TSNA’s and burley tobacco

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Outline

1. Chemistry of formation
2. Conventional curing conditions for flue and air-cured tobacco
3. Plant and cultural differences
4. Water loss and nitrite formation
5. Experimental manipulation of plant and curing conditions
6. Conclusions and Future

Nitrosamines

Potent family of carcinogens

Formed from diverse nitrogen compounds and nitrite (NO_2) or its derivatives

Why are nitrosamines important?

Are carcinogenic

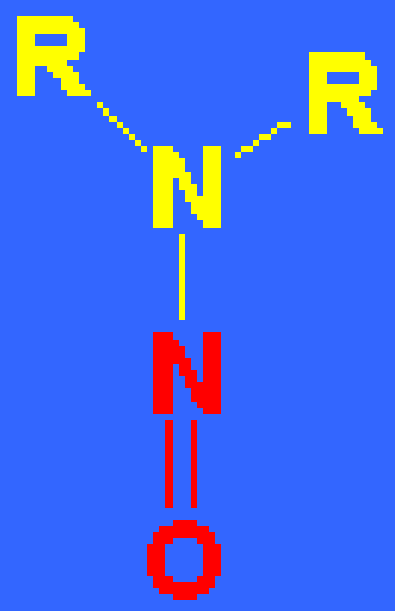
Have been identified in many everyday items including

- cosmetic products
- sunscreens
- nonfat dry milk
- beer
- rubber
- bacon

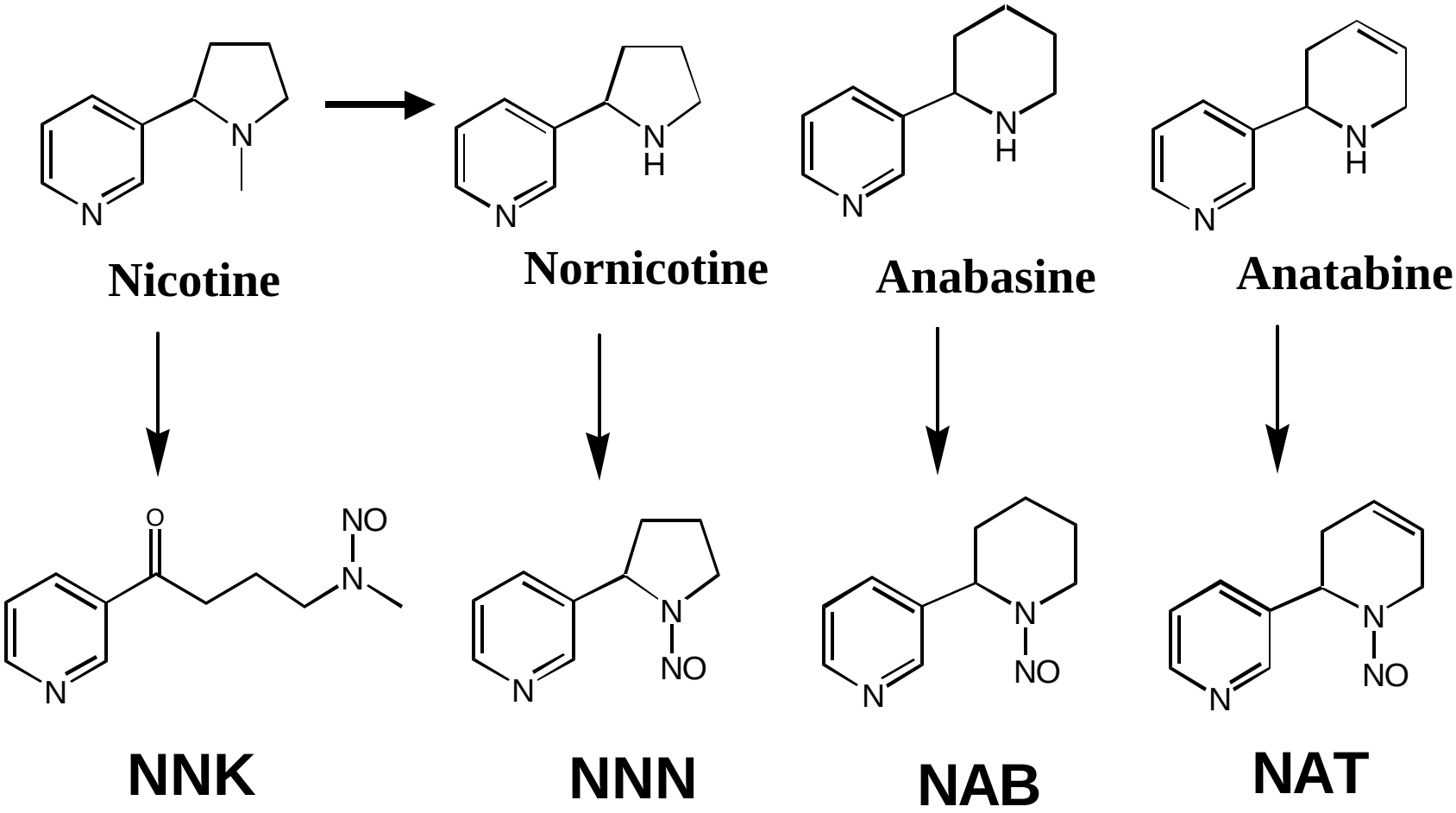
For TSNA formation need the

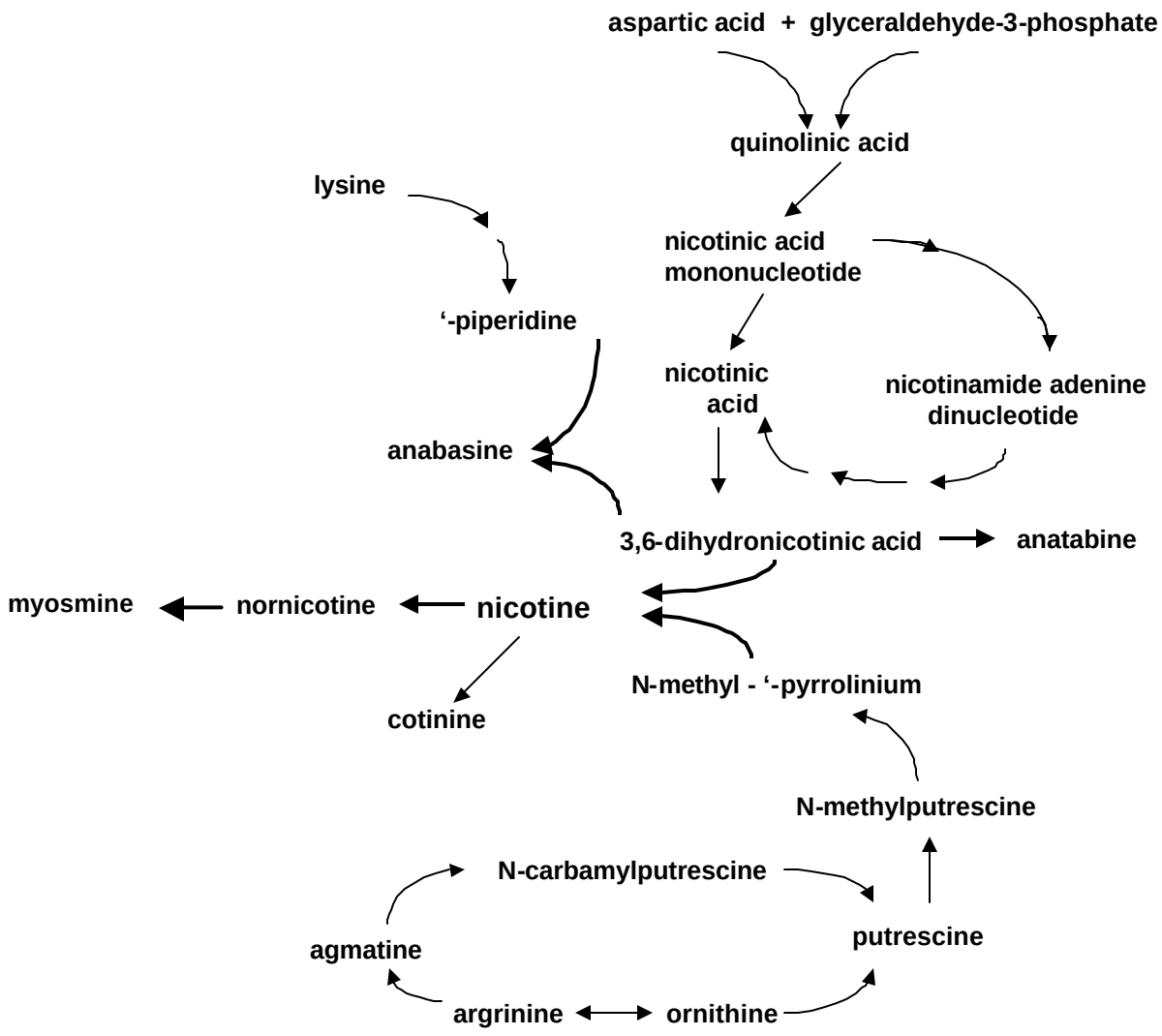
Alkaloid

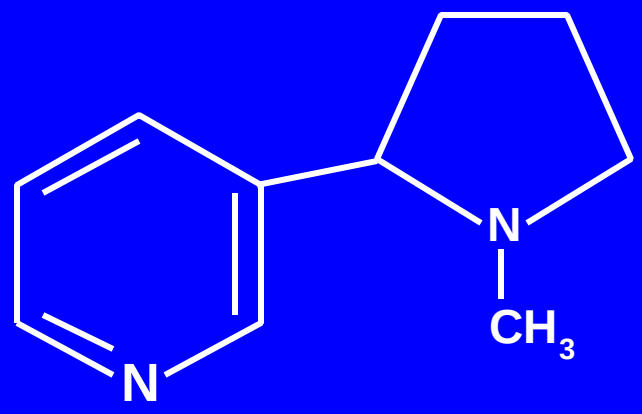
NO_2



TSNA = tobacco specific nitrosamine


Nicotine
Nornicotine
Anabasine
Anatabine
NNK
NNN
NAB
NAT


aspartic acid + glyceraldehyde-3-phosphate
quinolinic acid
lysine
nicotinic acid mononucleotide
'-piperidine
nicotinic acid
nicotinamide adenine dinucleotide
anabasine
3,6-dihydronicotinic acid
anatabine
myosmine
nornicotine
nicotine
N-methyl - '-pyrrolinium
cotinine
N-methylputrescine
N-carbamylputrescine
putrescine
agmatine
argrinine
ornithine



Nicotine

Nornicotine

Range of nitrosamines in burley tobacco air-cured in Kentucky.

Nitrosamine	Lamina	Midrib
	?g g^{-1}	?g g^{-1}
NNN	0.41 -18	0.43 - 43
NAT	1.20 -13	0.80 - 25
NNK	0.09 - 7	0.15 – 22
TSNA	1.70 – 38	1.38 - 89

Concentration of Leaf Constituents Important for TSNA Formation

Leaf Tissue	Alkaloids	NO_3-N	NO_2-N	TSNA
	Nominal Amount			
	%	%	ppm	ppm
Lamina	3	0.35	5	4
Midrib	0.6	1.05	50	15
	µmoles g^{-1}			
Lamina	185	250	0.3	0.02
Midrib	37	750	3	0.08

Examples of poor curing conditions

Low humidity/high temperature ? a drying and not a cure

Low humidity/satisfactory temperature ? piebald or green tobacco

Low or high humidity/low temperature ? green tobacco

High humidity/high temperature ? house burn, microbial growth and excessive weight loss

In a normal to wet curing season the midrib tissue has > amounts of TSNA than lamina

(more NO_2 in the midrib)

In a dry curing season lamina > amounts of TSNA than midrib

(NO_2 low in both and alkaloids are determinate factor)

Is it possible to alter TSNA in cured tobacco leaf?

Temperature and RH within a barn

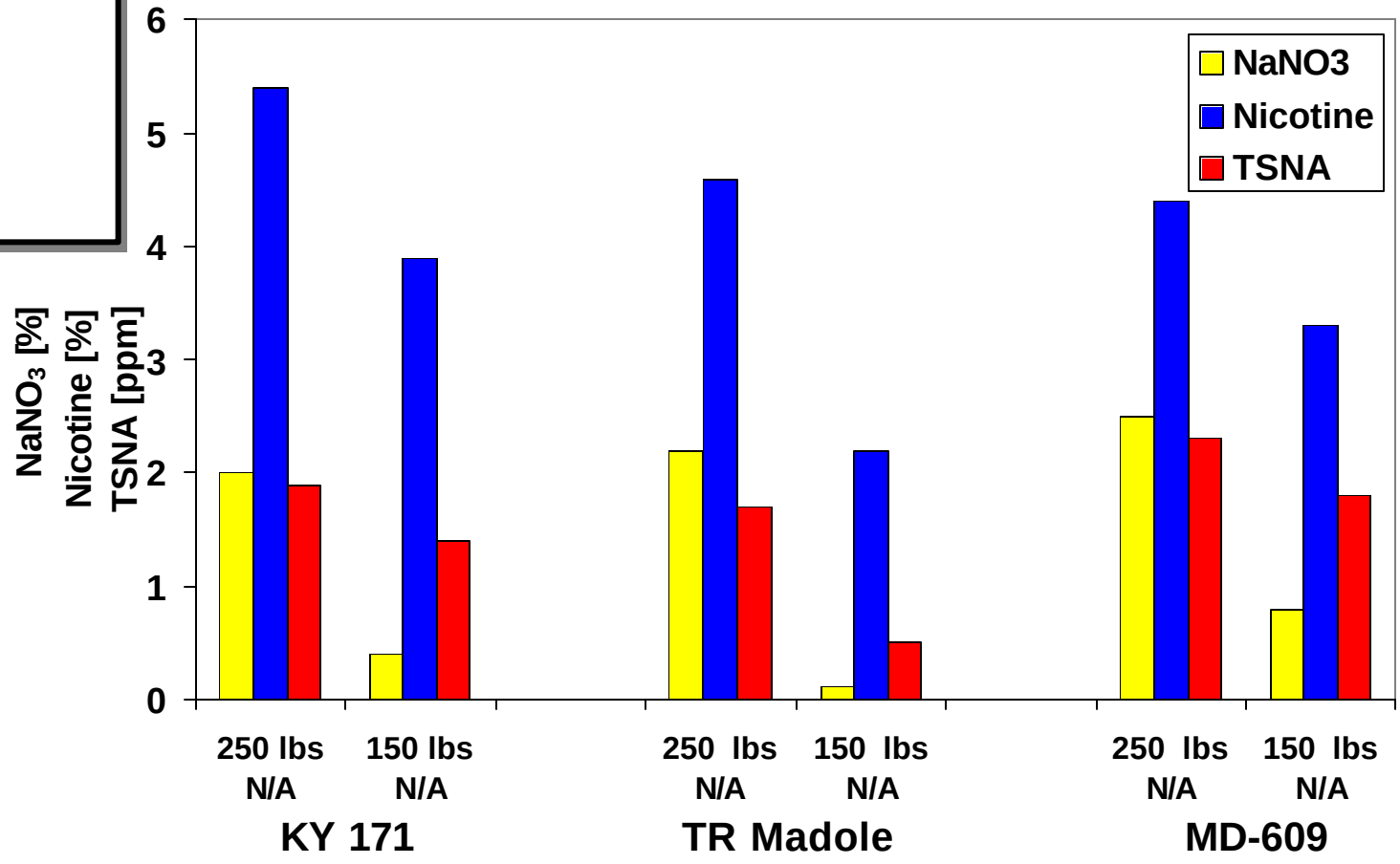
Rail	Temp	RH	TSNA	
	C	%	lamina ug g^{-1}	midrib ug g^{-1}
2	23.6	72.6	1.65	0.70
4	24.4	67.8	1.62	0.40
6	26.2	59.5	0.91	0.26

Effect of 280 kg of N ha^{-1} Fertilizer on Nicotine, Total Nitrogen, Nitrate, Nitrite and TSNA Accumulation in Air-Cured Tobacco Lamina

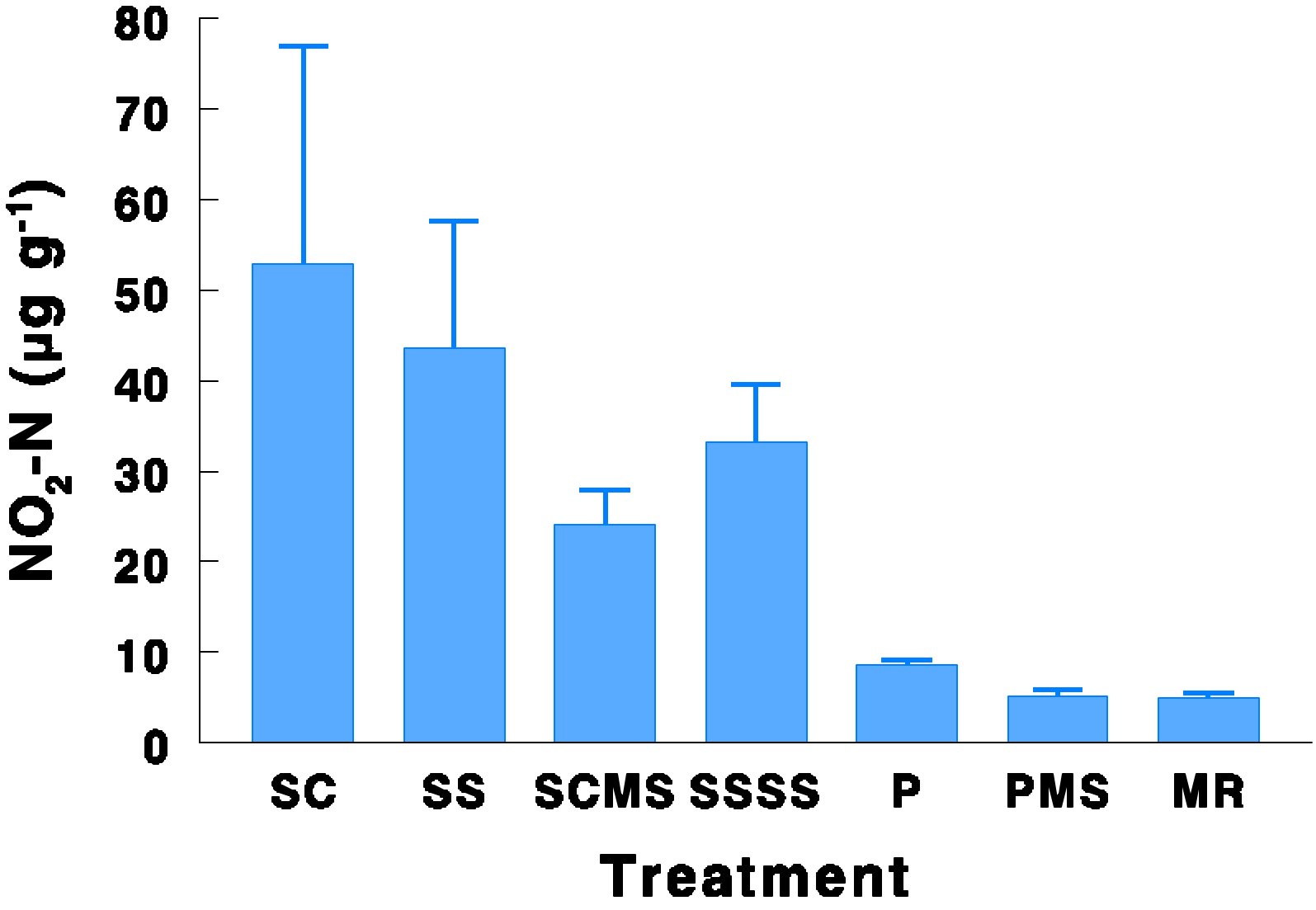
fertilizer treatment	nicotine	NO_3-N	NO_2-N	TSNA
	%	ppm	ppm	ppm
0 (control)	1.75	1710	3.5	2.49
transplant	1.99	5985	3.4	6.40
midseason	2.04	4265	2.7	6.68
topping	1.84	2945	3.2	5.40

TSNA-FERTILIZATION

1990 Reidsville, NC
Fert.rate:
•250 lbs N/A
•150 lbs N/A
Air-curing



Modified Plants Air-cured



Modified Plants Air-cured

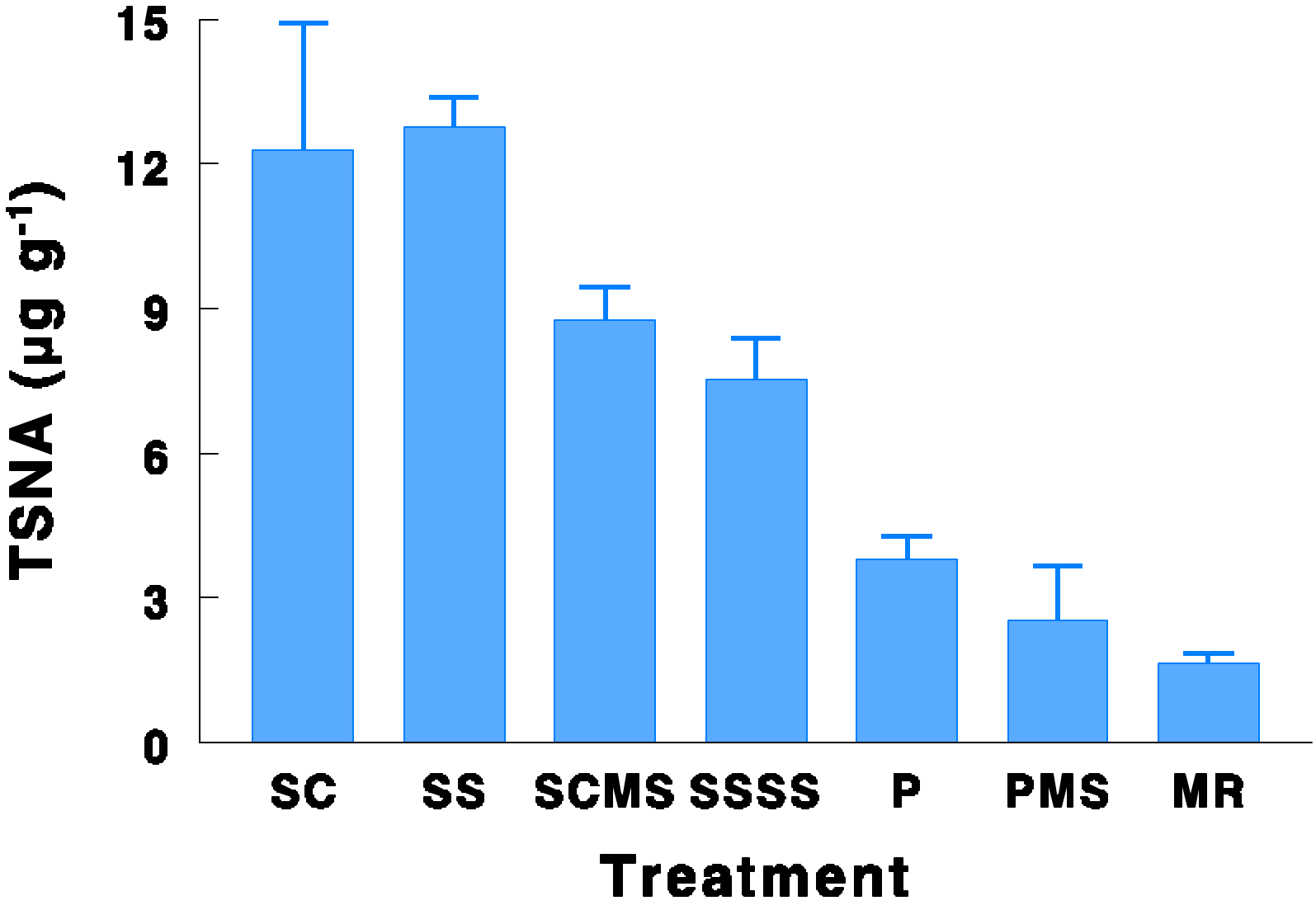
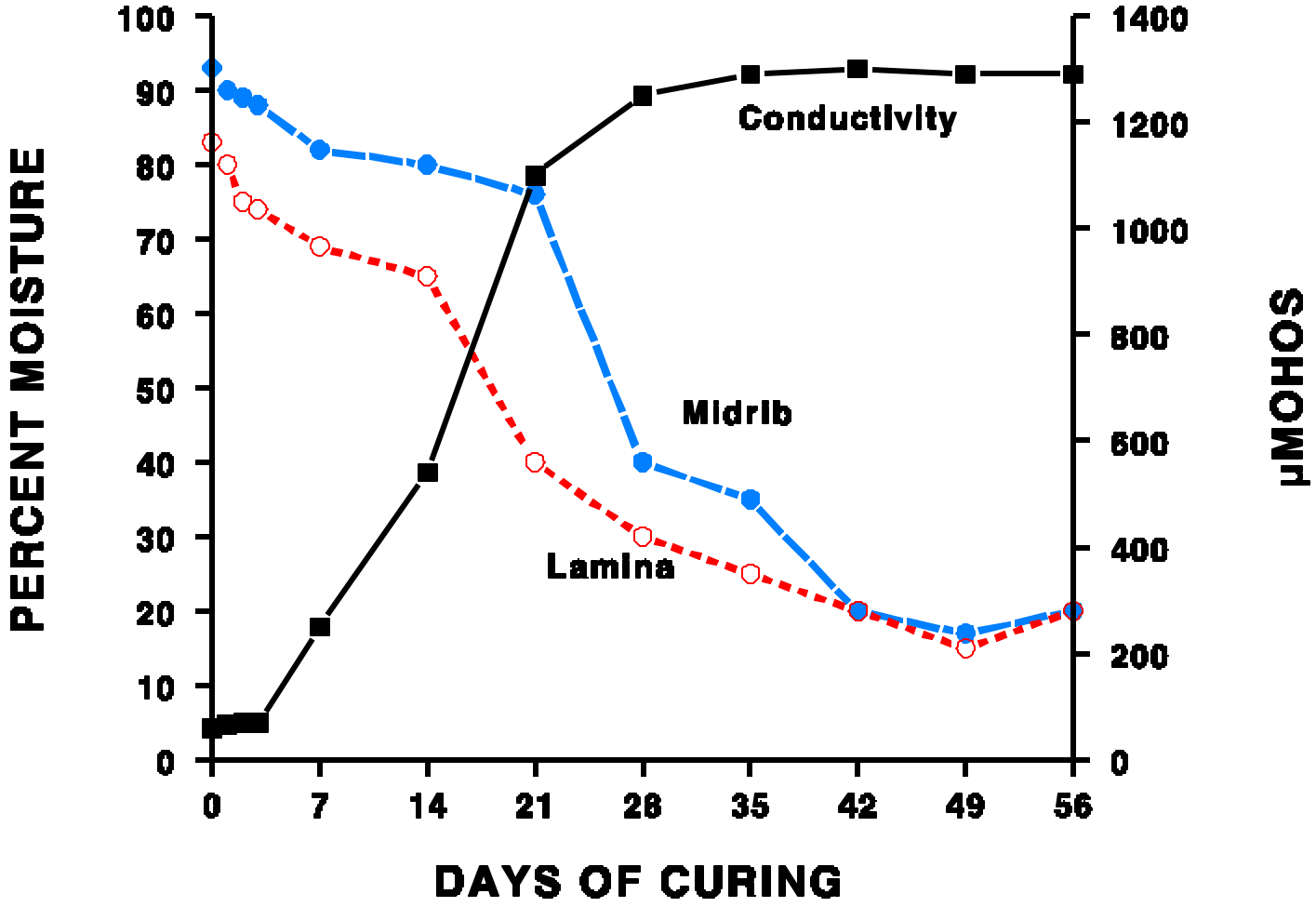


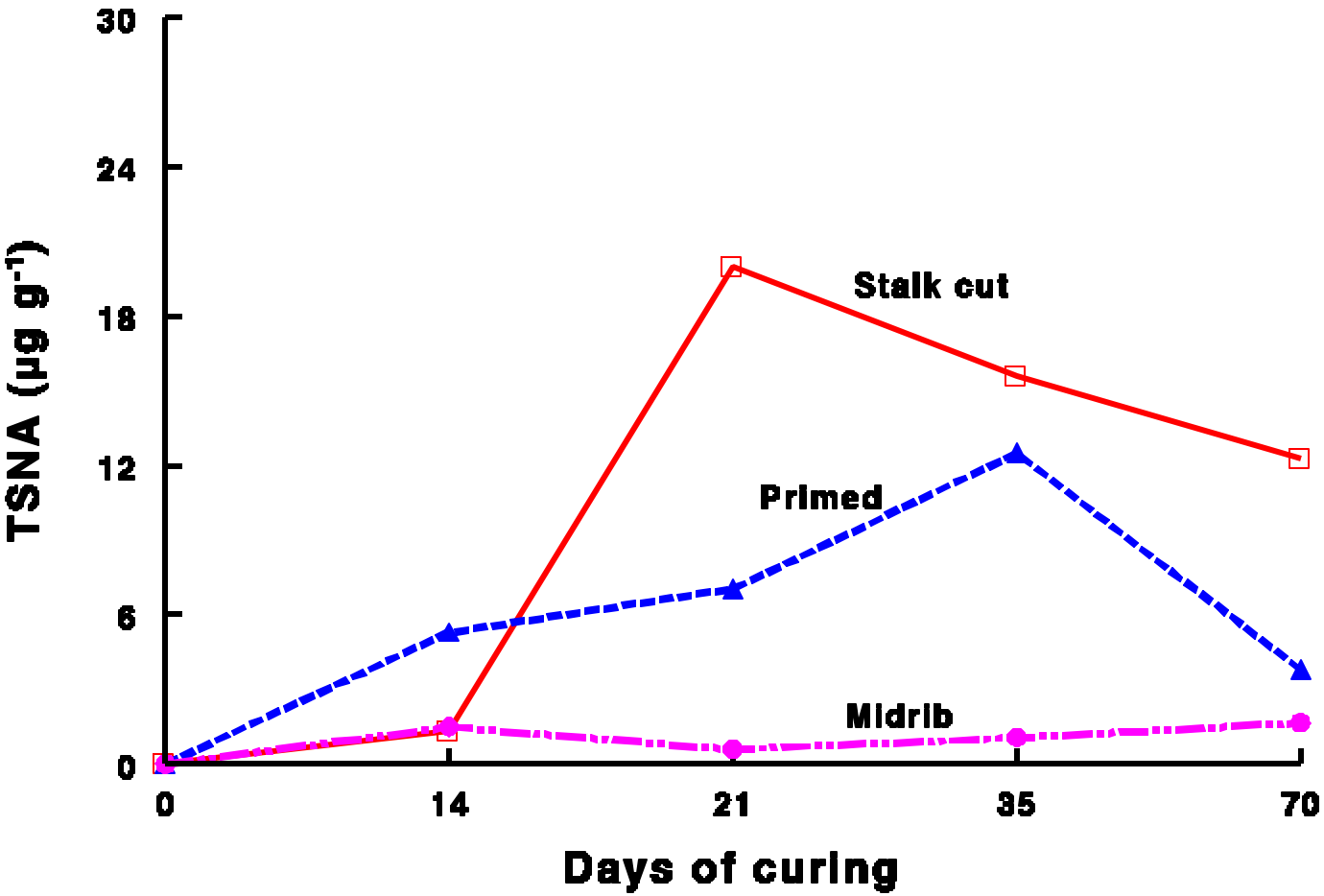
Table 5. Changes of Tobacco-Specific Nitrosamines and Nitrate during Air-Curing at 24°C/70% RH and 32°C/83% RH

	24°C/70 % RH		32°C/83% RH	
Days	TSNA ppm	NO_2-N ppm	TSNA ppm	NO_2-N ppm
	Harvested 4 Weeks After Topping			
0	2.5	0.6	2.5	0.6
1	3.3	0.7	3.3	0.7
7	7.6	0.8	42.9	30
12	12.3	3.5	24.8	50
14	12.1	3.4	100.6	110
16	5.9	2.7	153.1	630
19	15.8	5.1	912.9	1500
21	9.3	1.4	203.8	510

[a]RH=relative humidity



Leaf moisture and conductivity during air-curing of burley tobacco.



TSNA accumulation in midrib of primed leaf, midrib only and stalk-cured control.

Tobacco-Specific Nitrosamines in cured leaf from different harvest maturities

Harvest time	ppm TSNA
1 week after topping	5.8
4 weeks after topping	9.3
7 weeks after topping	9.5

R values for the lamina data of 30 cultivars from 4 locations in 2001

	Total Alkaloids	Total 2° Alkaloids	% 2° Alkaloids	Nornicotine to nicotine	NNN ng g^{-1}	TSNA ng g^{-1}
Nicotine	**0.82**	-0.35	-0.55	-0.52	-0.25	-0.14
Nornicotine	0.16	**0.995**	**0.96**	**0.94**	**0.84**	**0.72**
Myosmine	0.18	**0.98**	**0.93**	**0.91**	**0.87**	**0.77**
Anabasine	**0.79**	0.32	0.13	0.14	0.25	0.32
Anatabine	**0.52**	0.45	0.31	0.24	0.28	0.33

In this study of 30 cultivars there was a significant entry effect for

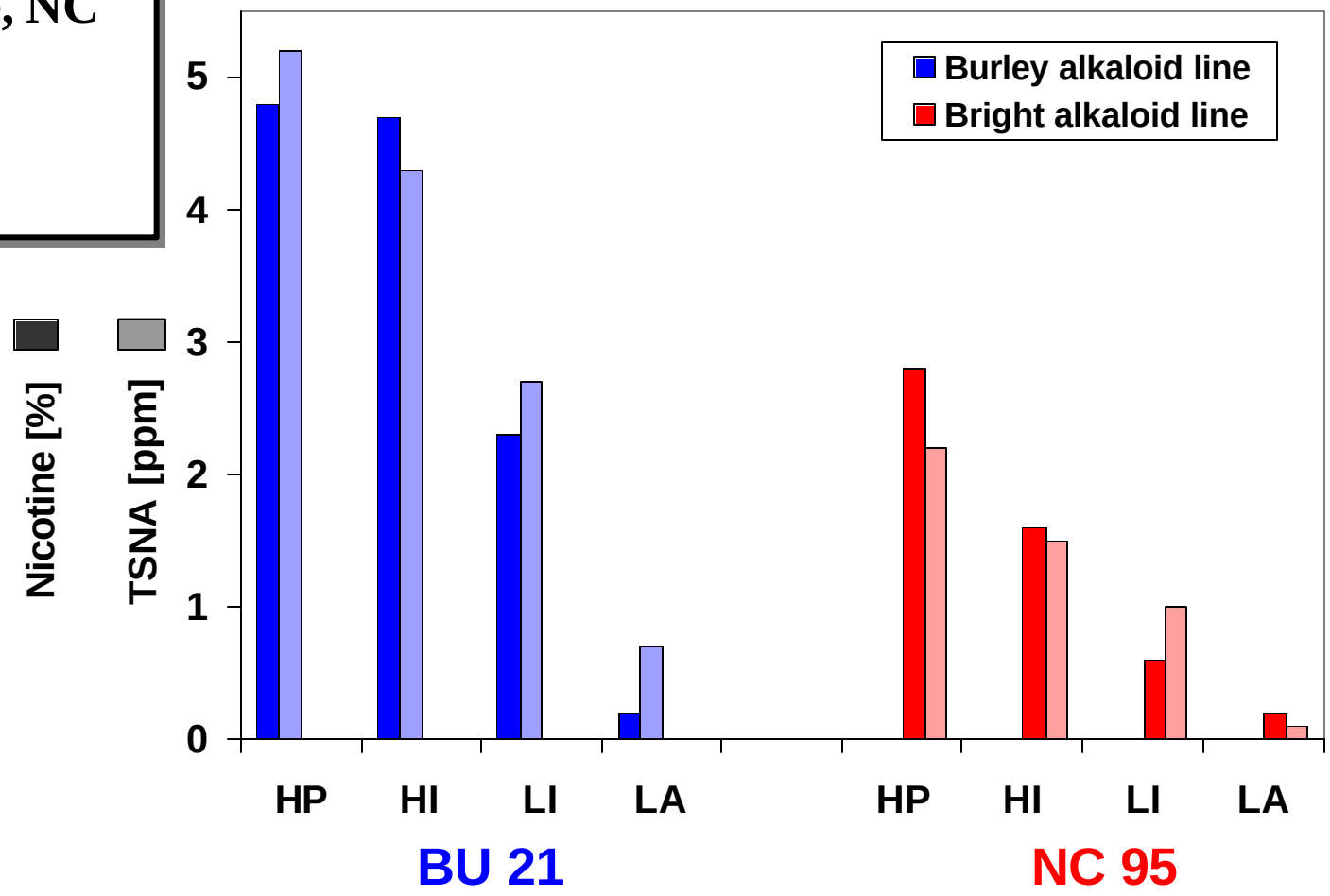
Nornicotine	Myosmine
Total 2° alkaloids	% 2° alkaloids
% nornicotine	Nornicotine to nicotine
NNN	TSNA

There was no entry effect for

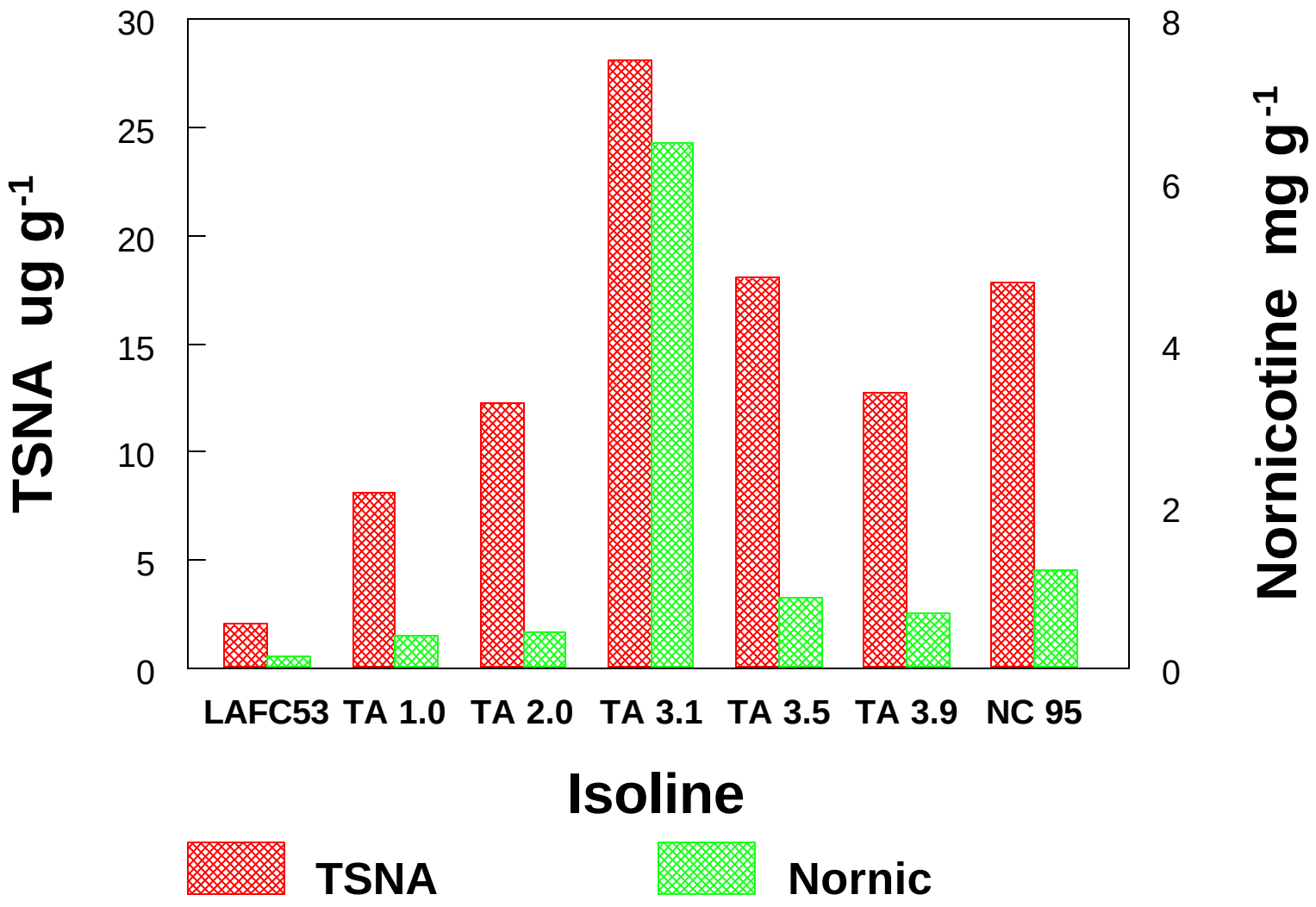
Nicotine	Anabasine
Anatabine	Total alkaloids
NAT	NAB
NNK	Nitrite

TSNA - GENOTYPES

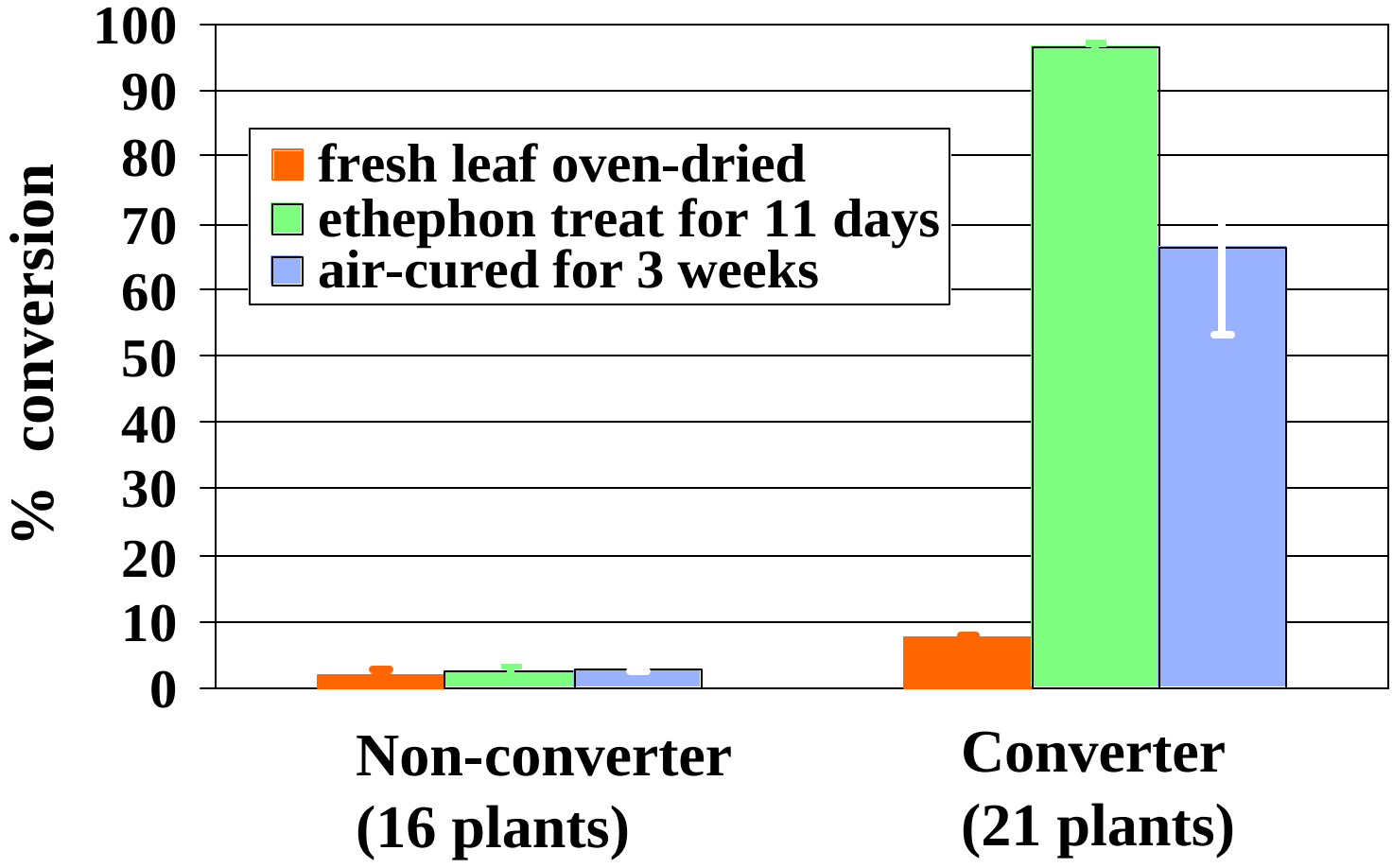
1991 Reidsville, NC
Fert. Rate:
250 lbs N/A
Air-curing



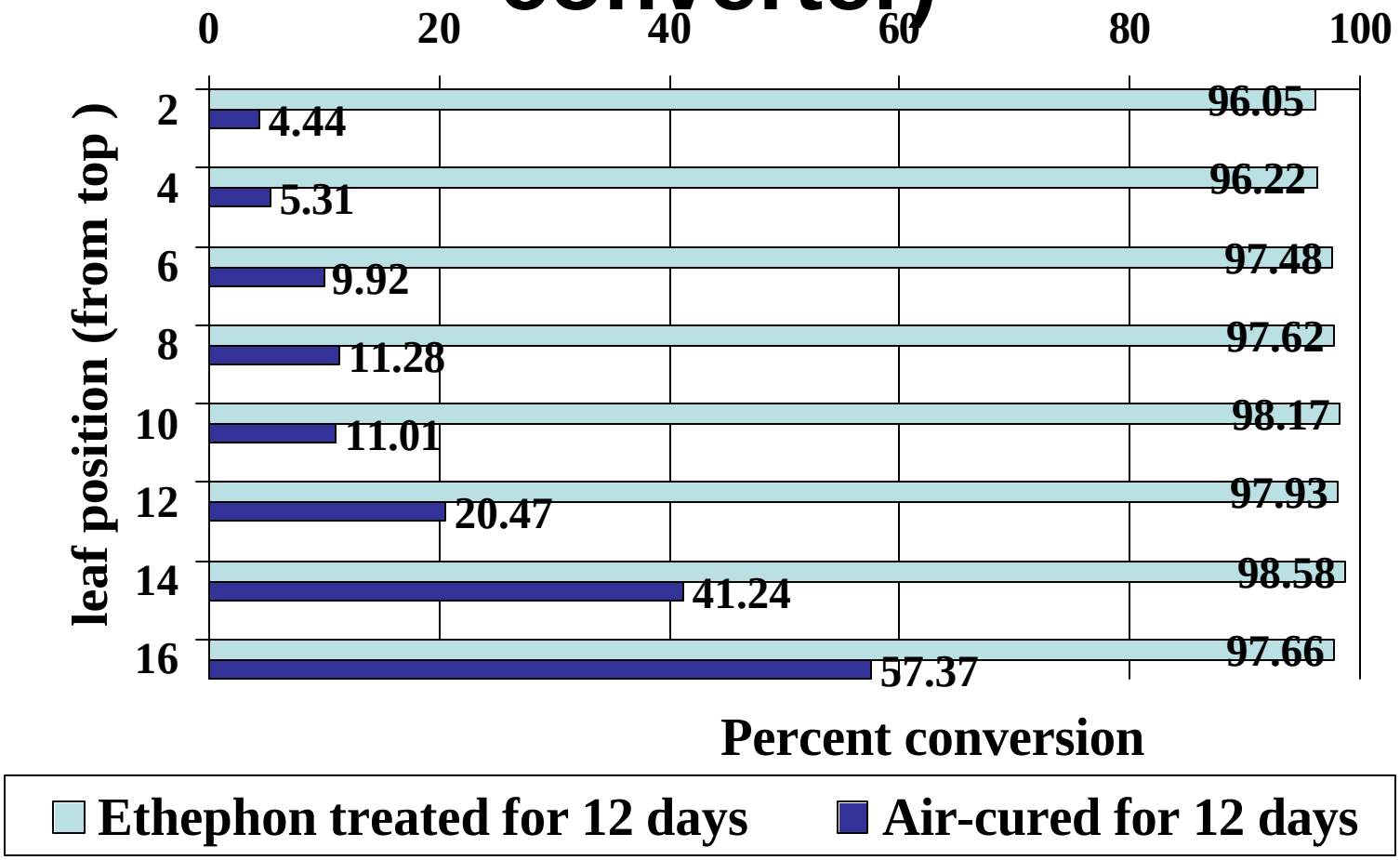
TSNA in NC 95 Alkaloid Isolines from 3 rd Priming



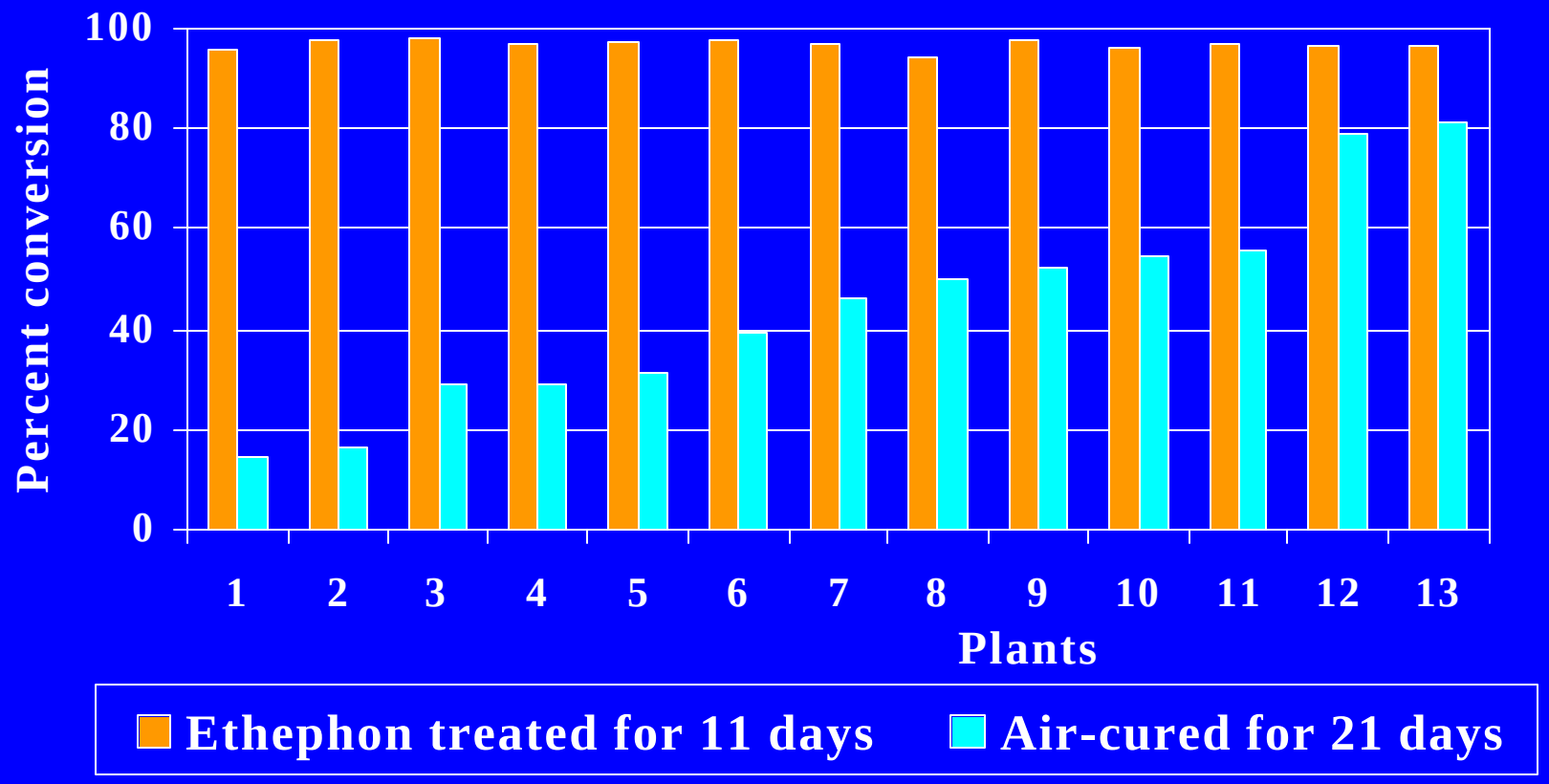
The effect of ethephon treatment on nicotine-nornicotine conversion (78379)

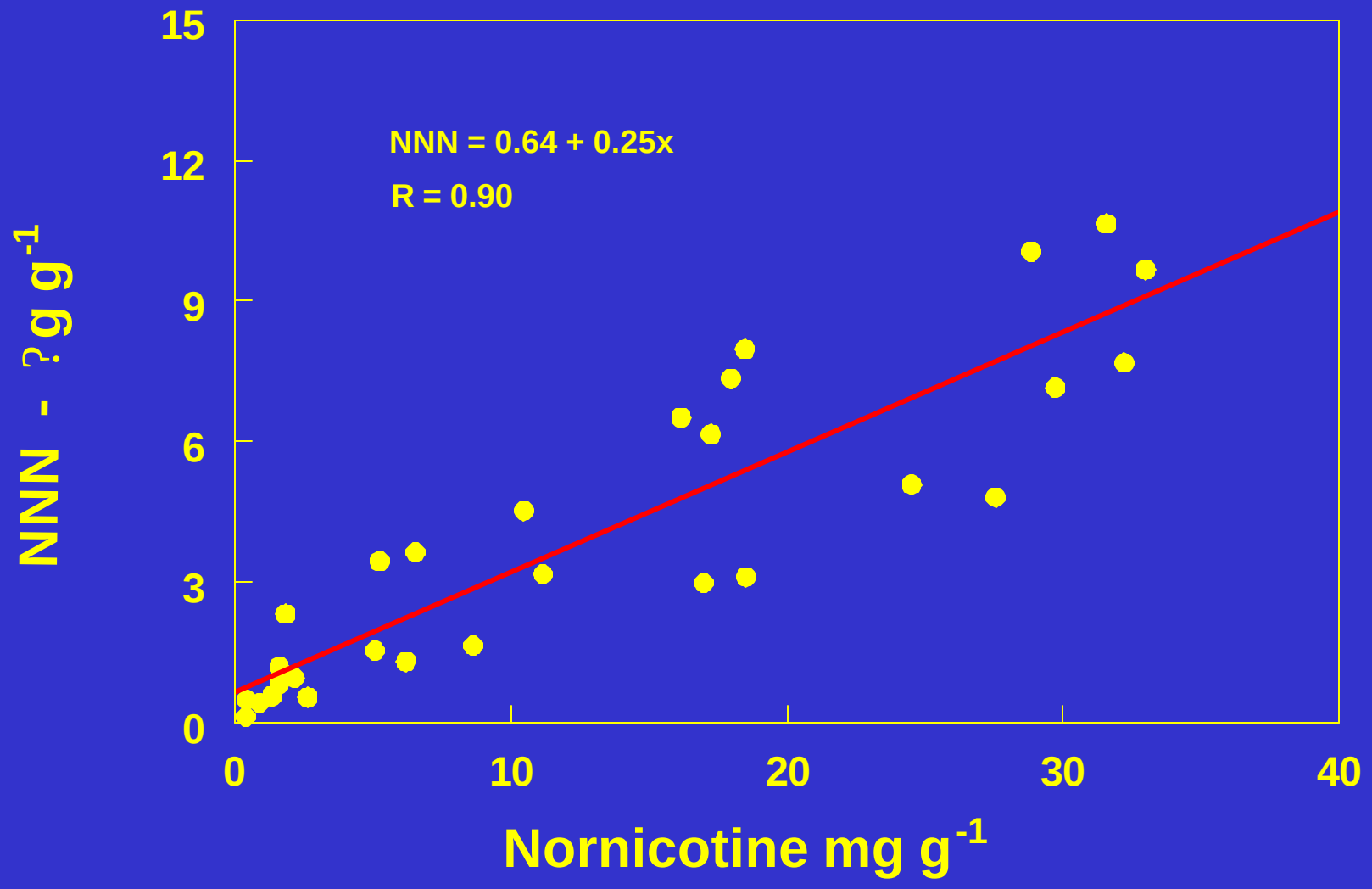


Effect of ethephon treatment on nicotine to nornicotine conversion in leaves of different position (TN90 converter)



Nicotine to nornicotine conversion in plants from converter seeds (TN90)




NNN = 0.64 + 0.25x
R = 0.90
NNN - ?g g-1
Nornicotine mg g-1
0
3
6
9
12
15
0
10
20
30
40

Nornicotine standards

Country	limit
France	15%
Japan	5% breeder's seed
US	20% ? (15%)
	UK Foundation seed <3%
Zimbabwe	3% breeder's seed

Nornicotine standards

University of Kentucky Foundation seed LC (Low Converter) seed

Both male and female parent plants must be less than 3% nicotine to nornicotine conversion

Conclusions for TSNA in cured leaf

1. Higher secondary amine alkaloids yields higher TSNA
2. Lower alkaloid content yields lower TSNA
3. Differences among genotypes may be for nornicotine
4. Increased nitrogen fertilizer increases TSNA
5. Greater maturity at harvest the higher the TSNA
6. Microbial reduction of NO_3 is source of NO_2 for TSNA formation
7. Faster the moisture loss the lower the TSNA

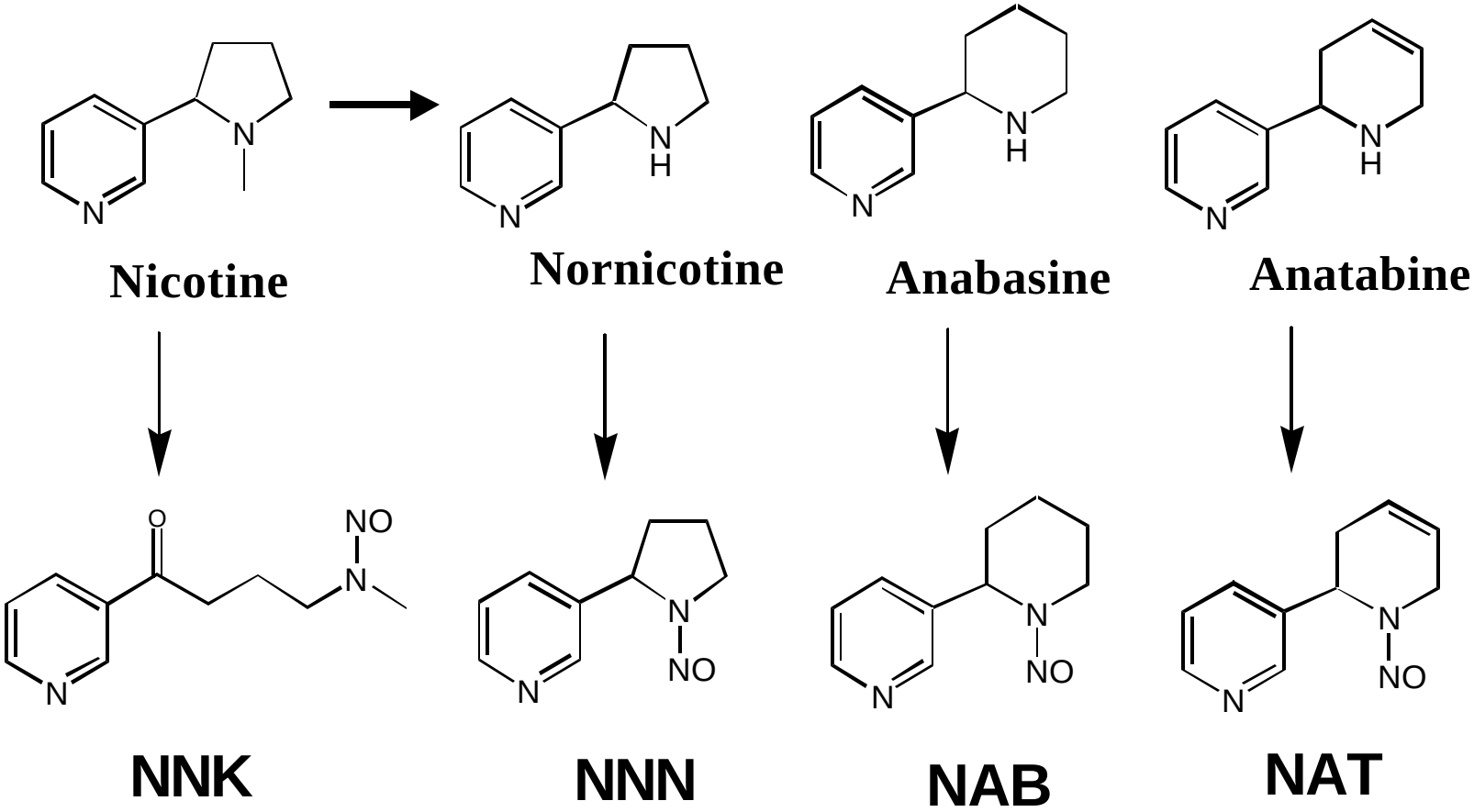
Future Direction for modifying accumulation of TSNA

Decrease accumulation of secondary amine alkaloids

- lower genetic potential
- inhibit nicotine demethylase

Decrease nitrite formation during curing

- inhibit dissimilatory NRA
- reduce fertilizer nitrogen
- use of modified curing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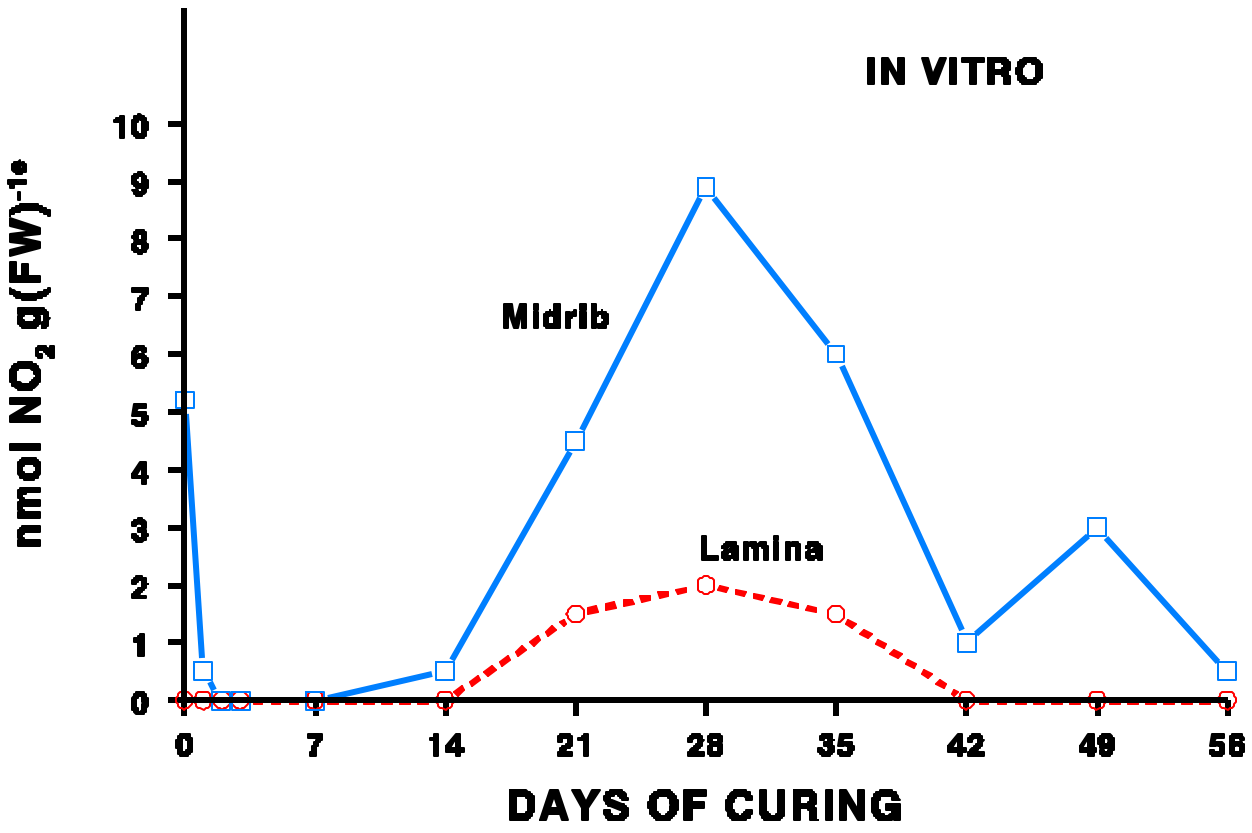
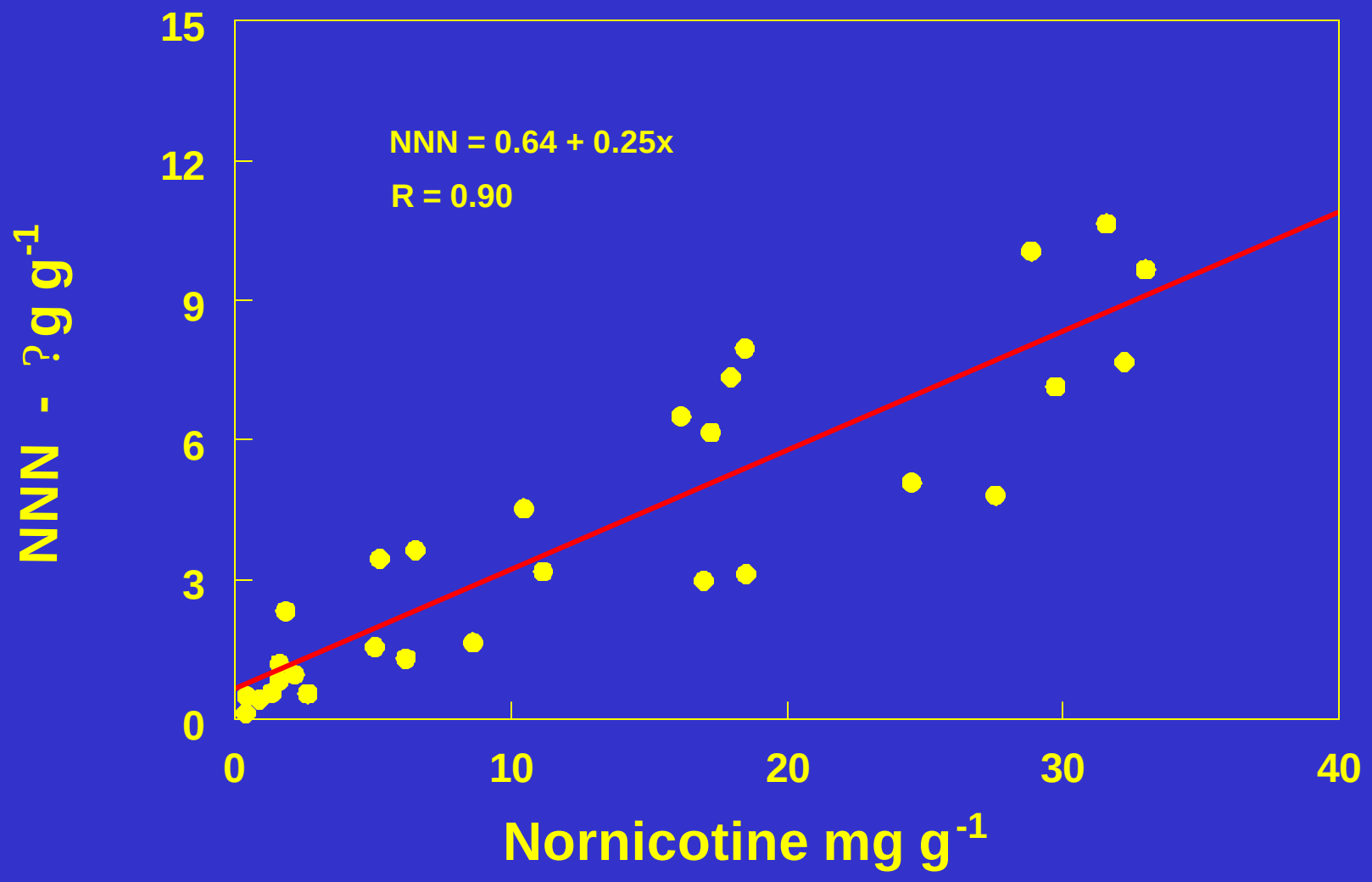


In vitro nitrate reductase activity in lamina and midrib during air curing.


NNN = 0.64 + 0.25x
R = 0.90
NNN - ?g g-1
Nornicotine mg g-1
0
3
6
9
12
15
0
10
20
30
40

Nornicotine content of juvenile leaves air-cured and ethephon treated for 14 days

Cultivar	air-cured % NN	ethephon % NN	R	# plants
KY 17	4.8	11.3	0.87	58
TN 90	5.2	11.1	0.92	57
KY 907	5.1	18.3	0.89	35
KY 14	4.7	9.2	0.94	57

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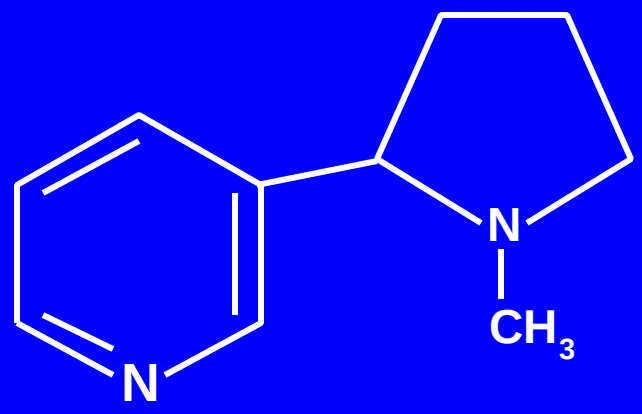

Nicotine

Nornicotine