

TSNA Accumulation in Two Burley Cultivars

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Coresta 2011

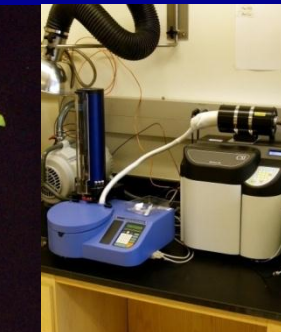
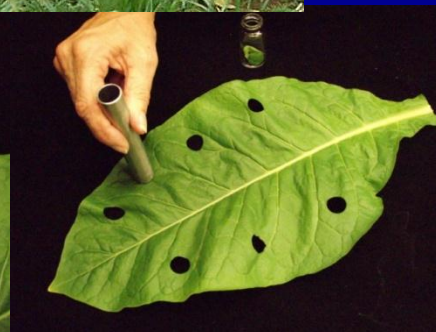
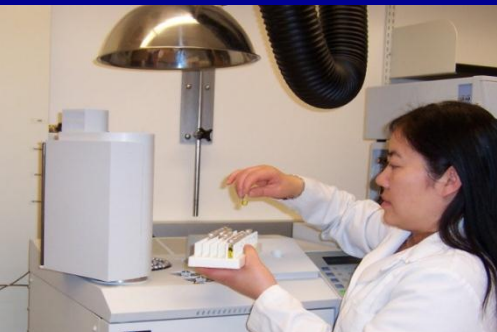
Background

- **TSNA differences reported between varieties with:**
 - similar conversion
 - similar total alkaloids
- **Literature**
 - speculates differential drying rate during curing
- **Previous work (Miller)**
 - TSNA_s
 - KT 204 < NCBH 129



Objective

- Compare these varieties
- Identify factors causing differential TSNA accumulation



Procedure



Procedure

- **2 varieties**
 - **KT 204**
 - **NCBH 129**
- **2 years**
 - **2009**
 - **2010**

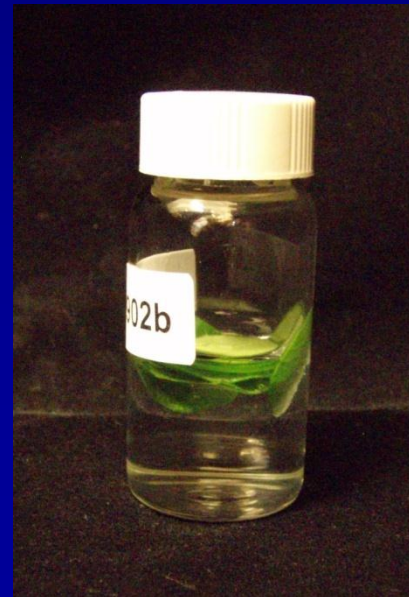


- **Normal production practices**
 - **Cured on railwagon, covered with plastic**
 - **dataloggers**



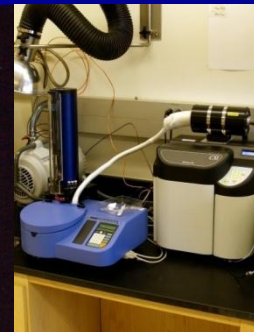
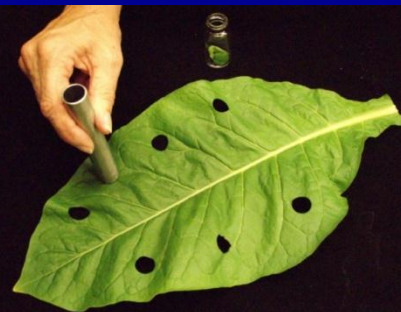
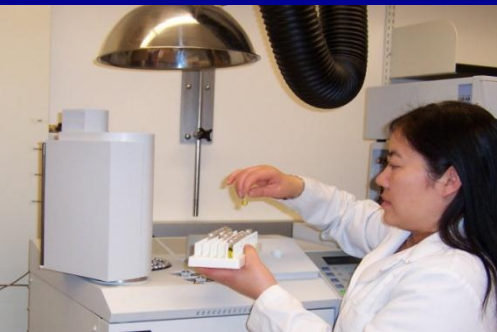
Variables

- **Sampled throughout cure:**
 - TSNAs
 - NO_2 N
 - Leaf moisture
 - Duvatrienediols (DVTs)
 - effect on leaf moisture loss



Variables cont

- **Sampled at stripping:**
 - Total alkaloids
 - Total N, NO_3N
 - Leaf thickness
 - Leaf specific weight
 - **TSNAs**
 - **NO_2N**
 - **DVTs**



Statistical Analysis

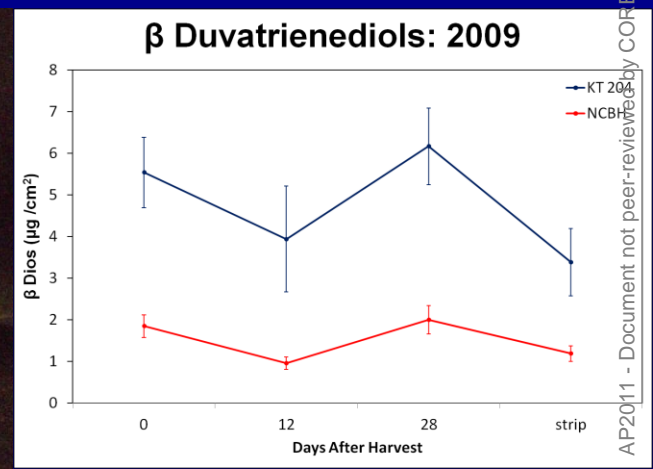
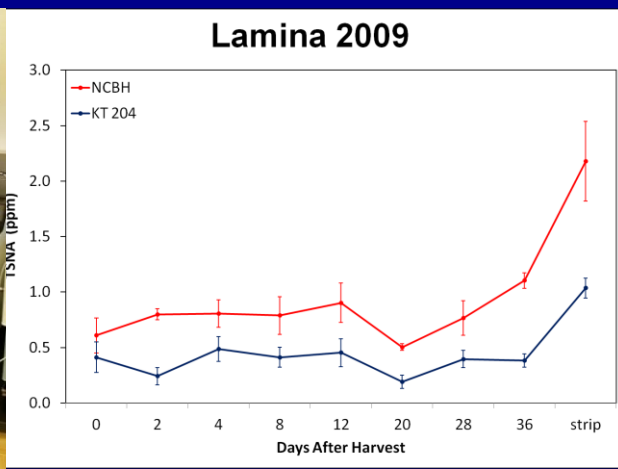
- **Time series plots**
 - samples through curing
- **ANOVA**
 - single sample variables (stripping)
 - SAS Proc Mixed
- **Verify model assumptions**
 - some variables – heteroscedasticity
 - transformations (sq rt, log, log-log, exponential) for means separation
 - untransformed data presented



Results

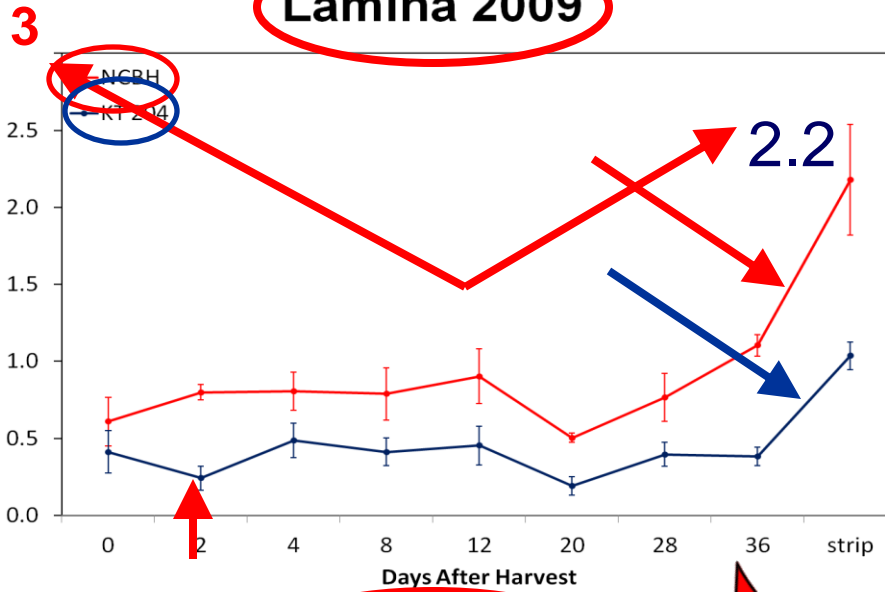


Time Series

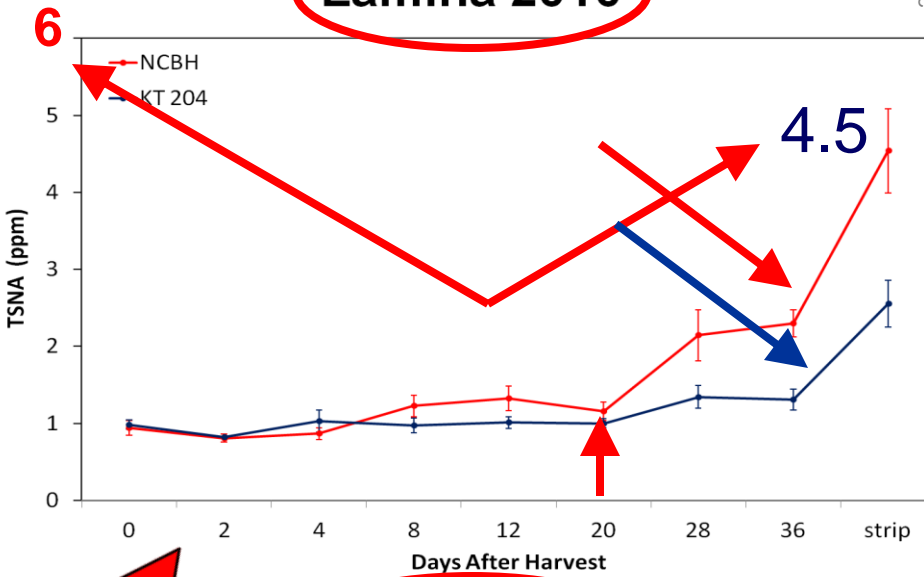


TSNAs

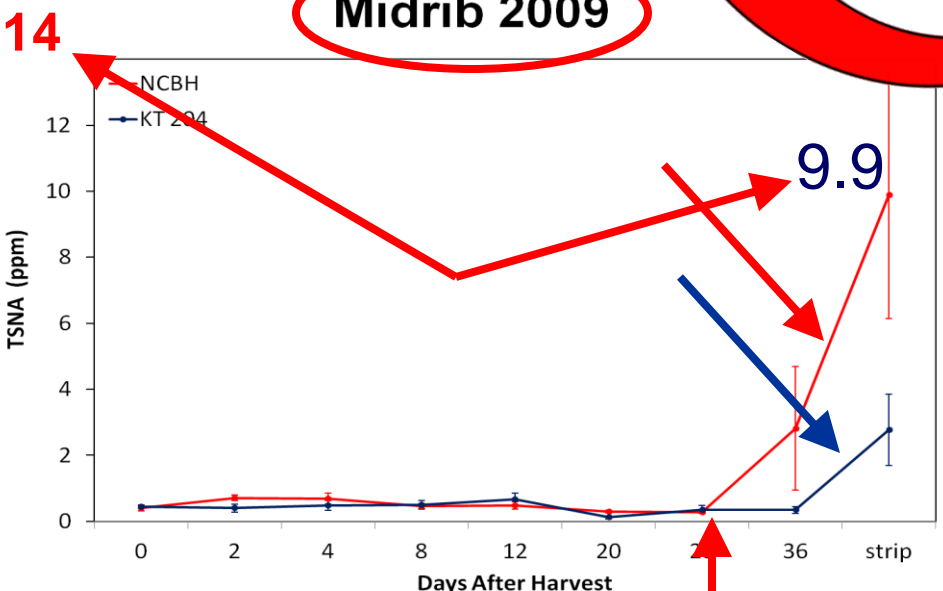
Lamina 2009



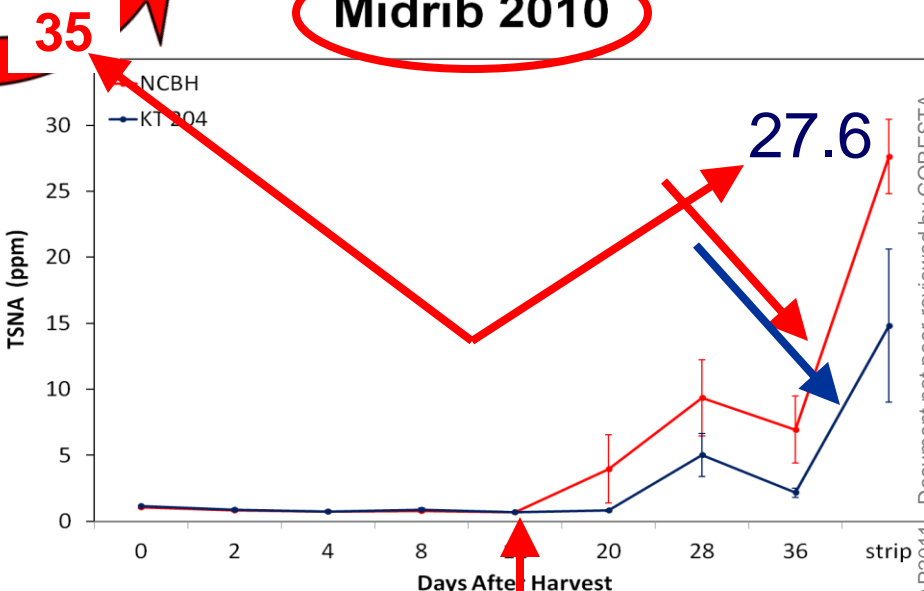
Lamina 2010



Midrib 2009

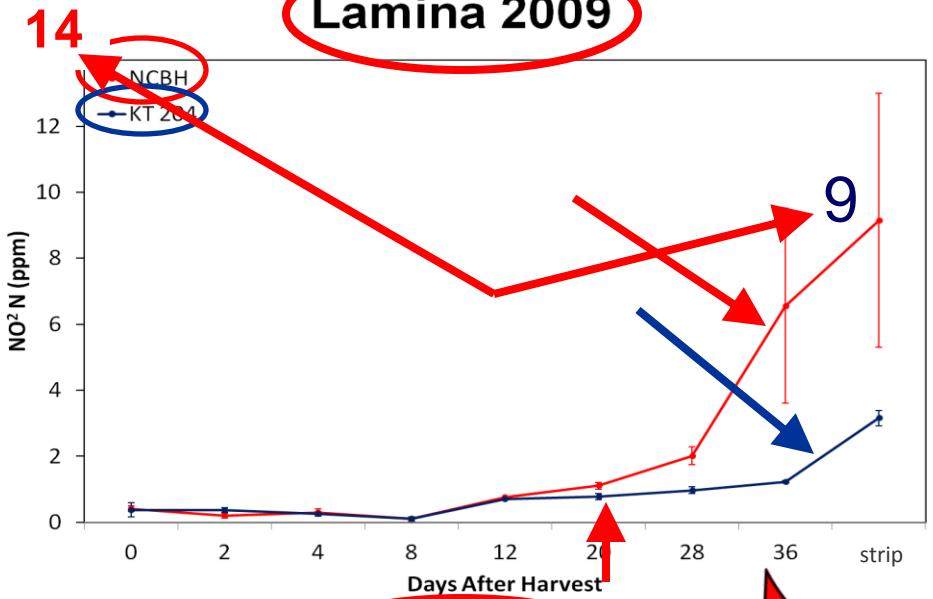


Midrib 2010

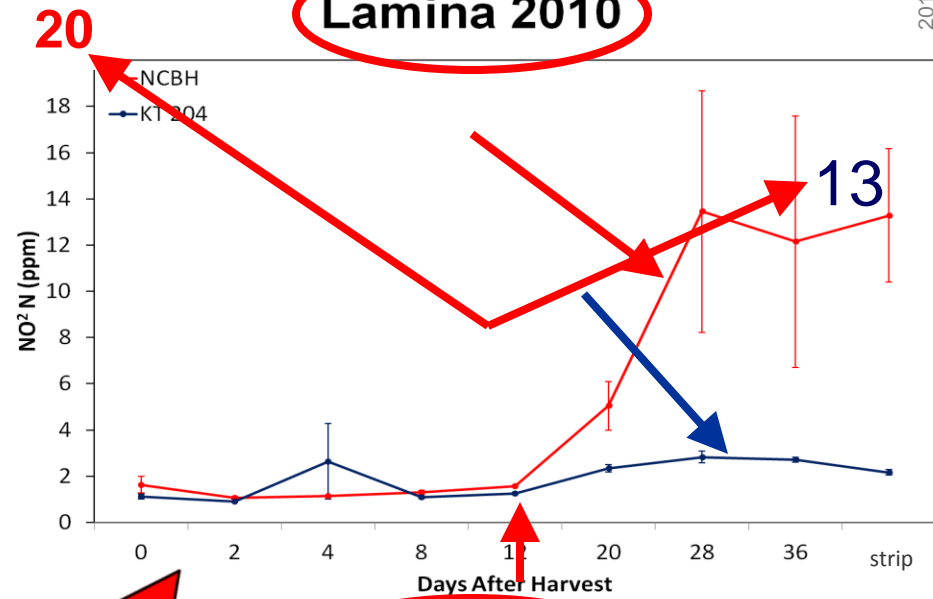


Nitrite N

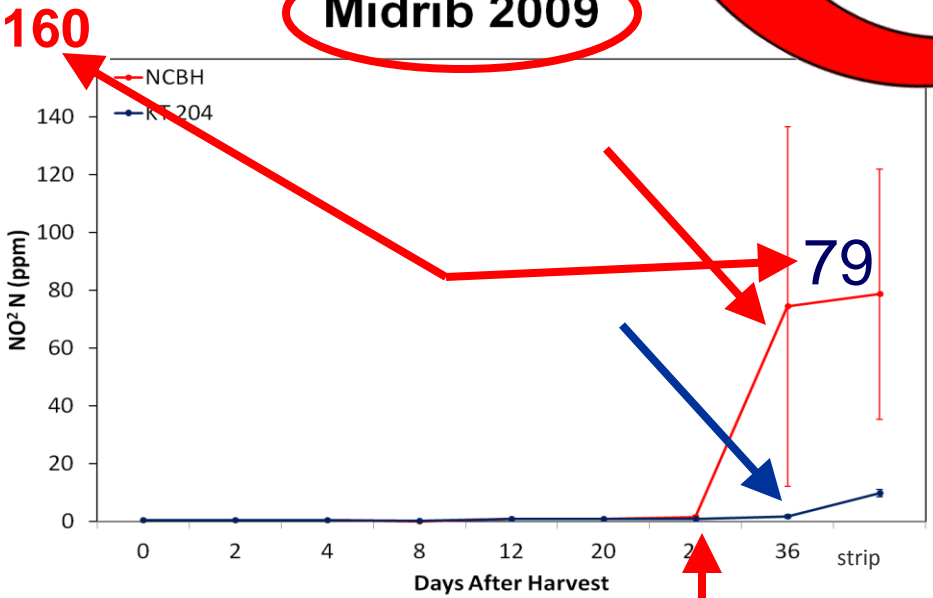
Lamina 2009



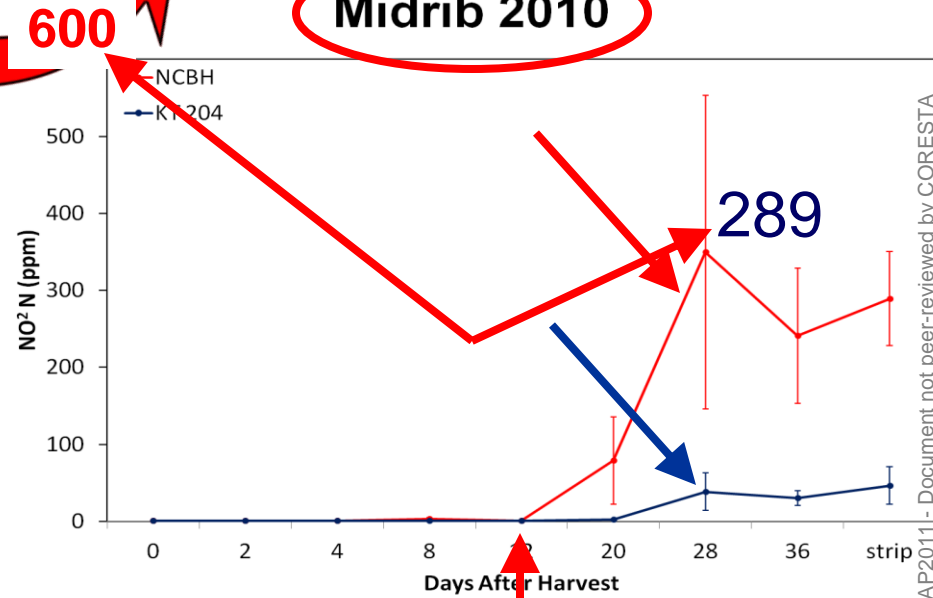
Lamina 2010



Midrib 2009

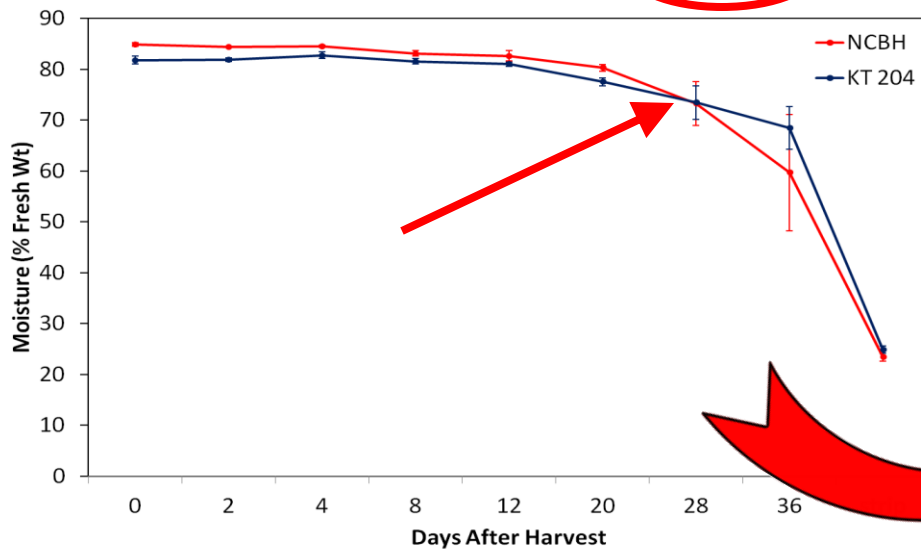


Midrib 2010

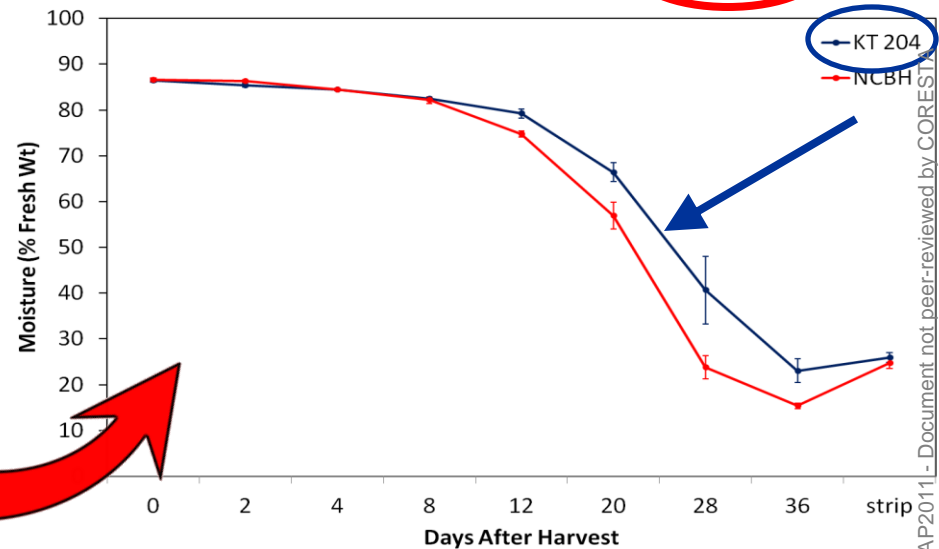


Leaf Moisture

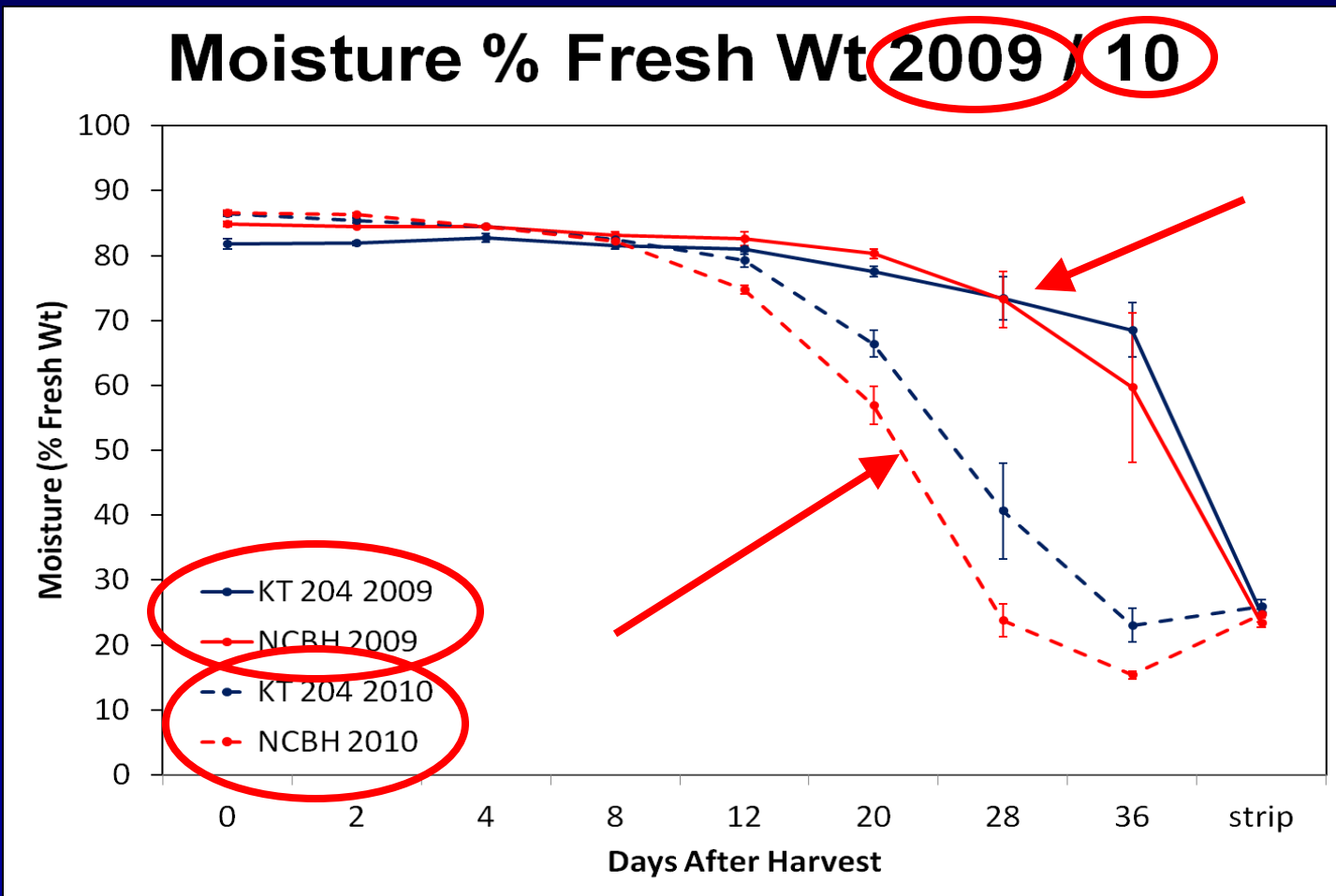
Moisture % Fresh Wt 2009



Moisture % Fresh Wt 2010

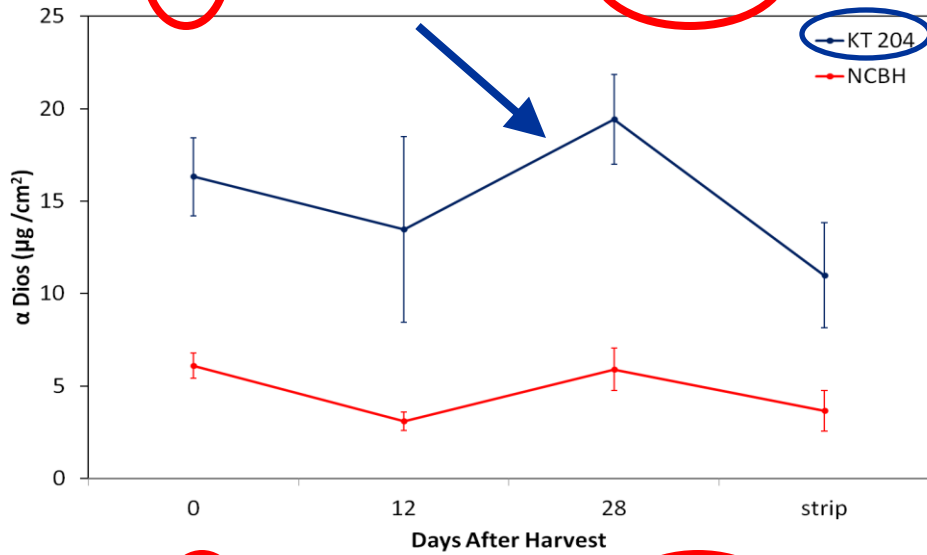


Leaf Moisture

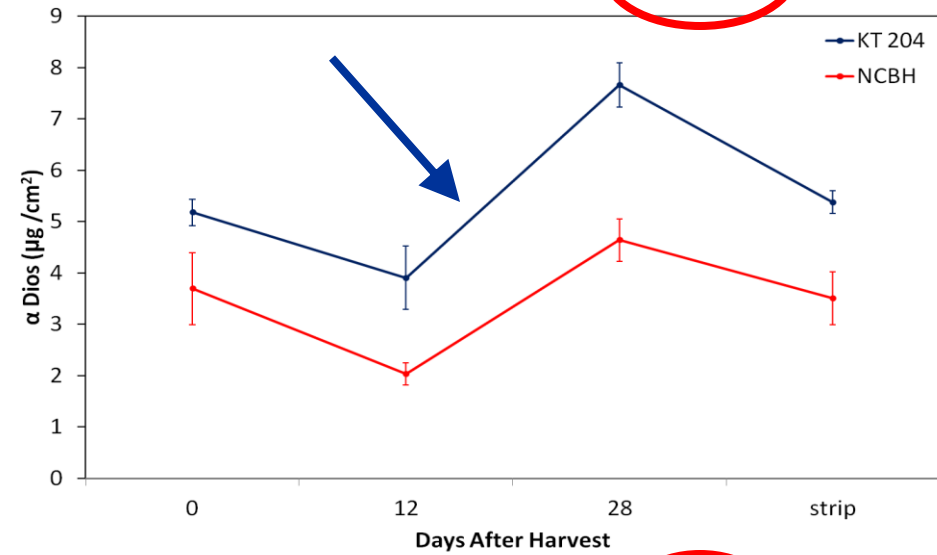


Duvatriendiols

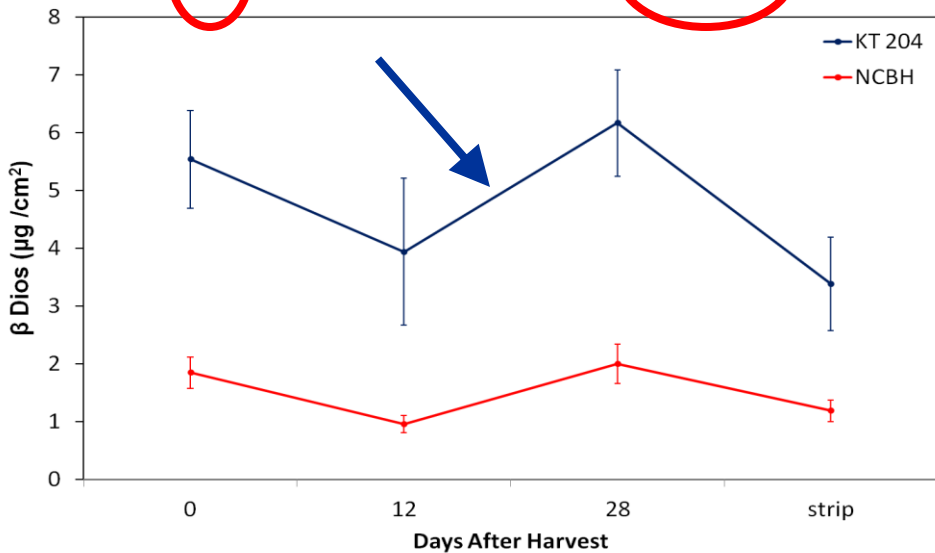
α Duvatrienediols: 2009



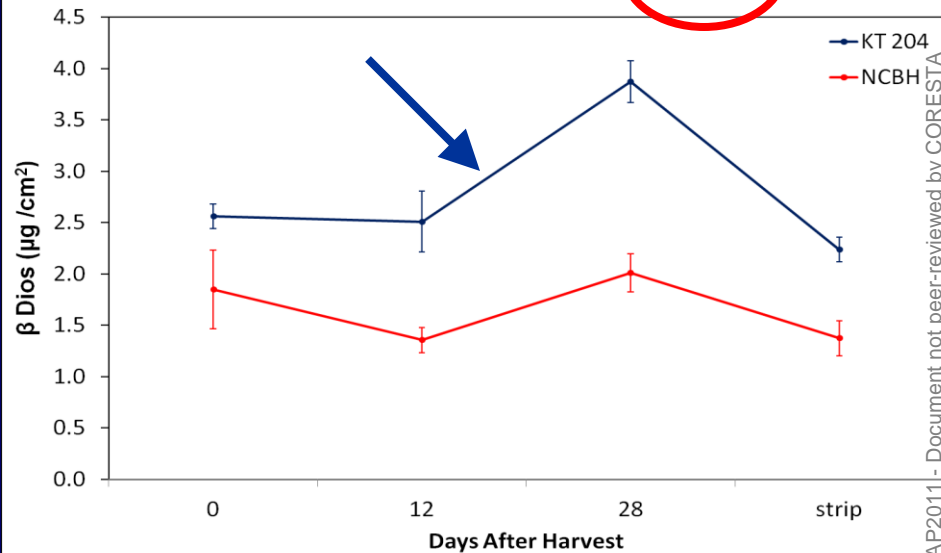
α Duvatrienediols: 2010



β Duvatrienediols: 2009

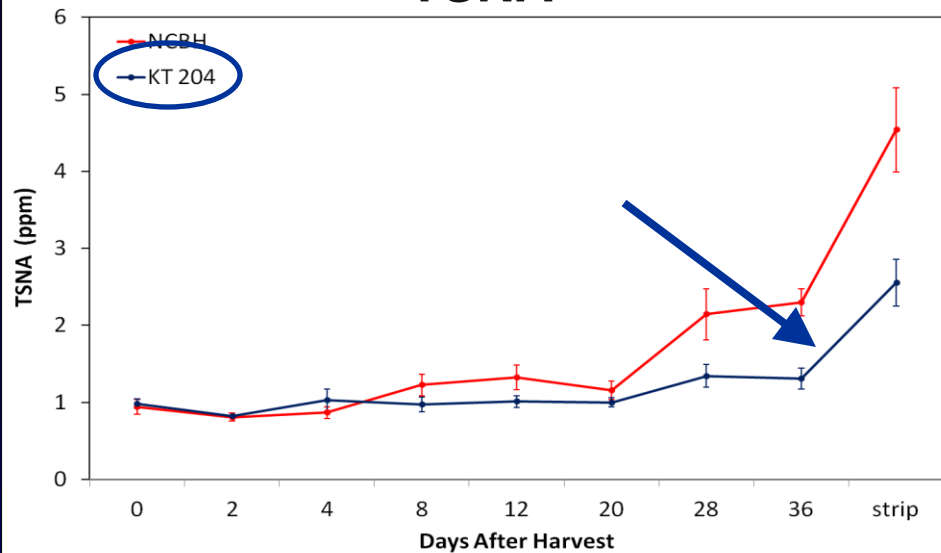


β Duvatrienediols: 2010

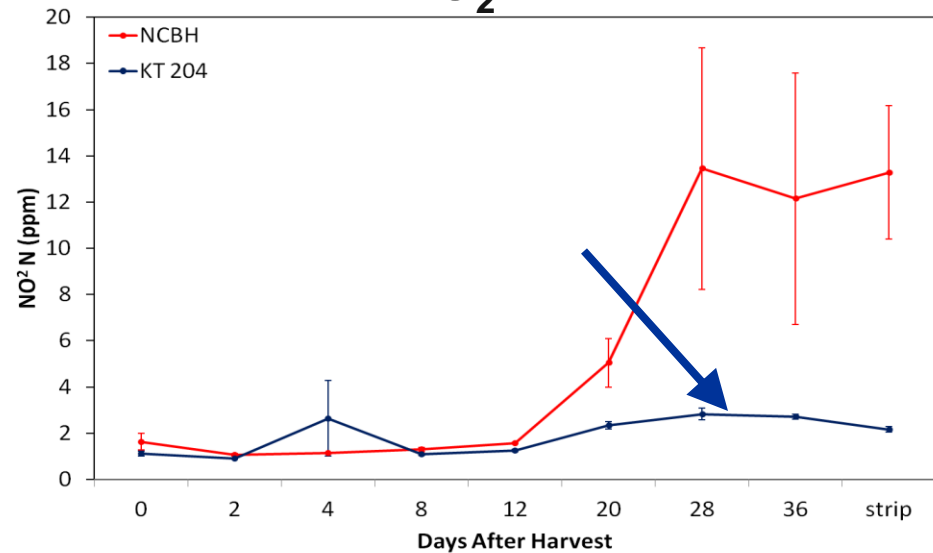


Summary – KT 204

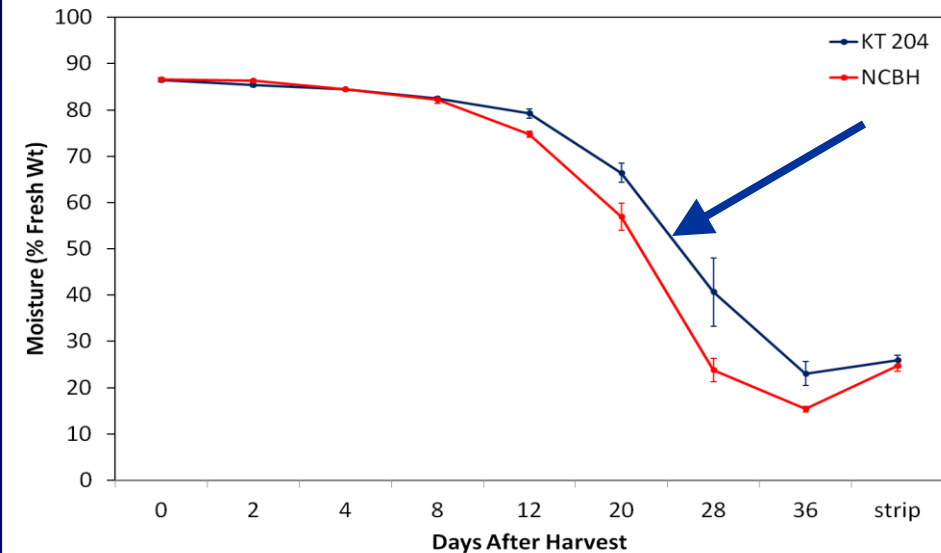
TSNA



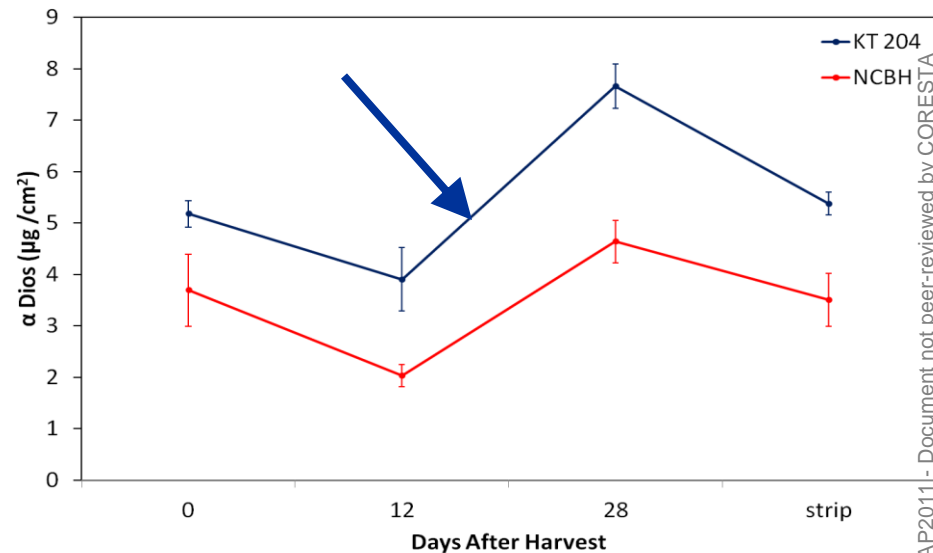
NO₂ N



Moisture % Fresh Wt



α Duvatriediols



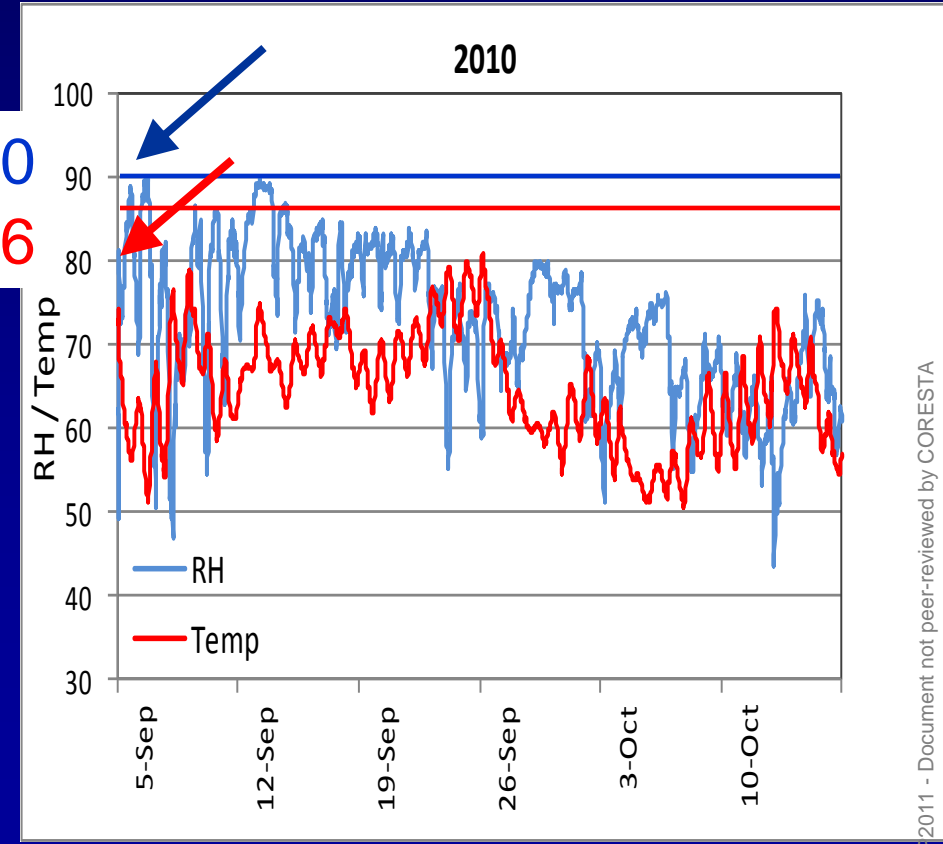
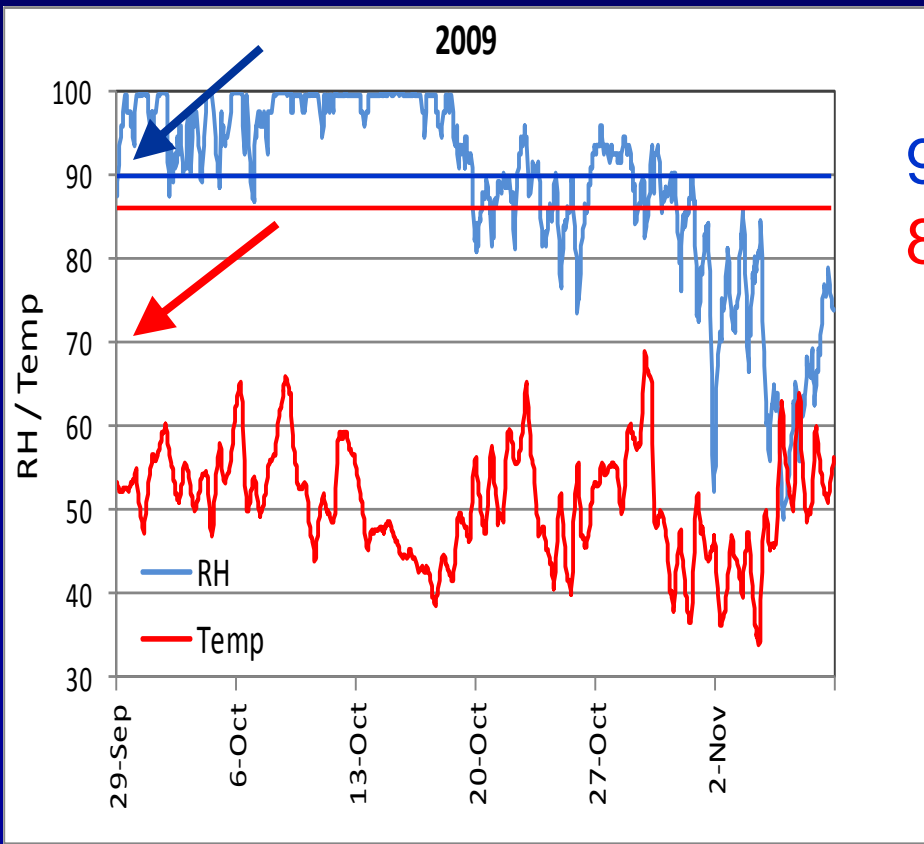


Dataloggers

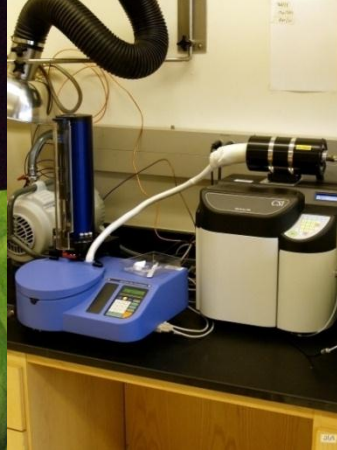
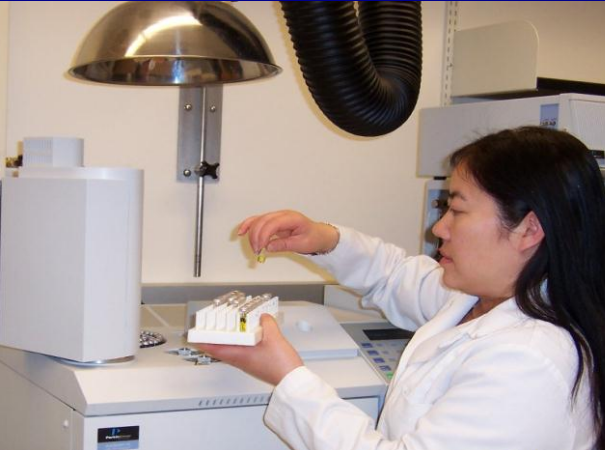


More humid
Cooler

Drier
Warmer



Samples at Stripping



TSNAs (lamina) : ANOVA

¹ loglog transformed, ² log transformed

	<u>NNN</u>	<u>NAT¹</u>	<u>TSNA²</u>
Variety	*	**	**
Year	**	***	***
Var x Yr	NS	NS	NS

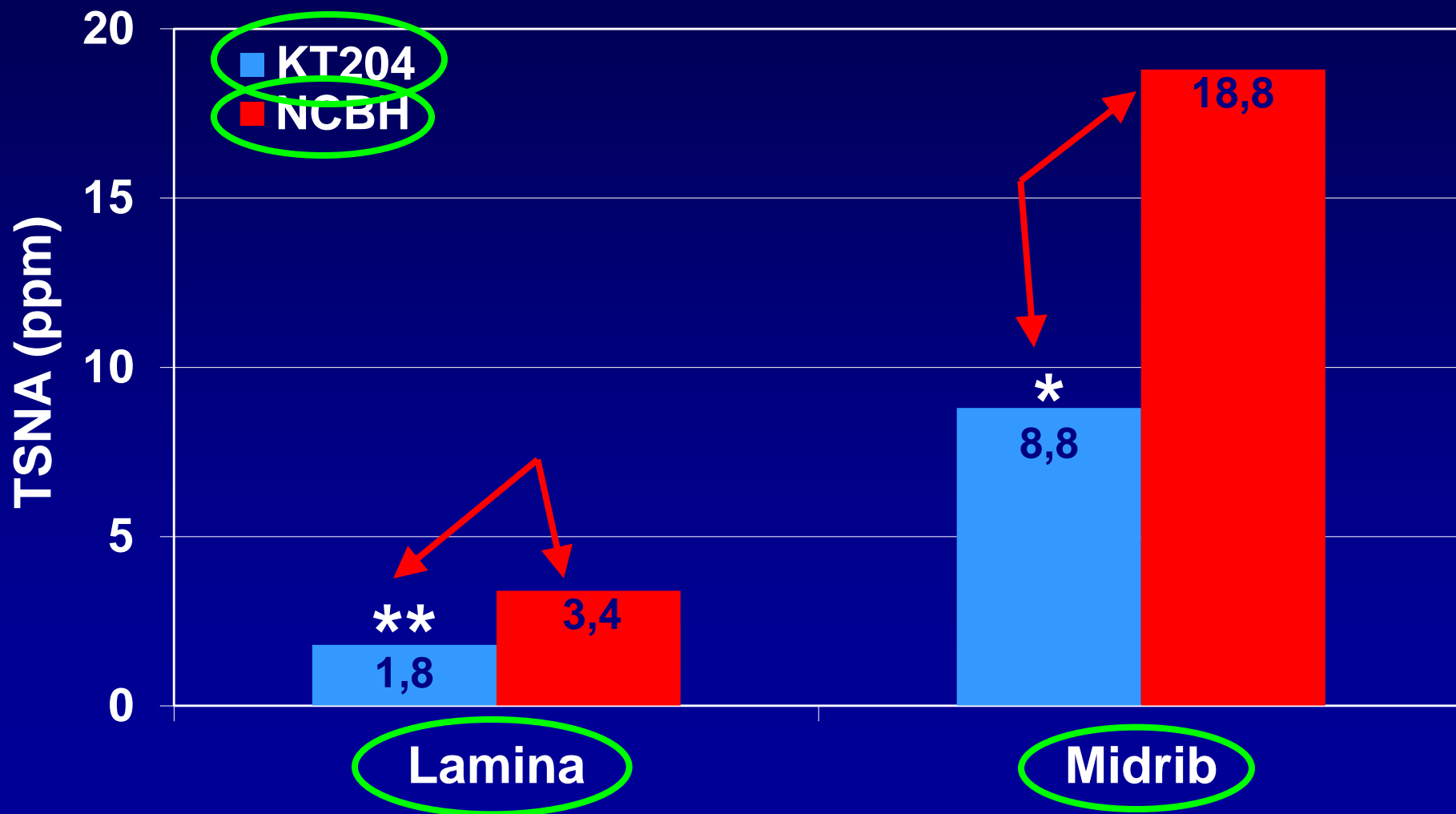
TSNAs (midrib) : ANOVA

¹ log transformed

	<u>NNN¹</u>	<u>NAT</u>	<u>TSNA¹</u>
Variety	NS	*	*
Year	**	*	**
Var x Yr	NS	NS	NS

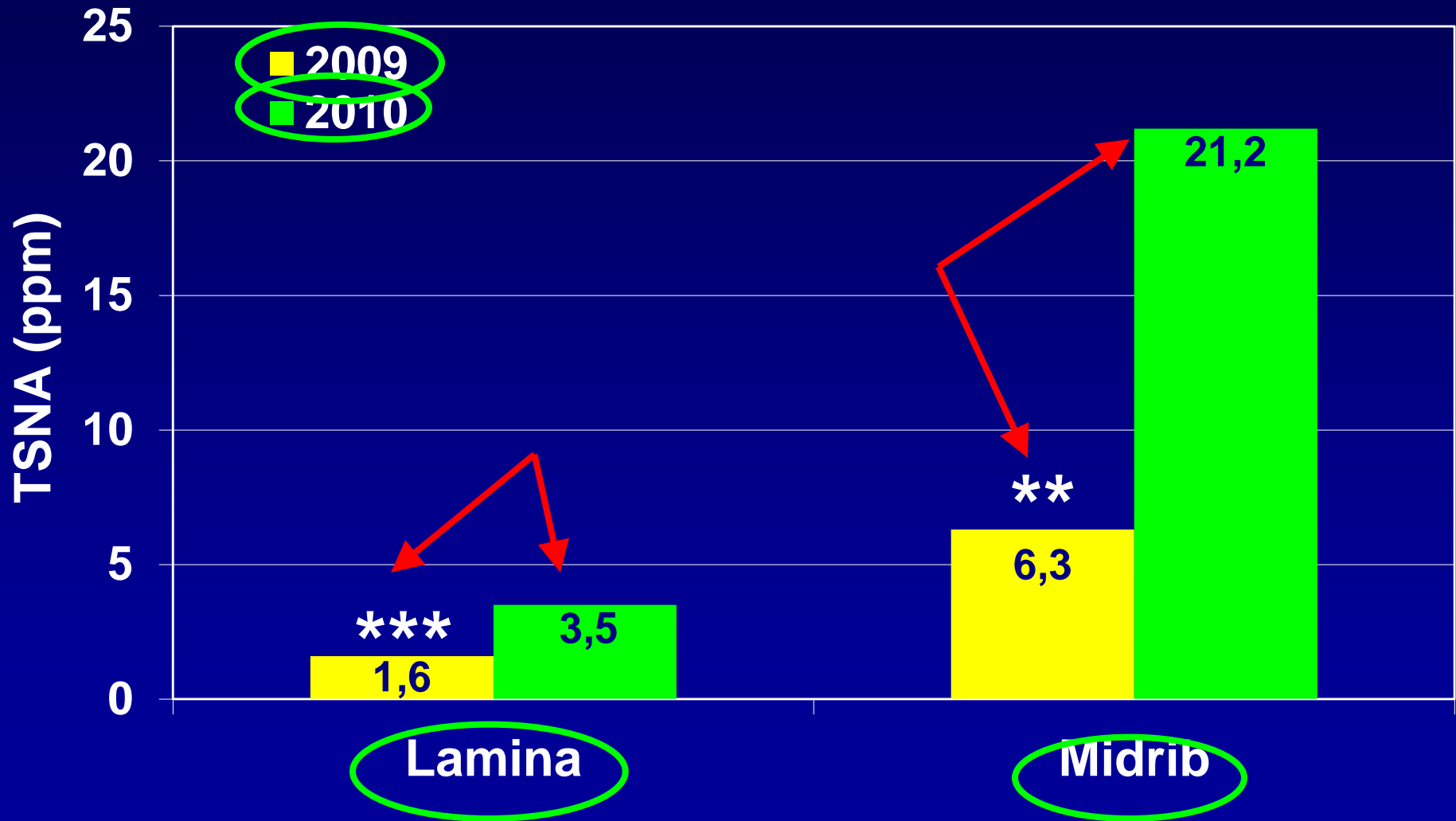
Total TSNA (ppm)

Variety Effects (pooled over years)



Total TSNA (ppm)

Year Effects (pooled over varieties)



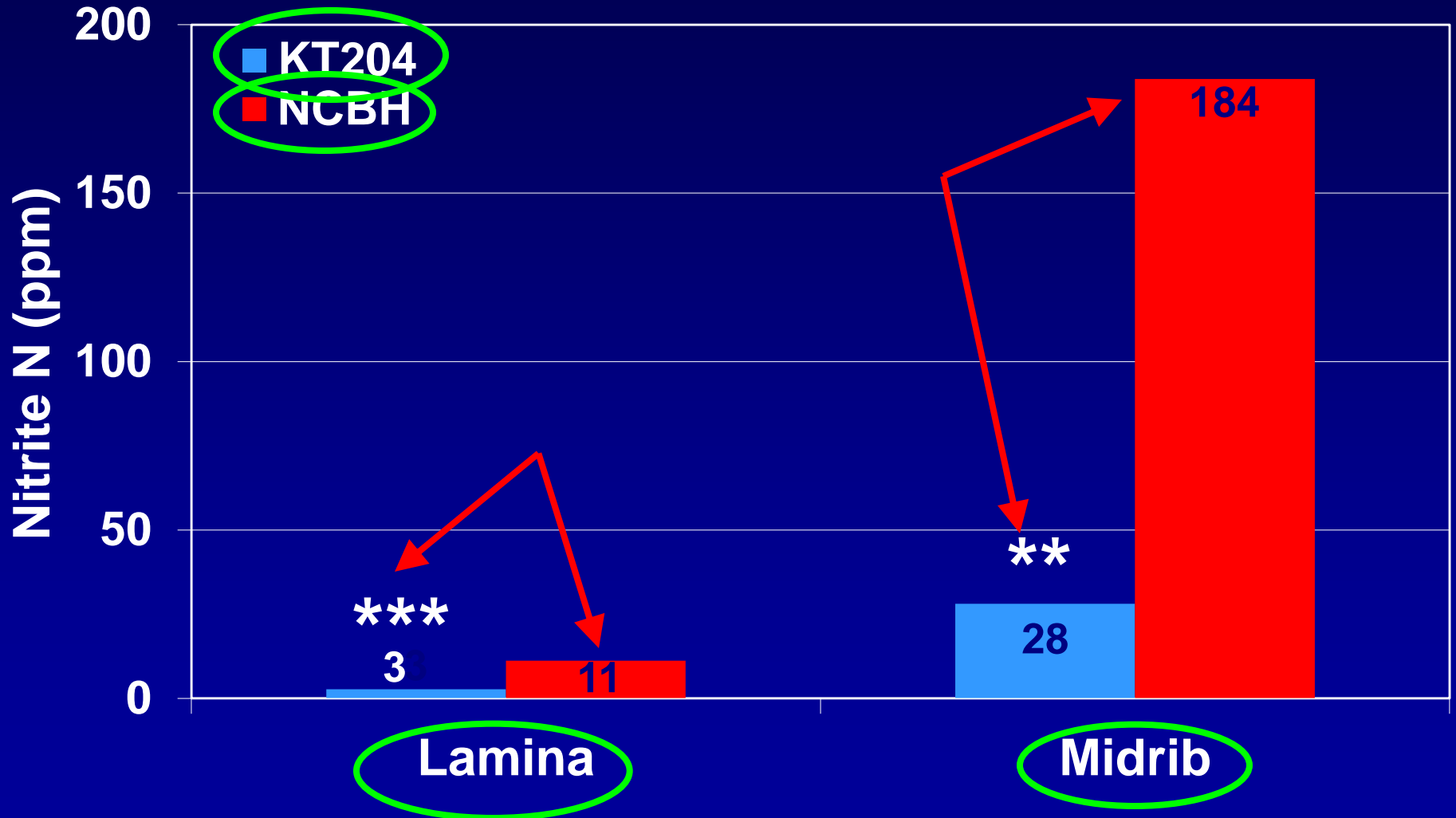
Nitrite N : ANOVA

¹ loglog transformed, ² sq rt transformed

	<u>Lamina¹</u>	<u>Midrib²</u>
Variety	***	**
Year	NS	*
Var x Yr	NS	NS

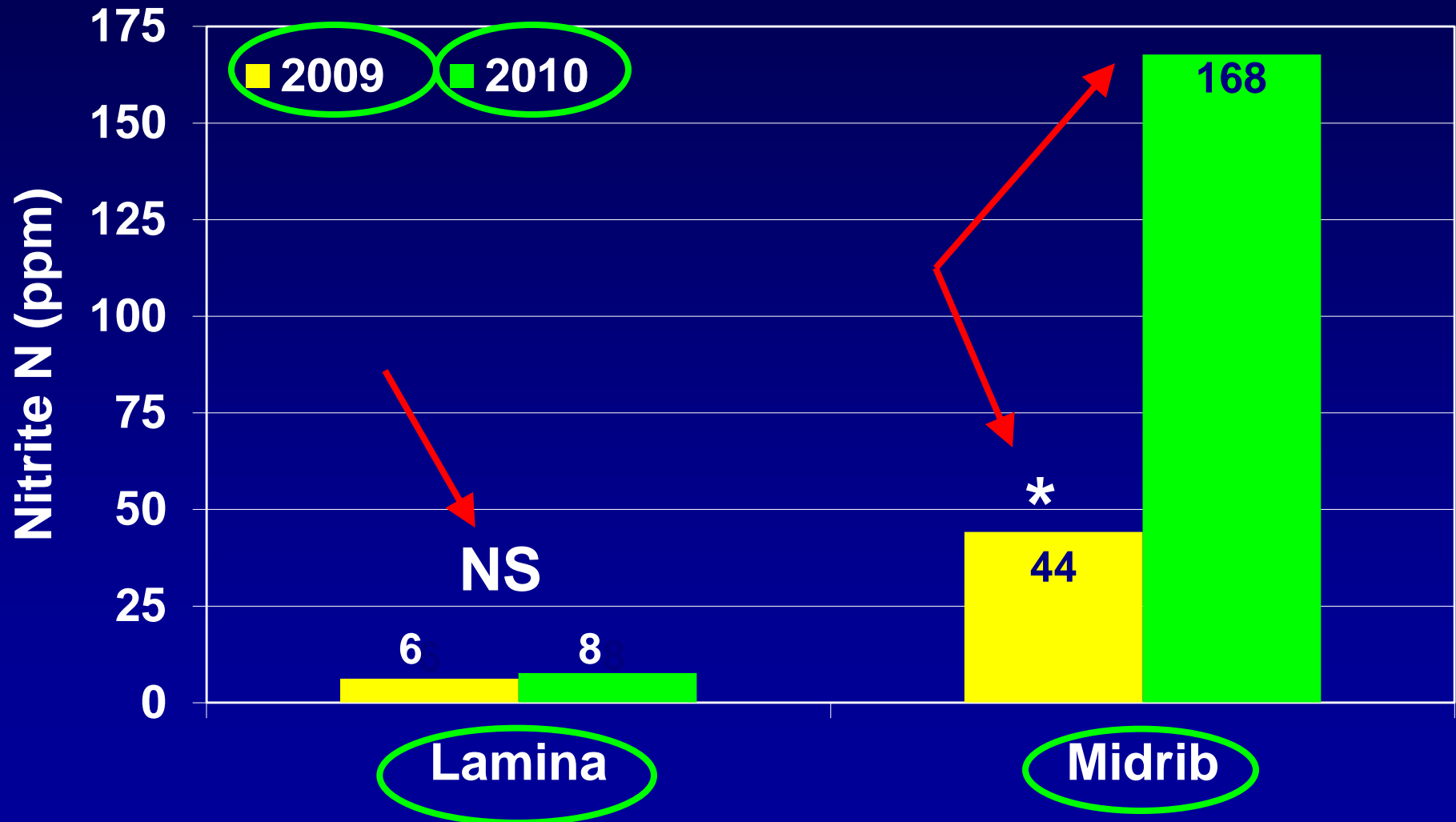
Nitrite N (ppm)

Variety Effects (pooled over years)



Nitrite N (ppm)

Year Effects (pooled over varieties)



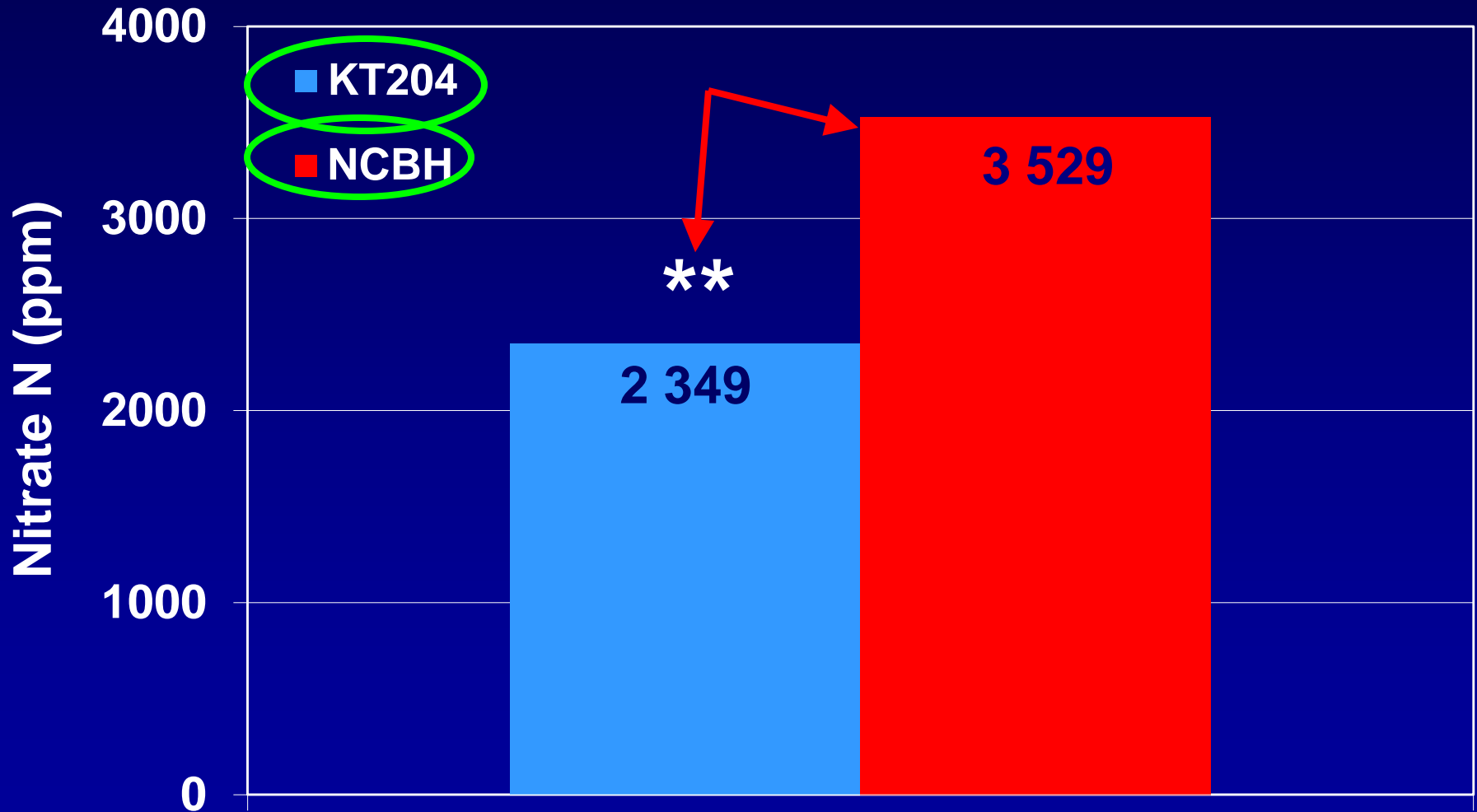
Nitrate N : ANOVA

¹ log transformed

	<u>Nitrate¹</u>
Variety	**
Year	***
Var x Yr	NS

Nitrate N (ppm)

Variety Effects (pooled over years)



Nitrate N (ppm)

Year Effects (pooled over varieties)



Tot Alk, Tot N : ANOVA

¹ exponential transformed

	<u>Tot Alk¹</u>	<u>Tot N</u>
Variety	**	**
Year	***	**
Var x Yr	***	*

Total Alkaloids, Total N (% DM)

Variety Effects (2010)



Total Alkaloids (%DM)

Year Effects (pooled over varieties)



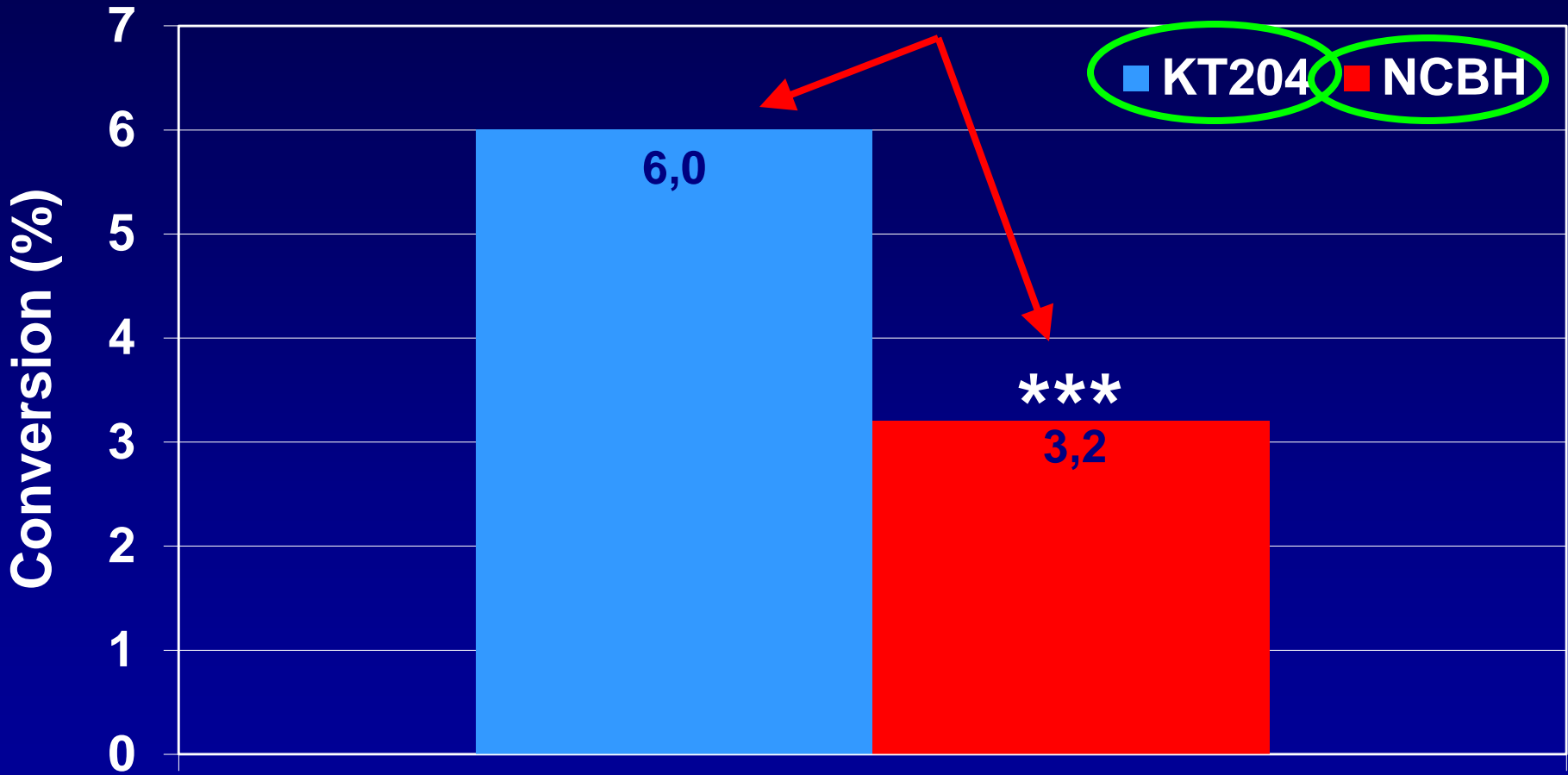
Nornic, Conversion : ANOVA

¹ log transformed

	<u>Nornic¹</u>	<u>Conv¹</u>
Variety	**	***
Year	*	*
Var x Yr	NS	NS

Conversion (%)

Variety Effects (pooled over years)



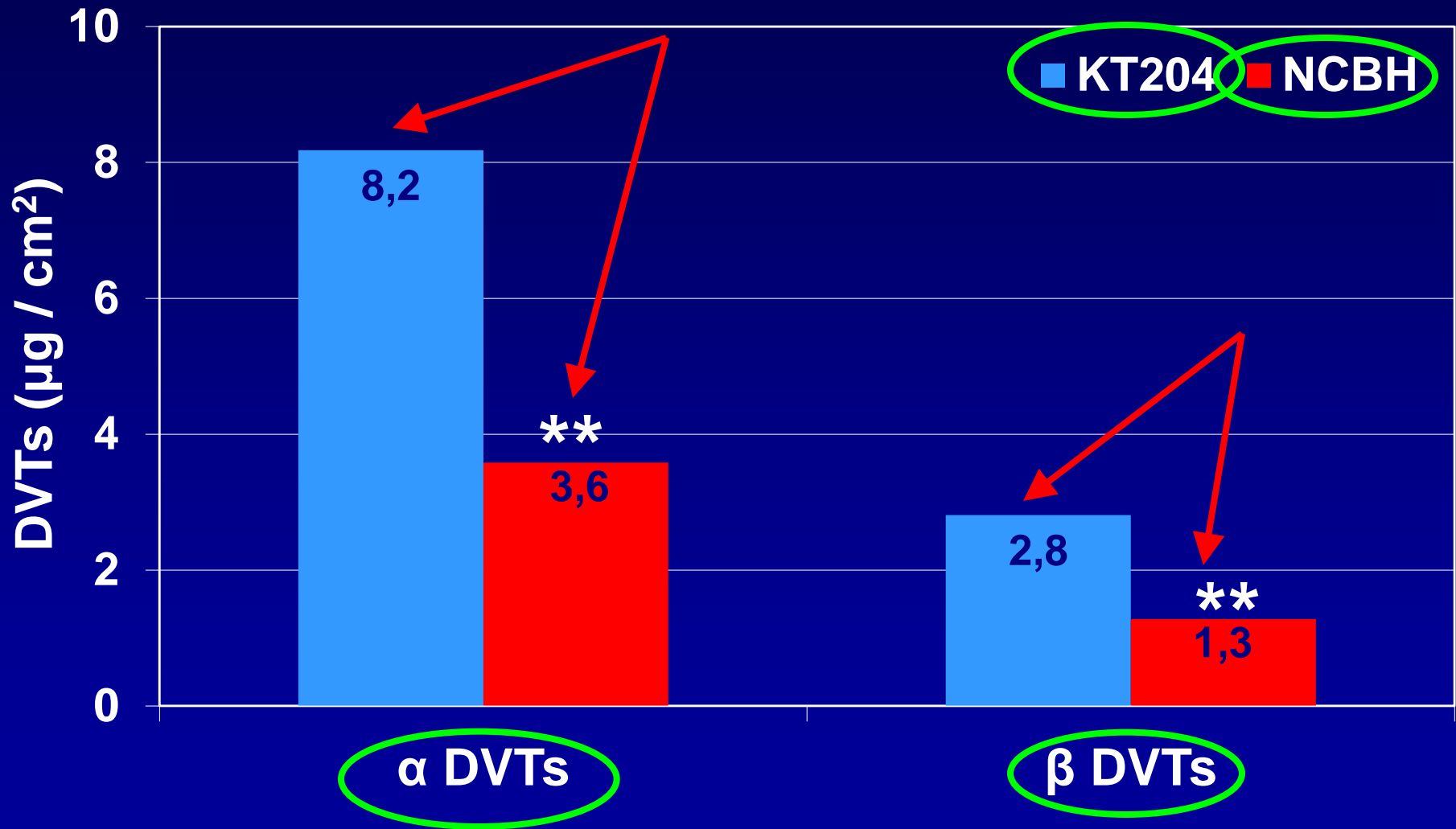
α DVTs, β DVTs : ANOVA

¹ log transformed

	<u>α DVT¹</u>	<u>β DVT¹</u>
Variety	**	**
Year	NS	NS
Var x Yr	NS	NS

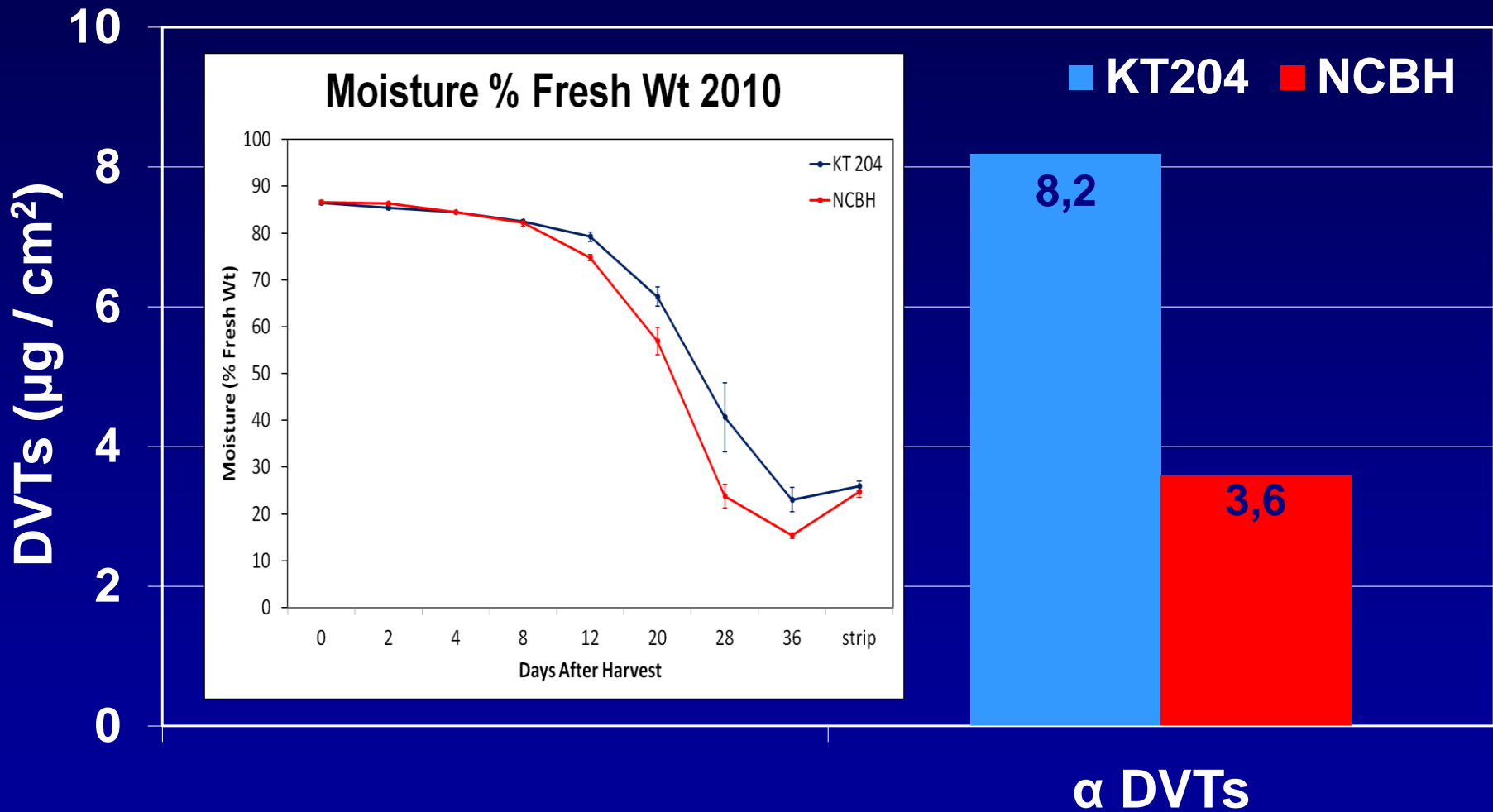
α DVTs, β DVTs ($\mu\text{g} / \text{cm}^2$)

Variety Effects (pooled over years)



α DVTs, β DVTs ($\mu\text{g} / \text{cm}^2$)

Variety Effects (pooled over years)



Leaf Thickness, Specific Wt : ANOVA

¹ loglog transformed

Sp Wt¹

Lf Thk

Fr

Dr

Variety

NS

NS

**

Year

**

Var x Yr

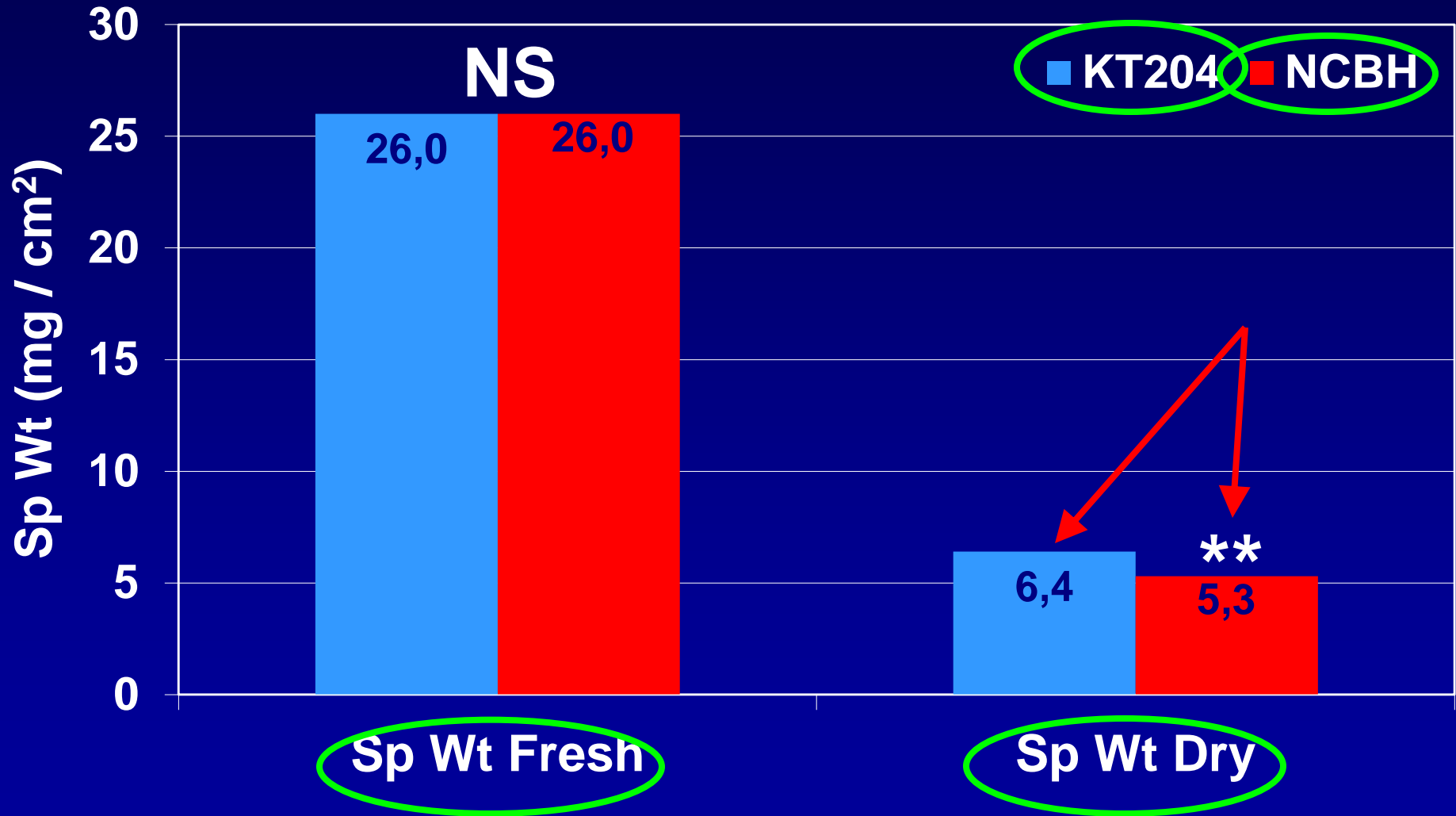
NS

NS

NS

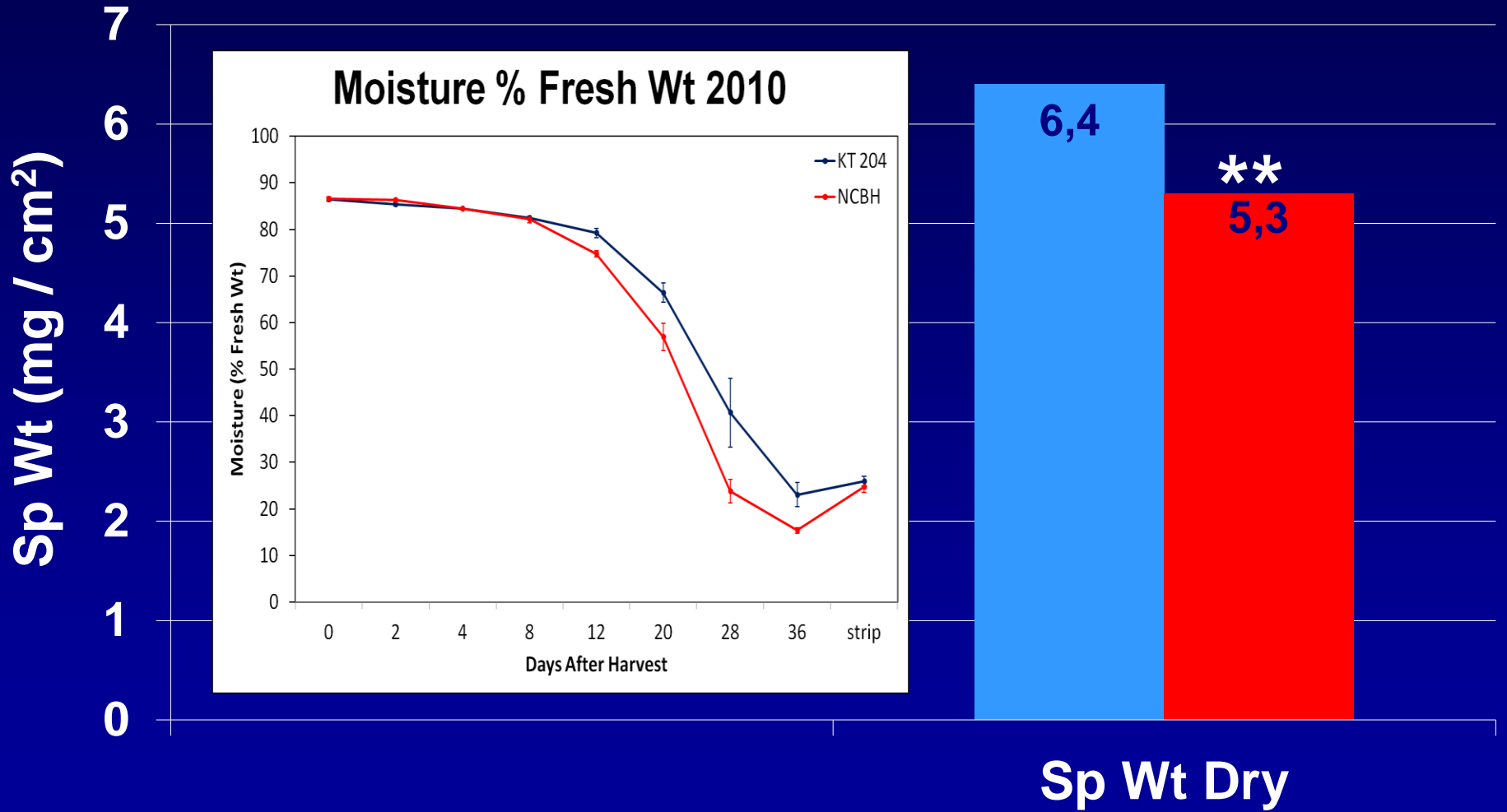
Sp Wt, Fresh & Dry (mg / cm²)

Variety Effects (pooled over years)



Sp Wt, Dry (mg / cm²)

Variety Effects (pooled over years)



Summary



Summary

	Variety	Year
TSNA	KT204 ↓	2010 ↑
NO ₂ N	KT204 ↓	2010 ↑
NO ₃ N	KT204 ↓	2010 ↑
Total Alkaloids	KT204 ↑	(2010 ↑)
Tot N	KT204 ↑	(2010 ↑)
Conversion	KT204 ↑	2010 ↓

Summary

	Variety	Year
TSNA	KT204 ↓	2010 ↑
NO₂ N	KT204 ↓	2010 ↑
NO₃ N	KT204 ↓	2010 ↑
Total Alkaloids	KT204 ↑	(2010 ↑)
Tot N	KT204 ↑	(2010 ↑)
Conversion	KT204 ↑	2010 ↓

Summary

	Variety	Year
TSNA	KT204 ↓	2010 ↑
NO₂ N	KT204 ↓	2010 ↑
NO₃ N	KT204 ↓	2010 ↑
Total Alkaloids	KT204 ↑	(2010 ↑)
Tot N	KT204 ↑	(2010 ↑)
Conversion	KT204 ↑	2010 ↓

Summary

	Variety	Year
TSNA	KT204 ↓	2010 ↑
NO₂ N	KT204 ↓	2010 ↑
NO₃ N	KT204 ↓	2010 ↑
Total Alkaloids	KT204 ↑	(2010 ↑)
Tot N	KT204 ↑	(2010 ↑)
Conversion	KT204 ↑	2010 ↓

Summary cont

	Variety	Year
TSNA	KT204 ↓	2010 ↑
DVTs	KT204 ↑	-
Sp Wt Dr	KT204 ↑	2010 ↓
Moisture	KT204 ↑	2010 ↓
Sp Wt Fr	-	2010 ↓
Leaf Thick L	-	2010 ↓
Leaf Thick M	-	2010 ↑

Conclusions



Conclusions

- **KT 204 consistently lower TSNAs**
 - ↑ very little during cure
 - NCBH 129 starts ↑ after 3-4 weeks
- **KT 204 consistently lower nitrite**
 - same accumulation pattern as TSNAs
 - other researchers similar results
 - likely KT 204 ↓ nitrite partly explains ↓ TSNAs
- **KT 204 ↓ nitrate: ↑ alkaloids, tot N**
 - not totally consistent
 - differential efficiency in N assimilation?

Conclusions cont

- **Possible reasons for KT 204 ↓ nitrite**
 - endogenous anti-oxidants
 - unique microbial profile
 - internal anatomy of leaf
- **KT 204 ↓ nitrite not because of differential drying in cure**
 - slower drying rate
 - higher DVTs
 - ↑ specific weight
 - no difference in leaf thickness

Conclusions cont

- **Current work**

- compare varieties under controlled curing conditions

- hot, humid / cool, dry / ambient

- study microbial profile

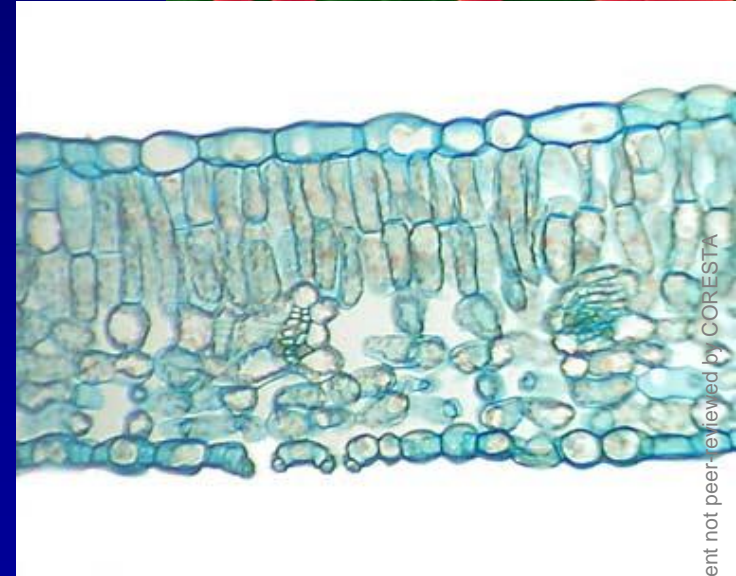
- metagenomics approach
 - nitrate reducers

- **Future work**

- study histology

- metagenomics

- nitrite reducers, TSNAs metabolizers



Acknowledgements

- **Financial support**
 - **PMI, Altria**
- **Lab staff**
- **Farm staff & student helpers**

