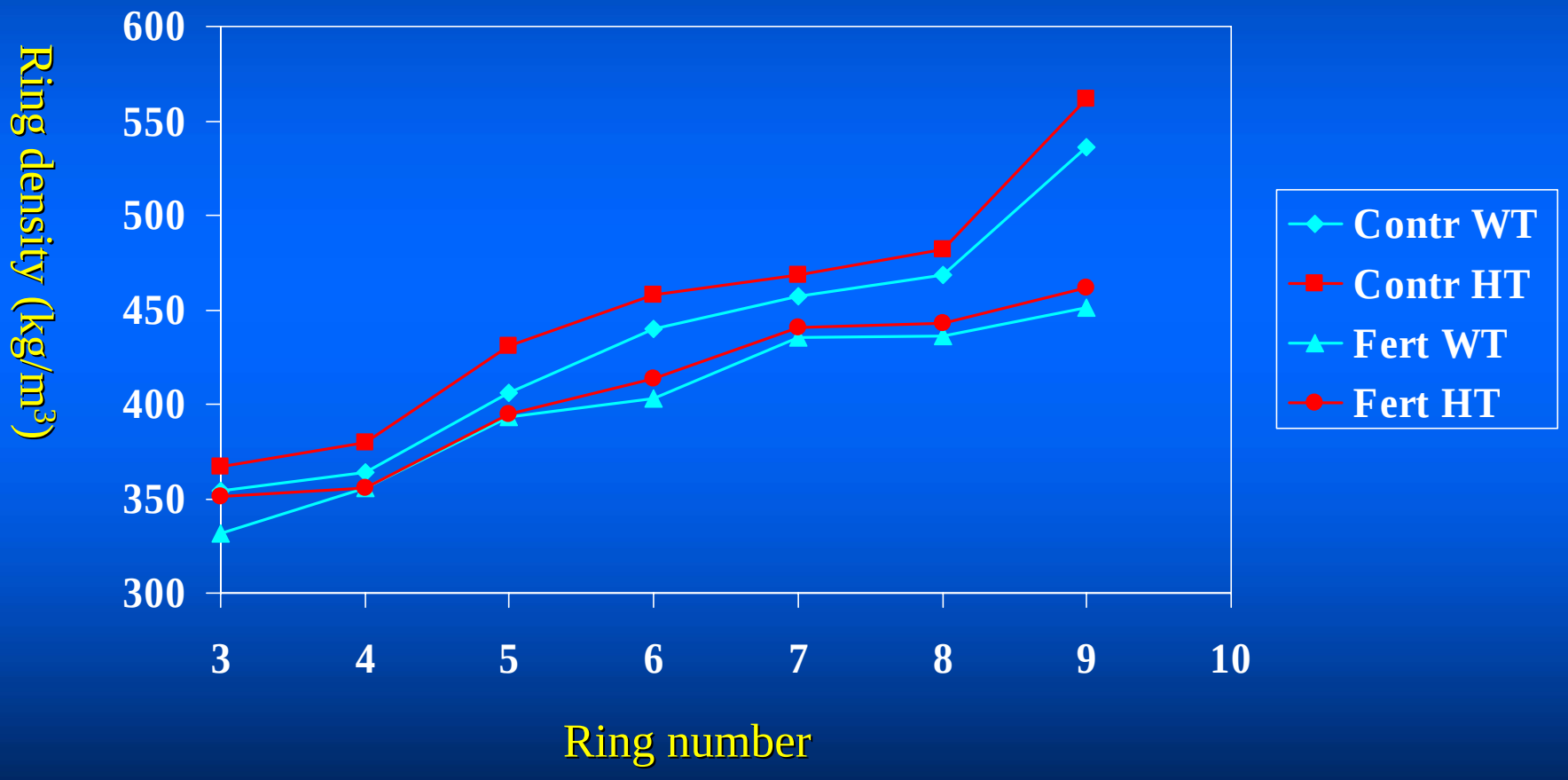
Control



Fertilized



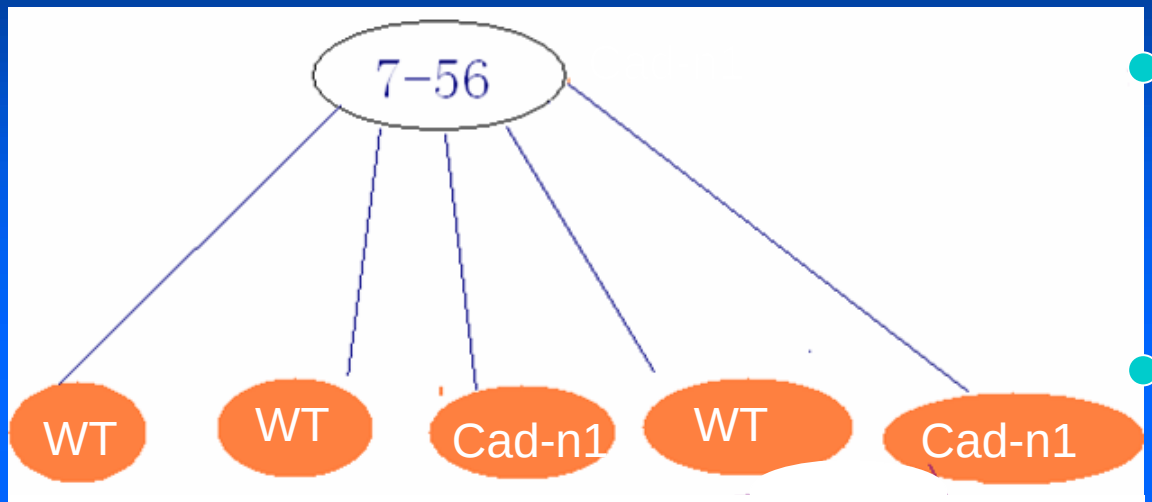
Treatment - Genotype Means
Ring Density, Years 3-9



Wood Density Difference

- Cad-nl heterozgotes have significant higher wood density (+2.6 %) than wild type.
- This is due to the higher percentage of latewood in heterozygotes.
- Ring density, latewood density and percentage increase from pith outwards.

Study the Difference in Other Genetic Background related to 7-56

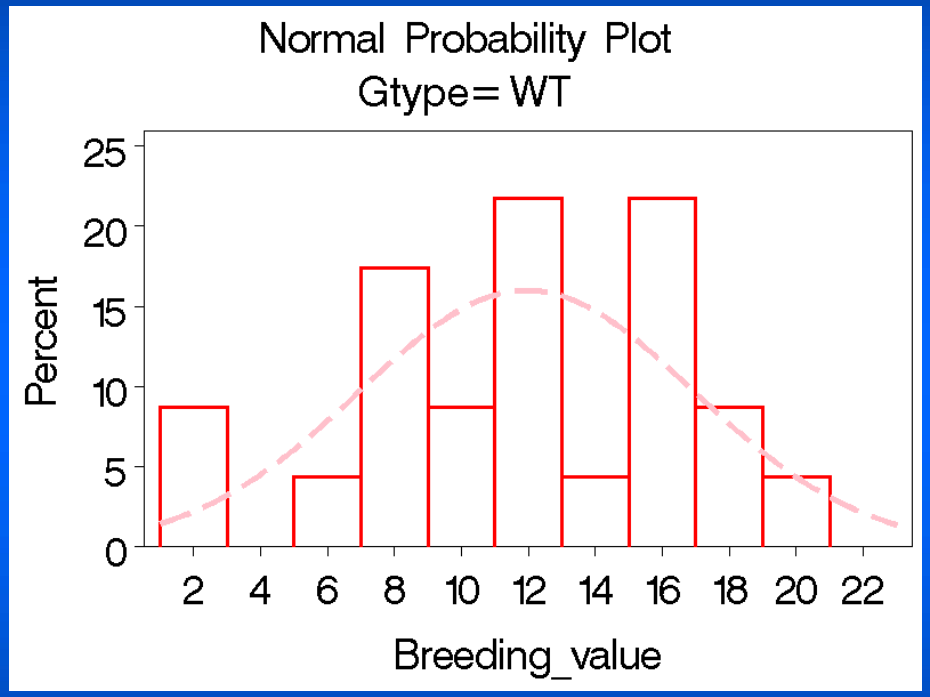


Wild type: ***23***
cad-n1 heterozygotes: ***7***

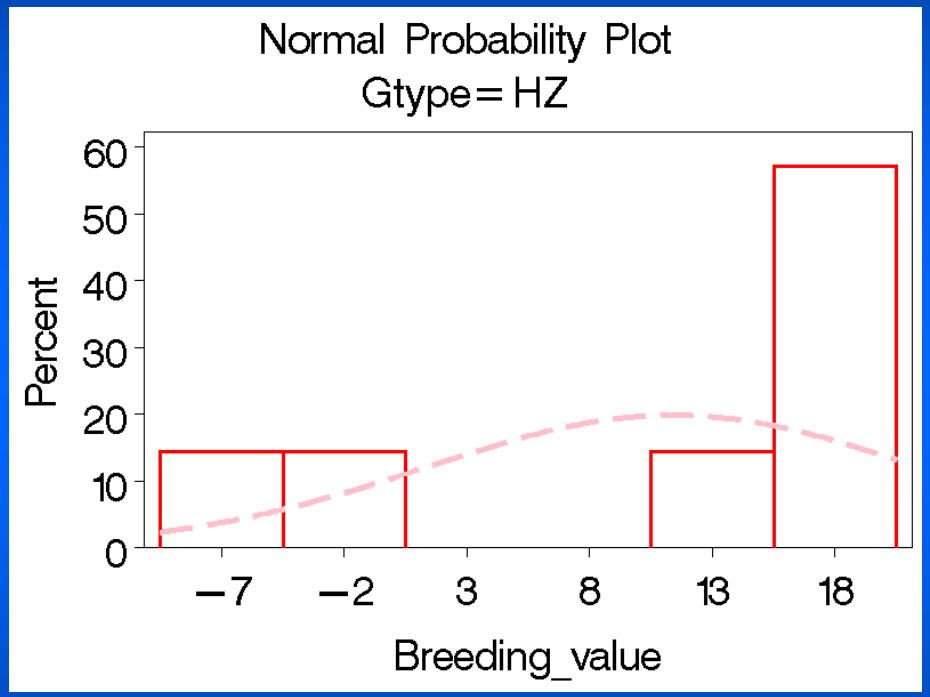
- **1st generation selections**
- **2nd generation selections**

Breeding Value of 2nd Generation Selections

Wild-type

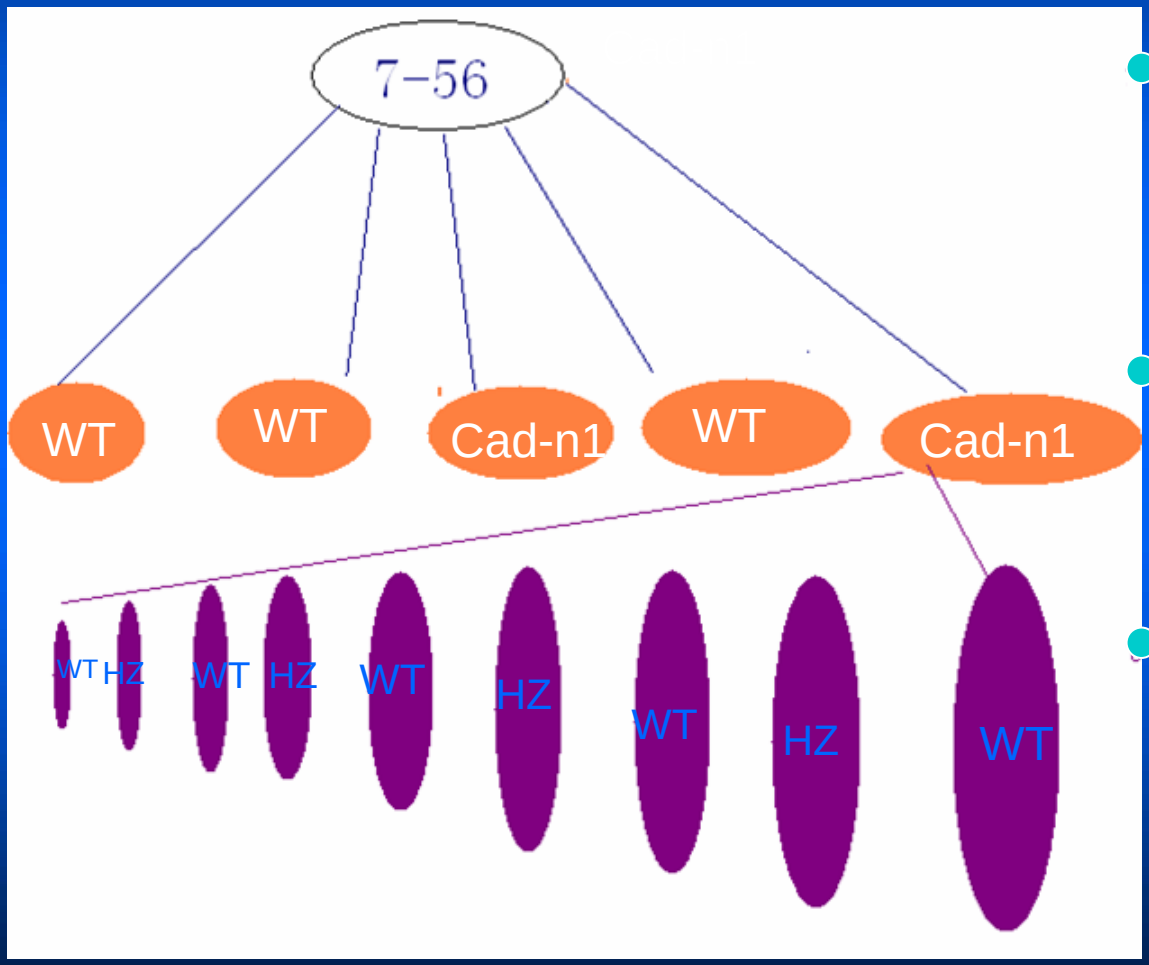


Cad-n1 heterozygotes



The high BV of 75-6 for growth may not associated with *cad-n1* alone, other gene loci are likely involved

Study the Progenies of 2nd generation Parents in several experimental pedigrees



- 1st generation parent
- 2nd generation parents
- Progenies of 2nd heterozygous parents In half-diallel tests

Cad-n1 Heterozygous (HZ) and Wild-type (WT) Trees for Growth Traits in Four Genetic Backgrounds.

Genetic background	6 years					
	Height (m)		DBH (cm)		Volume (dm^3)	
	WT	HZ	WT	HZ	WT	HZ
S1-A	6.0±0.1	6.1±0.1	9.4±0.2	9.6±0.2	28.0±0.9	28.6±0.9
S1-B	5.9±0.1	5.8±0.1	9.5±0.2	9.2±0.2	28.2±0.8	27.1±0.8
S2_C	5.9±0.1	6.0±0.1	10.7±0.2	10.9±0.2	46.5±1.4	47.2±1.3
S3-A	6.9±0.1	7.2±0.1	12.3±0.2	12.7±0.2	47.3±1.7	51.4±1.7
Average	6.2	6.3	10.5	10.6	37.5	38.6

Cad-n1 Heterozygous (HZ) and Wild-Type (WT) Trees for Growth Traits in Four Genetic Backgrounds At Age 15.

Genetic background	15 years					
	Height (m)		DBH(cm)		Volume (m^3)	
	WT	HZ	WT	HZ	WT	HZ
S1-A	17.5±0.1	17.8±0.1	18.4±0.3	18.8±0.3	0.23±0.01	0.24±0.01
S1-B	16.9±0.2	16.9±0.2	18.3±0.3	18.0±0.3	0.22±0.01	0.21±0.01
S2_C	18.6±0.2	19.2±0.2	21.3±0.5	21.9±0.5	0.38±0.02	0.40±0.02
S3-A	19.1±0.2	20.1±0.2	21.8±0.5	22.7±0.5	0.37±0.02	0.41±0.02
Average	18	18.5	20	20.4	0.30	0.32

Cad-n1 Heterozygous (HZ) and Wild-type (WT) Trees for Wood Traits in Four Genetic Backgrounds at Age 6.

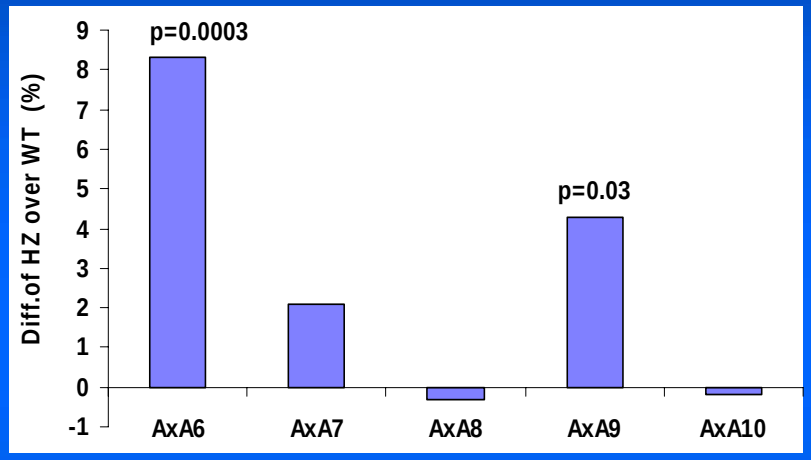
Genetic background	Wood density (kg/m^3)		Latewood (%)	
	WT	HZ	WT	HZ
S1-A	536±3.05	536±3.13	50±0.56	50±0.58
S1-B	529±3.21	526±3.16	50±0.62	50±0.61
S2-C	487±3.62	492±3.49	43±0.72	44±0.69
S3-A	498±3.83	511±4.03	46±0.83	49±0.88
Average	513	516	47	48

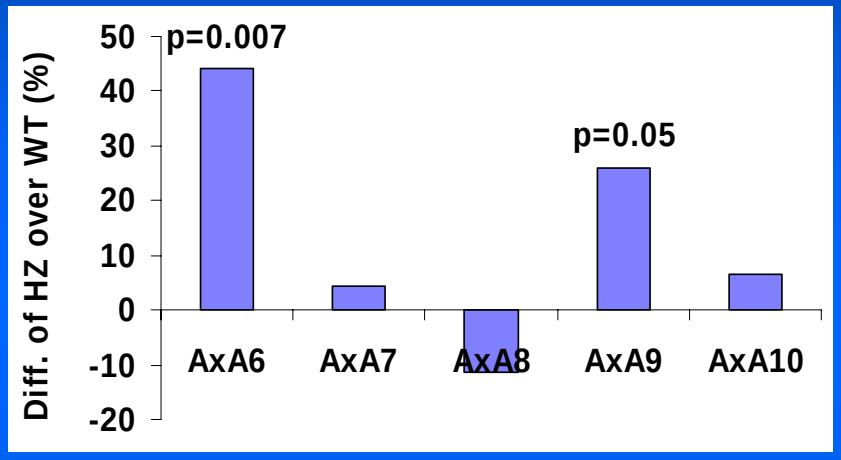
Difference Among Full-sib Families in Genetic Background A-S3 and A-S1

Wood density (kg/m3)

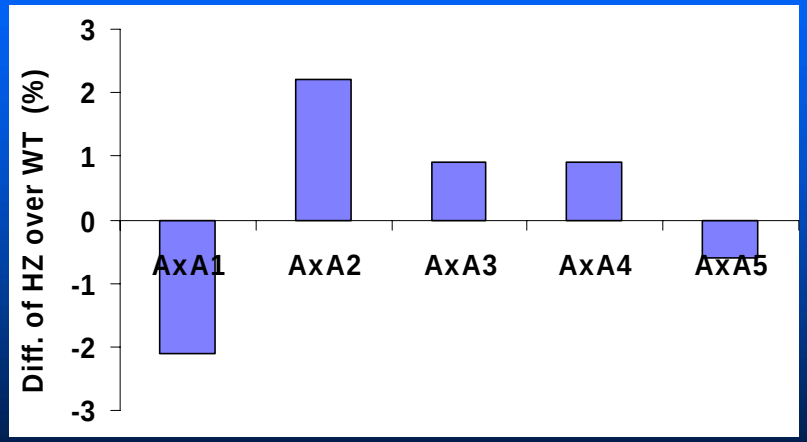
Volume (m3)

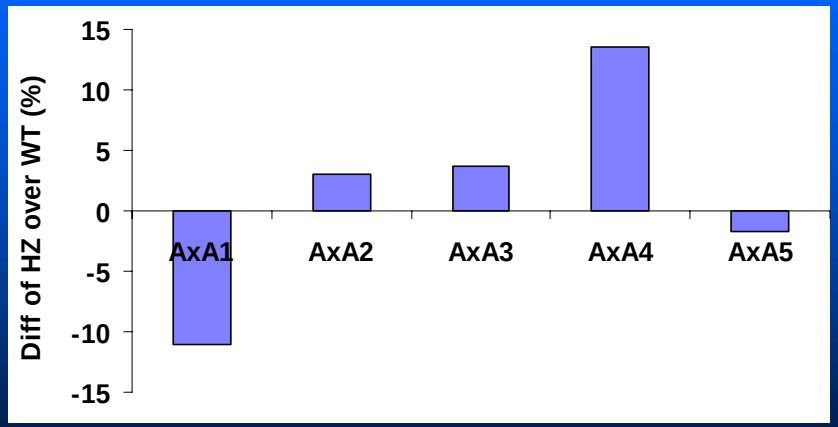
A-S3





A_S1



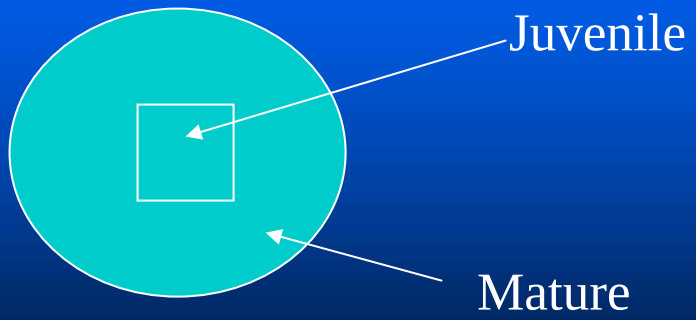


Effects of Genotype and Background

- Cad-n1 effect may associate with increasing growth and wood density, but depending on genetic backgrounds and specific full-sib families
- A epistatic and pleiotropic effect appear to be involved.
- No strong interactions of age x genotype, site x genotype, and genetic background x genotype

Pulping Behavior

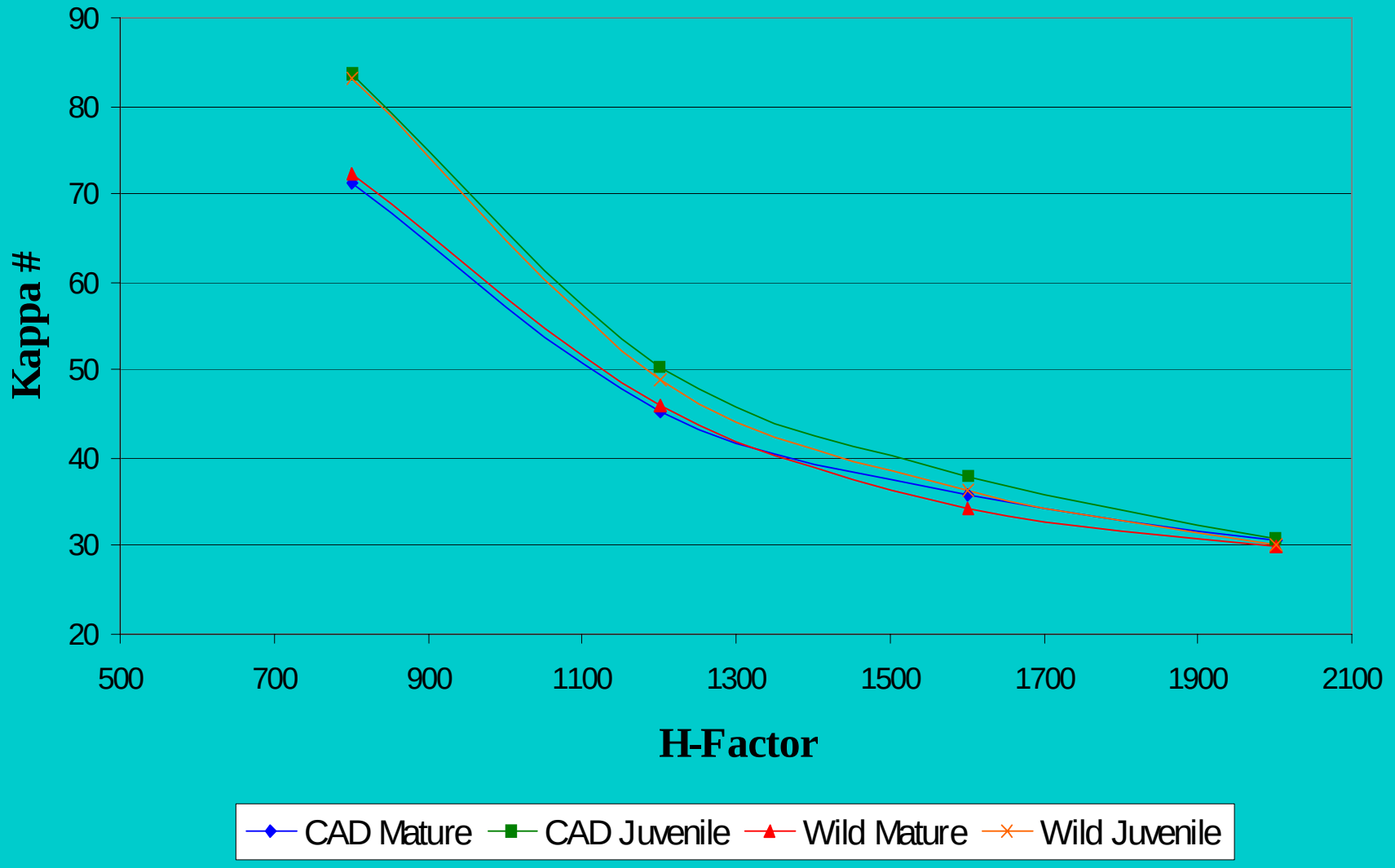
- Ten trees from each group were randomly sampled
  - CAD
  - Wild
- The trees were cut to separate the juvenile (0-12 years) from the mature wood (15+ years)
  - CAD Mature
  - CAD Juvenile
  - Wild Mature
  - Wild Juvenile



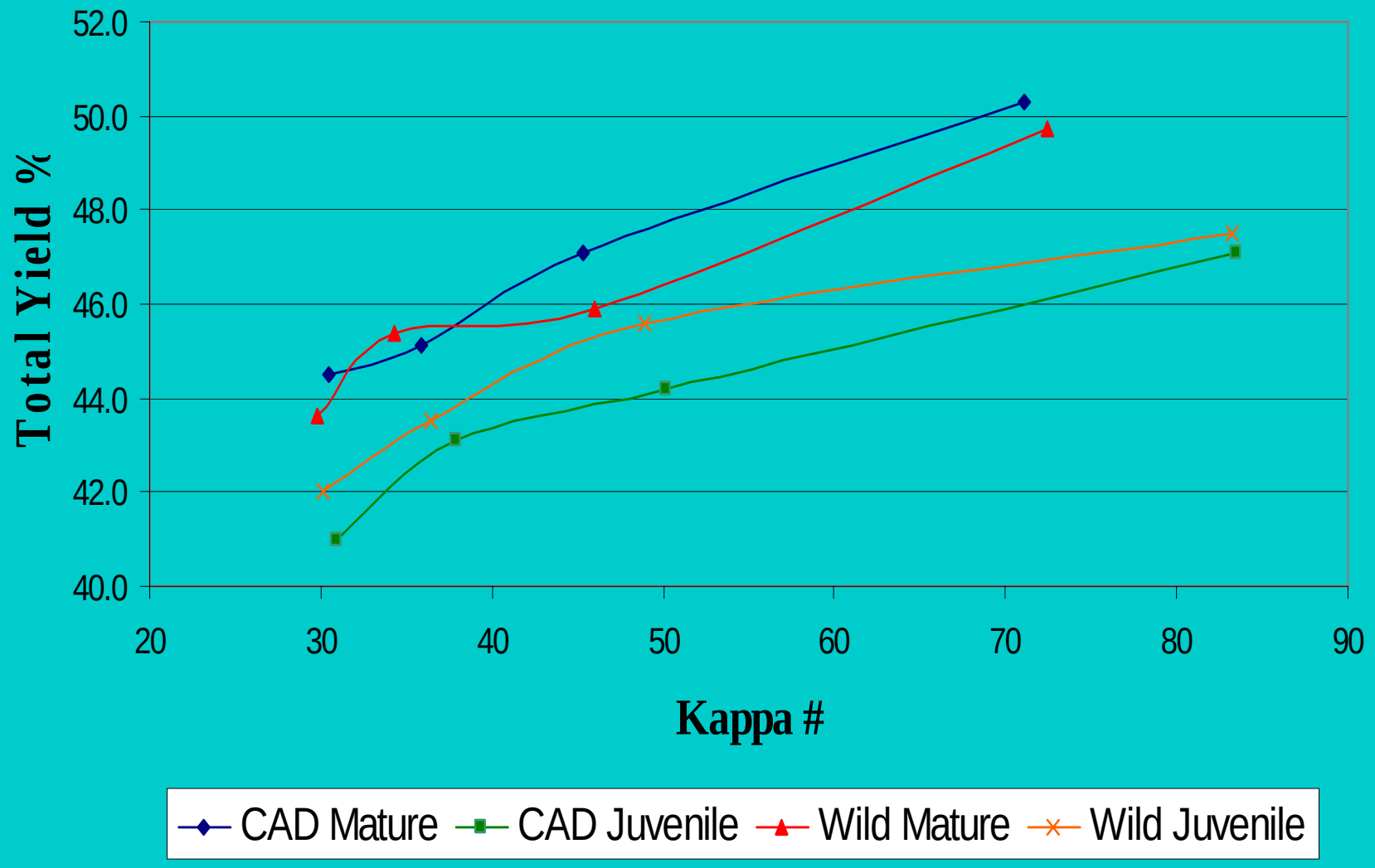
Pulping Behavior

- Chips were screened
  - Accepts: less than 1”, greater than 3/8”
- Pulping Conditions
  - Active alkali = 19%
  - Sulfidity = 25%
  - Cooking temperature = 170°C
  - H Factor = 800-2000
- All cooks were done in duplicate

Kappa Vs. H Factor



Total Yield vs. Kappa



Pulping Results

- The difference in pulping rate between the CAD and wild samples was very small for both the mature and juvenile wood
- The mature wood was easier to pulp for both the CAD and Wild
  - The difference was greater than 10 kappa at the low H Factor
  - At high H Factors the difference was very small

Yield Results

- The mature wood had a higher yield than the juvenile wood
  - Mature CAD – Juvenile CAD =2.9% yield difference
  - Mature Wild – Juvenile Wild =1.5% yield difference
- The difference in yield between CAD and Wild not as conclusive
  - Mature CAD – Mature Wild =0.6% yield difference
  - Juvenile CAD – Juvenile Wild = -0.8% yield difference

Yield Results

- The difference in yield between mature CAD and Wild was further evaluated at high kappa numbers

	%AA	Sulfidity, %	H Factor	Kappa	Total Yield, %
CAD Mature	15	25	900	99.3	55.3
Wild Mature	15	25	900	101.9	54.6

* Average of three pulping trials

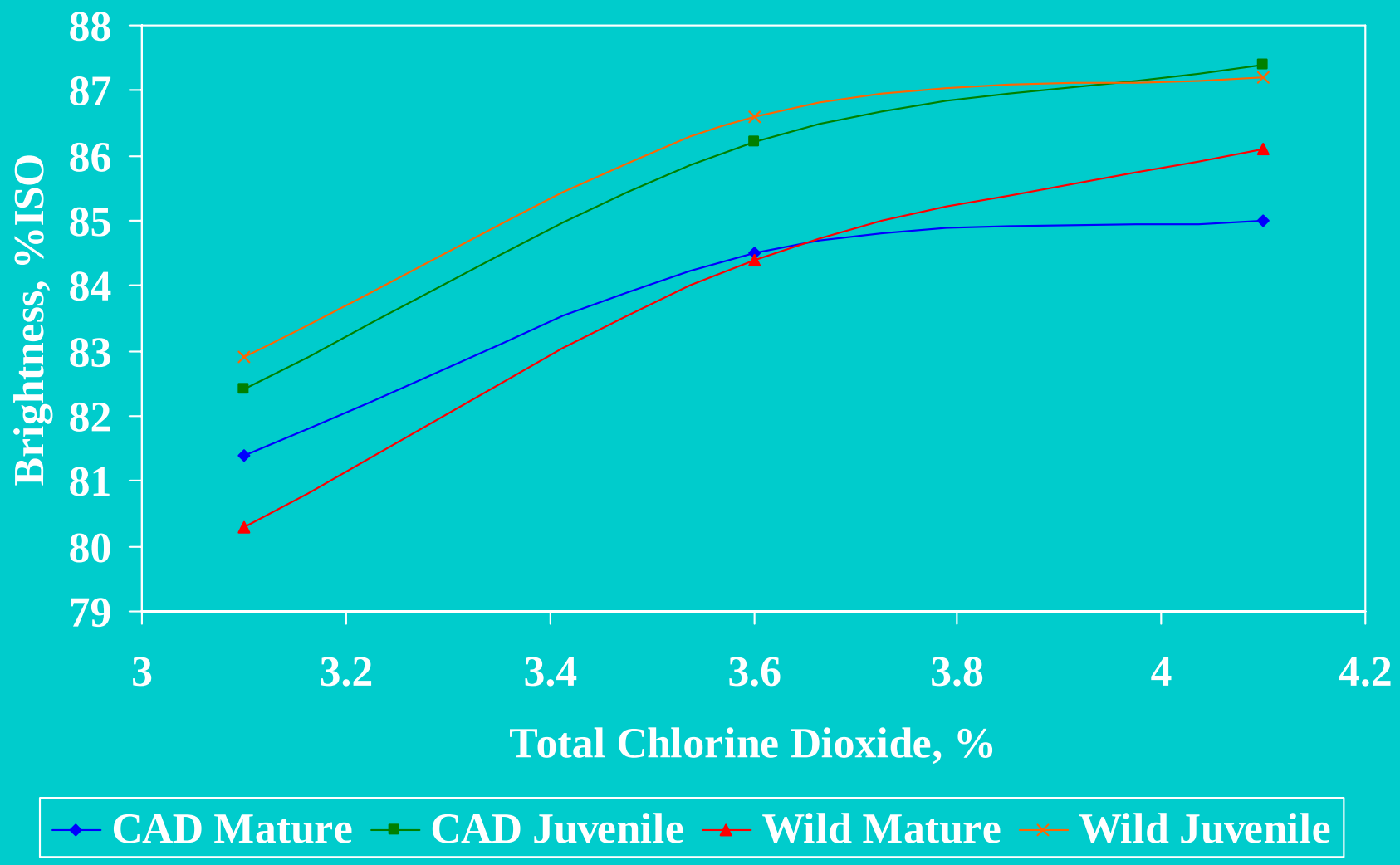
- There is no difference in kappa number, but a slightly higher yield was measured with the CAD mature as compared to Wild Mature

Bleachability and Strength Properties

- Pulps with ~30 kappa were bleached and the strength properties measured
  - $D_o(EP)D_1$ bleaching sequence
    - » Kappa factor = .225
    - » Three levels of ClO_2 in D_1 stage
- The mid points pulps were refined and the strength properties measured

Brightness Response of Pulps

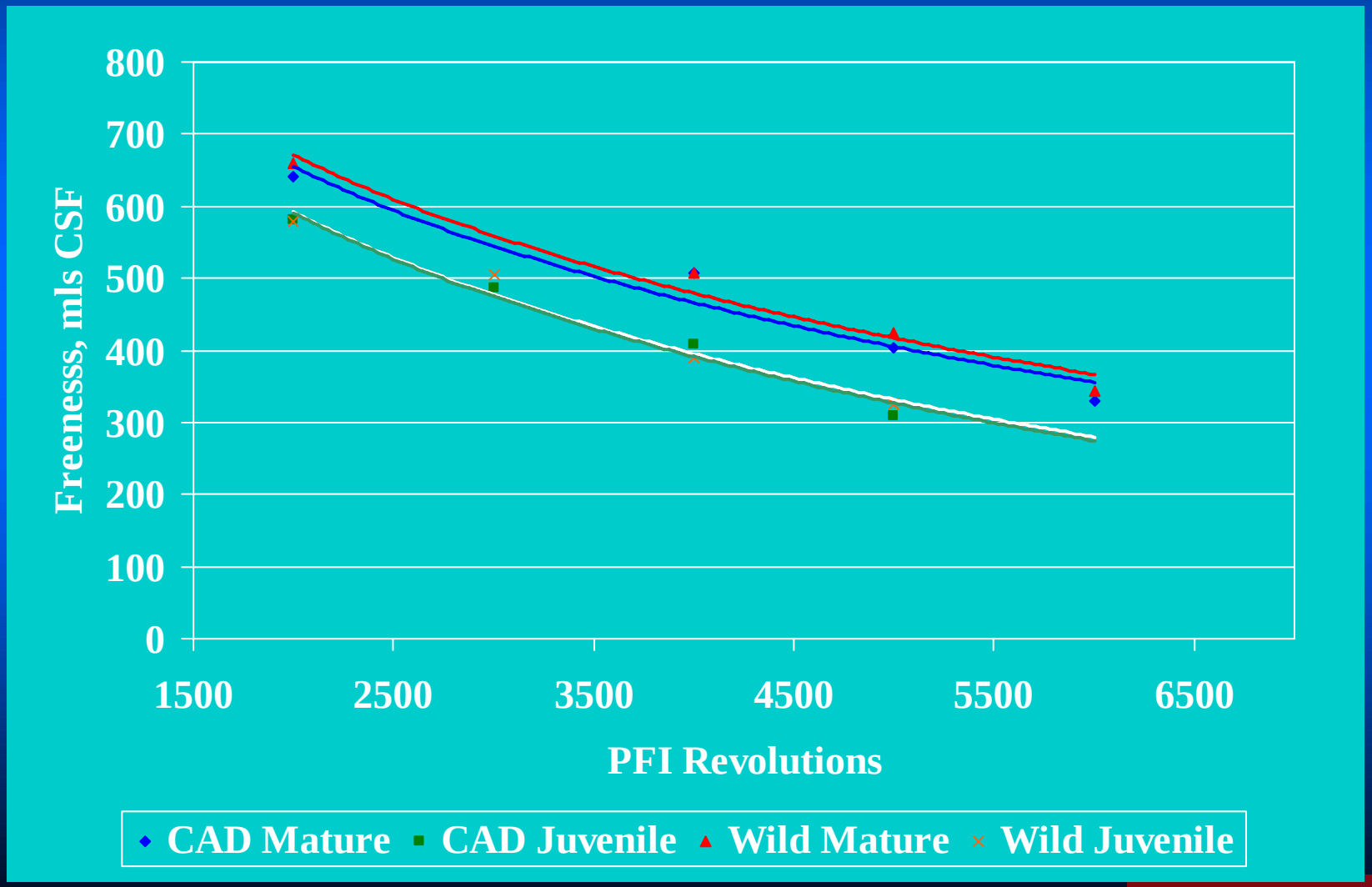
D(EP)D Sequence



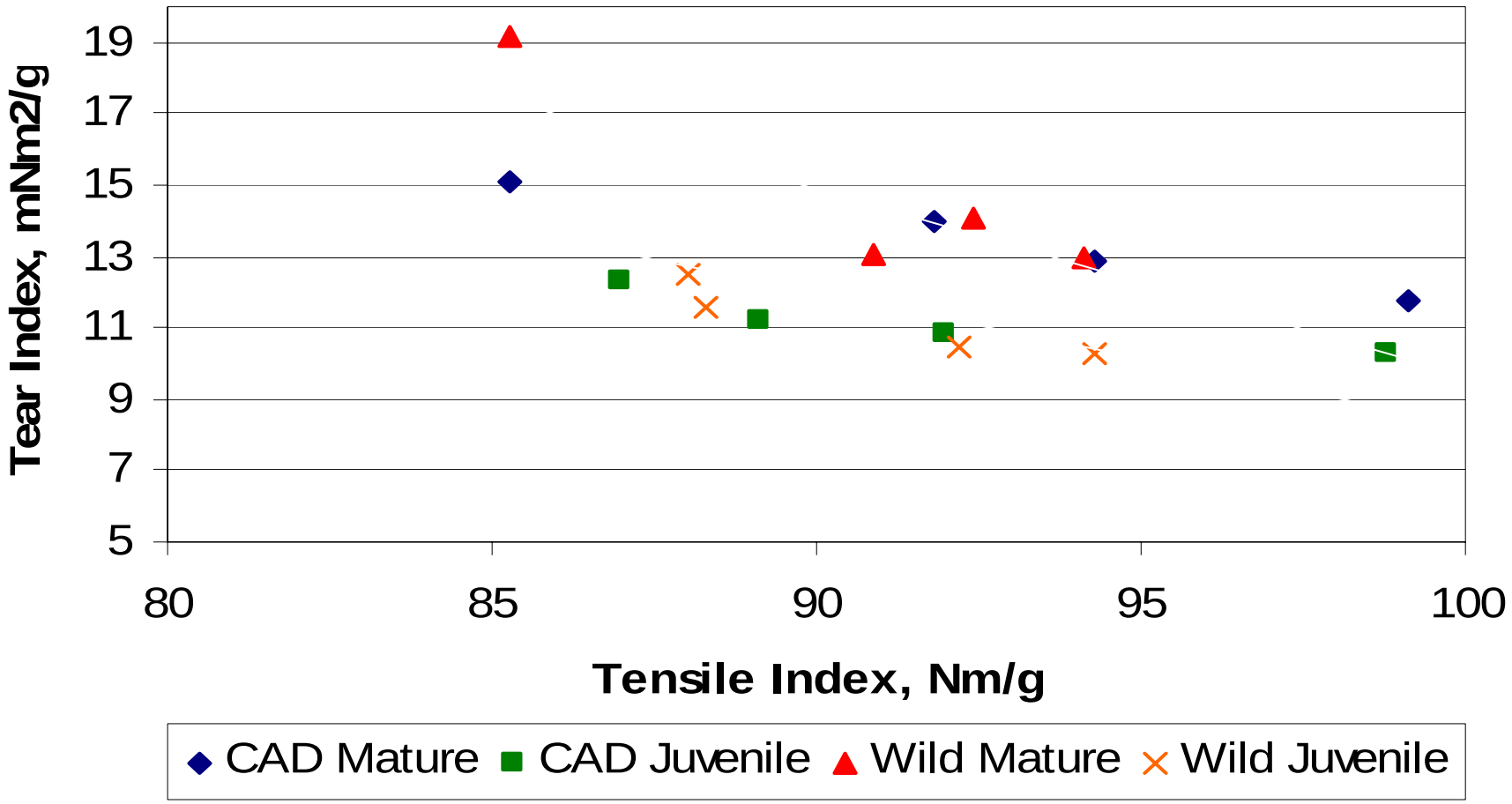
Bleachability

- Difference in bleachability between CAD and wild samples was insignificant
- The juvenile wood was easier to bleach for both the wild and CAD samples
  - There was a 1.5-2% ISO brightness difference

Refining Response of Bleached Pulps



Strength Properties of Bleached Pulps



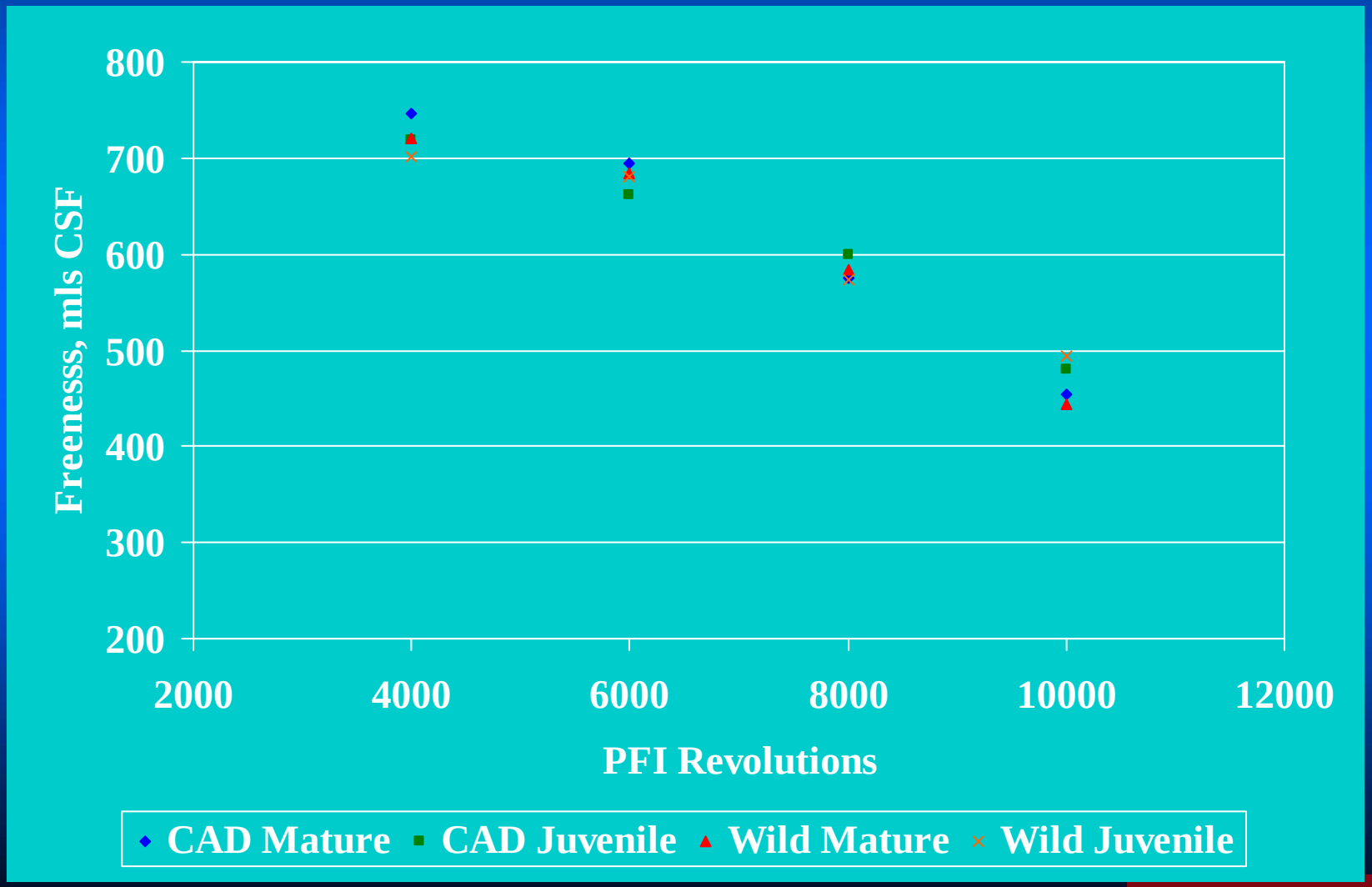
Bleached Paper Strength Properties

- No difference in refining energy required between CAD and wild samples
- The juvenile wood required less energy to refine
- No significant difference in tear-tensile relationship between CAD and Wild
- The mature wood had about ~15% higher tear at equivalent tensile

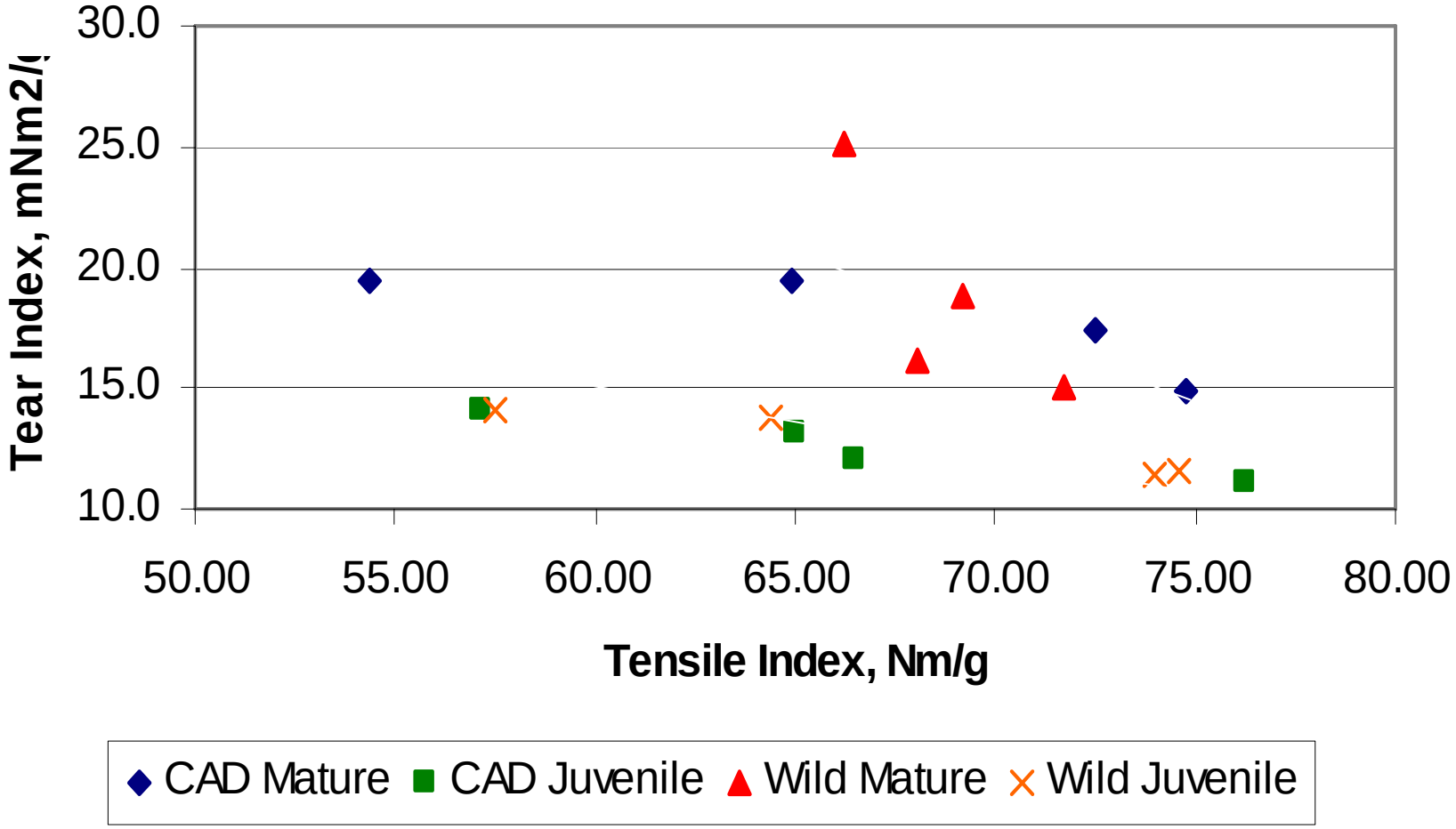
Linerboard Properties

- Pulps with a kappa number of 100 were refined and tested for strength properties

Refining Response of Linerboard Pulps



Strength Properties of Linerboard Pulps



Linerboard Properties

- The refining energy required for all the pulps were comparable
- No significant difference between the CAD and wild samples as measured by tear vs tensile
- The mature samples had a ~20% higher tear at the same tensile

Summary

- Pulping
  - No difference in pulping rate between the CAD and wild samples
  - Mature wood was easier to pulp for both the CAD and Wild
  - Mature CAD had a slightly higher yield at high kappa numbers
  - Mature wood had a ~2% higher yield than juvenile wood
  - No difference in bleachabilty between CAD and wild
  - Juvenile wood was easier to bleach than mature wood

Summary

- Strength properties
  - Strength properties were compared for bleached and linerboard grade pulps
  - No significant difference in tear-tensile relationship between CAD and Wild
  - The mature wood had about 15-20% higher tear at equivalent tensile

PULPING

28-year-old trees

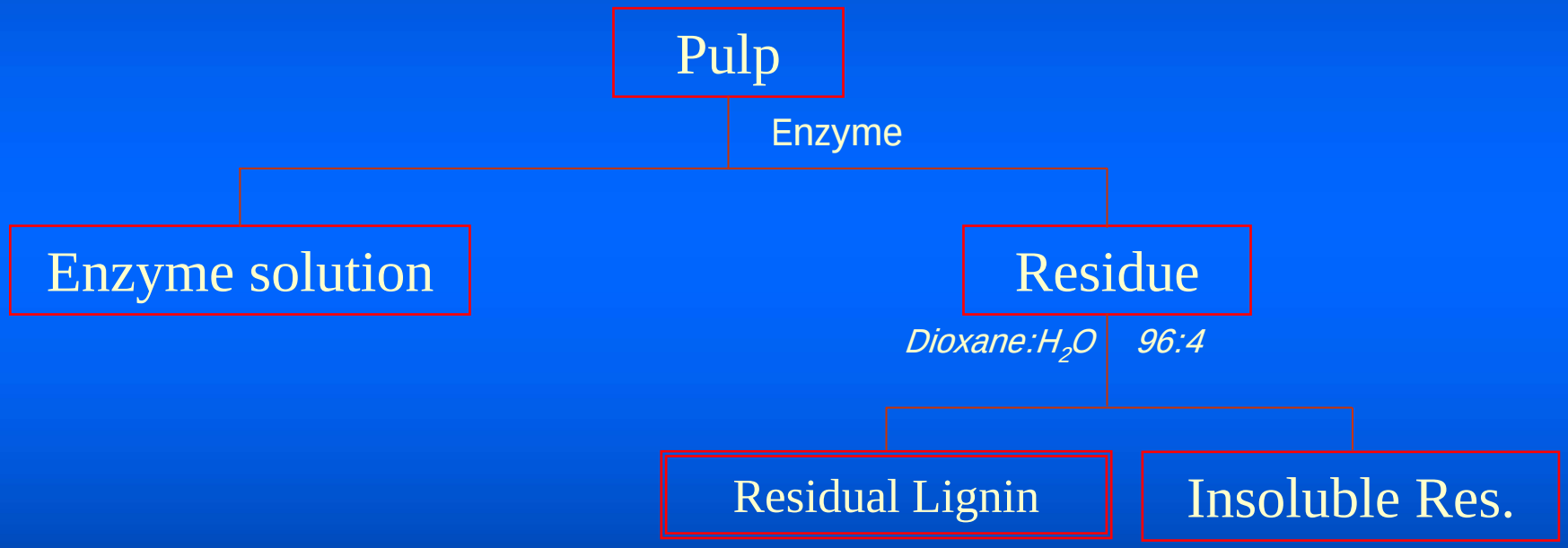
- Wood Samples:
  - Combined wood samples from 20 Trees: 10 CAD and 10 Wild type
    - Mature Wood: Rings 15-28 Yr
    - Juvenile Wood: Rings <12 Yr
- Pulping conditions
  - 19% AA;
  - 4:1 Liquor-to-wood ratio;
  - 170°C;
  - 25% sulfidity

Lignin Samples

kraft lignin (dissolved lignin)

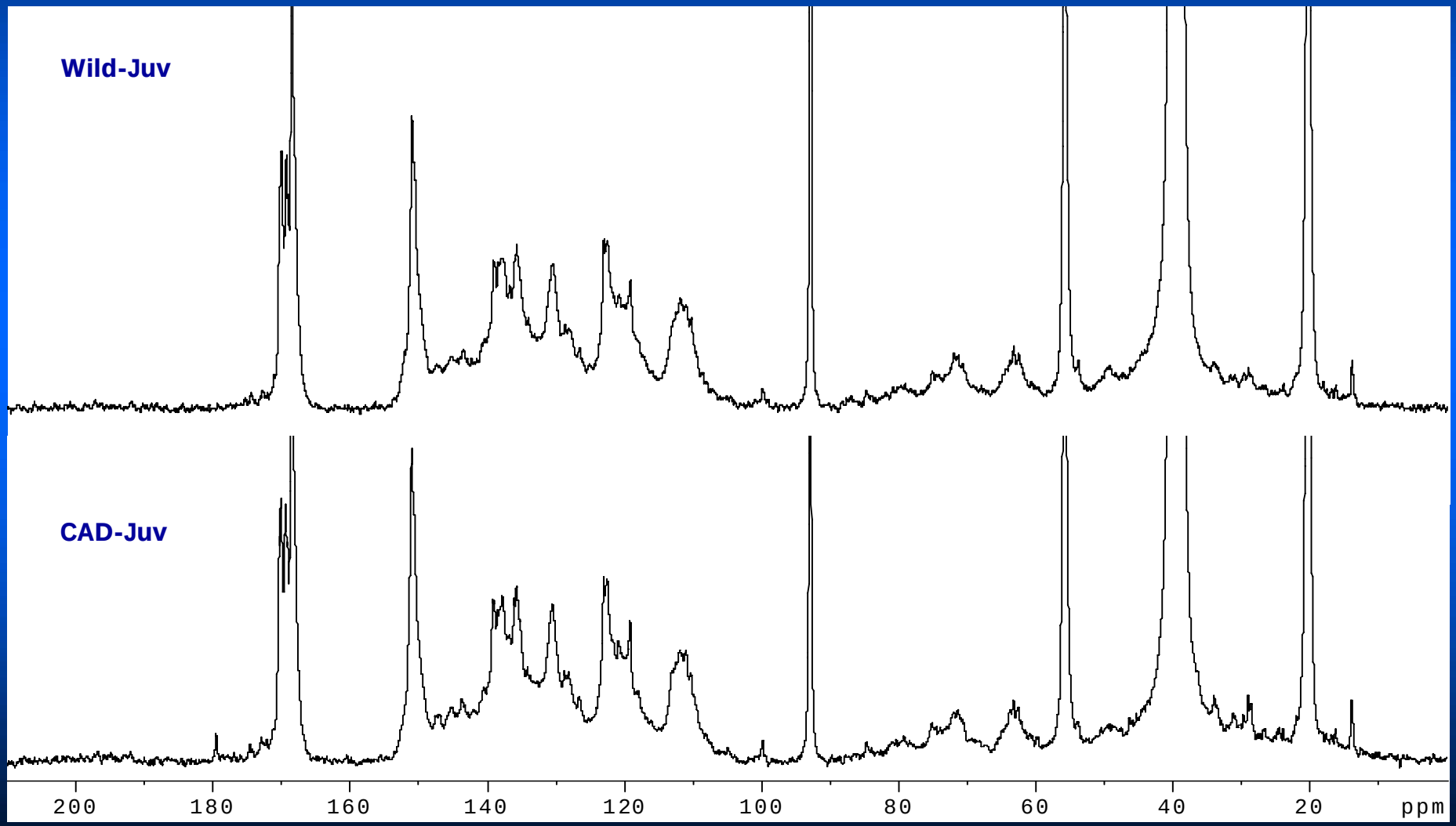
Residual lignin from pulps

ISOLATION OF RESIDUAL LIGNIN



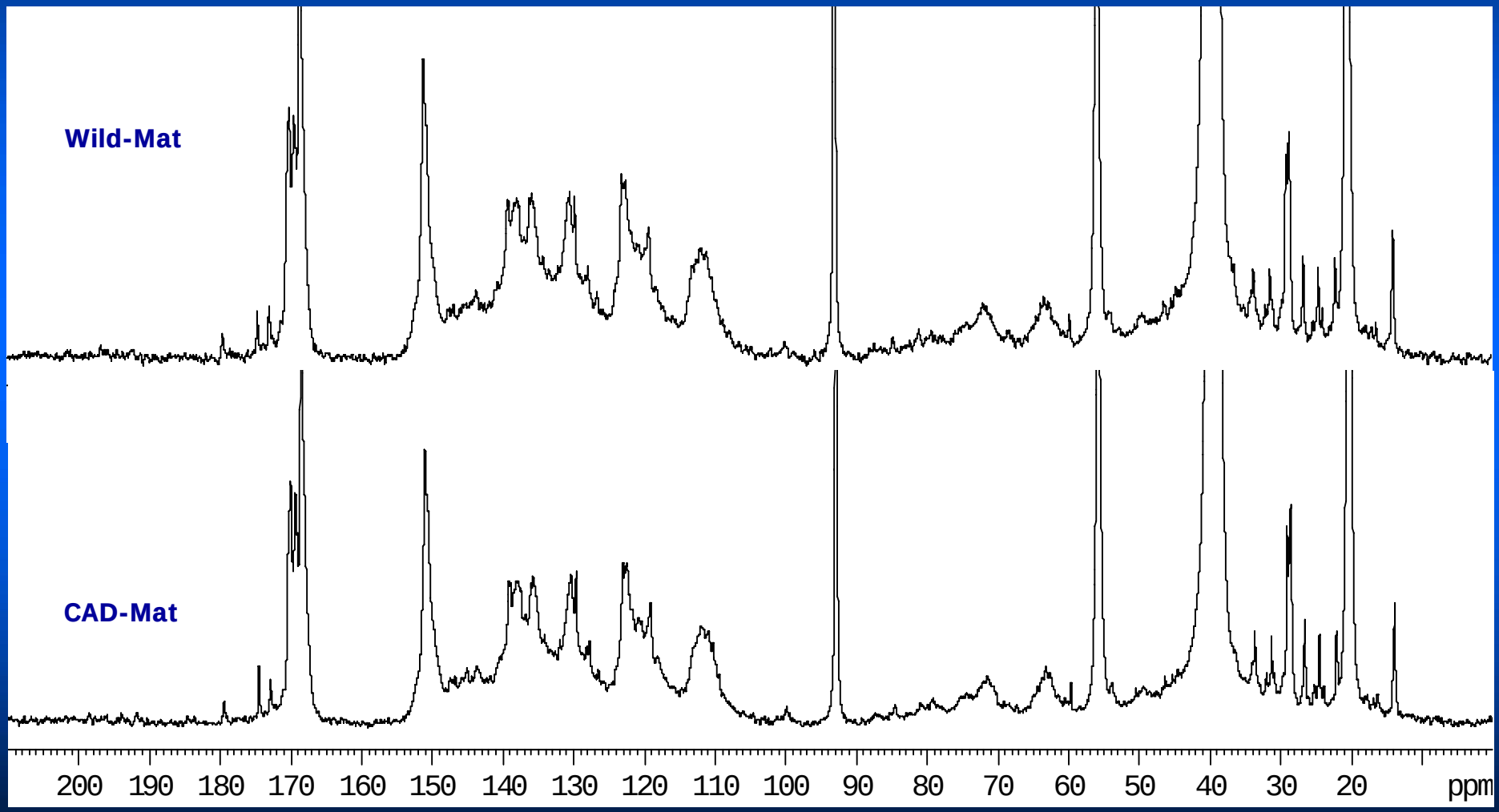
QUANTITATIVE ^{13}C NMR SPECTRUM

KRAFT LIGNIN



QUANTITATIVE 13C NMR SPECTRUM

KRAFT LIGNIN



DISSOLVED LIGNIN STRUCTURAL CHARACTERIZATION

Moieites	Integral Value (per 100 AR)			
	CAD-Juv	Wild-Juv	CAD-Mat	Wild-Mat
Total CO	9	6	11	10
Conjugated CO	6	3	6	5
Non-Conjugated CO	3	3	5	5
Total COOH	20	19	27	23
Aliphatic COOH	19	18	24	22
Aromatic COOH	1	1	3	1
Total OH	123	120	121	119
Aliphatic OH	51	49	50	49
Primary	26	23	27	24
Secondary	25	26	23	25
Phenolic OH	72	71	71	70
H-units total	4	4	5	4
H-units etherified	2	1	1	1
Ar-H	217	220	216	212
OMe	82	81	80	79
Oxygen. aliphatic (90-58 ppm)	103	105	102	106
90-77 ppm	23	24	23	22
77-66 ppm	46	46	44	47
66-58 ppm	34	35	35	37
β-5	2	3	3	4
β-β	4	4	3	3
β-O-4	3	2	3	3
Saturated aliphatic	101	88	148	149
Sugars	5	5	5	5

RESIDUAL LIGNIN STRUCTURAL CHARACTERIZATION

Moieites	Integral Value (per 100 AR)			
	CAD-Juv	Wild-Juv	CAD-Mat	Wild-Mat
Total CO	11	12	11	11
Conjugated CO	7	8	6	7
Non-Conjugated CO	4	4	5	4
Total COOH	38	36	48	42
Aliphatic COOH	36	33	46	40
Aromatic COOH	2	3	2	2
H-units total	7	5	6	7
Ar-H	196	210	199	195
OMe	97	100	101	98
Oxygen. aliph.	175	175	179	161
90-77	33	33	34	31
77-66	98	98	101	89
66-58	44	44	44	41
Saturated aliphatic (35-10 ppm)	86	74	86	100
Sugars	11	11	12	13

CONCLUSIONS

- No significant differences have been detected between lignins (dissolved and residual) isolated after pulping of composed wood samples of CAD- and Wild type.
- There are some differences between lignins isolated after pulping of juvenile and mature wood (both CAD and Wild type).

  Mature lignins have slightly higher amounts of COOH groups and significantly higher amounts of saturated aliphatic structures than the corresponding juvenile lignins (especially in the case of dissolved lignin).