

**Studies of Barn and Field Curing Environments on TSNA Formation  
in Burley Tobacco<sup>1</sup>**

**Abstract:**

The formation of tobacco specific nitrosamines (TSNAs) in various tobaccos has become a topic of importance in recent years. The significant formation of TSNAs in burley tobacco occurs after the yellowing phase of curing and is dependent on several factors, notably the fertility level during growth and the curing environment during the browning and drying phase. Several field curing studies at the University of Kentucky Agricultural Experimental Station have focused on the relationships of curing environment and the resulting TSNAs of lamina and midrib components. The environmental treatments have included whole plant harvest and natural air curing in a barn and field curing frames, field curing structure with supplemental fan circulation and laboratory chamber curing of 'bulk' leaves in an accelerated eight to ten day schedule. Results show TSNA levels ranging from under 1 ppm up to 22 ppm for the various treatments. Generally, the lower levels of TSNA have resulted from curing in a more rapid drying regime than normal with the resultant leaf quality not as desirable to the buying representatives. Studies are continuing on a combination of cultural practices and curing environments that will produce desirable burley quality and the lowest possible TSNA levels.

**Introduction:**

Unusual variations in certain chemical constituents have been detected by the industry in naturally air-cured burley from different climatic conditions and regional locations for curing. These variations can have significant impact on blending and final product quality and evaluations. Past studies of the chemical constituents of burley and the health related aspects reveal certain tobacco specific nitrosamines (TSNAs) as being important in the cured product and have components that may be manipulated in the curing process. Studies by industry and university scientists have shown that freshly harvested burley contains very low levels of TSNA components but these tend to increase after the yellowing phase (two to three weeks of air curing) and are highly influenced by the curing environment during the latter phase of curing. High temperatures are known to be conducive to TSNA formation during this phase.

**Objectives:**

The series of curing studies reported in this paper are a part of several ongoing projects to study the effects of selected cultural practices, harvesting and curing methods, and curing environments

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on the resulting TSNA of stalk and leaf harvested burley while striving to obtain the best quality ('useable') burley possible.

### **Experimental Methods:**

The 1999 study compared several methods of field and barn curing on the TSNA formation of stalk harvested and cured burley. Several types of plastic covered field structures are becoming more prevalent for curing burley in the central United States due to lower construction costs, reduced manual labor and generally equivalent curing quality compared with conventional air cure barns (Figs. 1-3). The black plastic covering is installed and managed to provide tobacco protection from rain but allow ventilation by raising and lowering the sides as needed. The 1999 study focused on the effect of two locations of the field curing frames (upland with good air movement vs. bottom location with higher humidity), three densities of stick spacing (30, 45 and 60 sticks per 4.3 m frame length in a two stick wide arrangement, each stick containing 6 plants), three plastic covering methods (top protection only, top and one side, top and both sides), and conventional air cure barn (Figs. 1-4). Burley variety TN 90 was grown on the Ky. Agricultural Experiment Station farm using normal cultural practices of fertility (approximately 280 kg/ha of N), pesticide and sucker control management. The growing and curing season was exceptionally dry. Two irrigation applications were applied to the growing crop.

The 2000 study compared two levels of fertility, the field structure and conventional barn curing methods for stalk harvested tobacco and a leaf harvested treatment with four leaf primings cured in an eight day schedule in laboratory chambers. The field frames had 52 sticks on each 4.3 m long frame with a set of frames having top plastic covering only and a set with top and both sides covered. Burley variety TN 90 was grown with one plot having an N fertilizer rate of 250 kg/ha broadcast before transplanting and a second plot having an additional rate of 60 kg/ha N side dressed one month after transplanting. These rates comply with soil test results and general burley production practices.

Temperature and relative humidity data were recorded with HOBOTM H8 Pro two-channel data loggers having an accuracy of  $\pm 0.2$  °C in the range -40 °C to +75 °C and  $\pm 3\%$  RH in the range 0% to 100% (ONSET Computer Corp., Bourne, MA 02532 USA). Validation checks of the relative humidity accuracy were made before and after use and adjustments made in the final data when a data logger deviated more than 2% from a standard value. A data logger was suspended at the mid-stalk position in the curing tobacco of each curing frame treatment. The data logger in the barn was located near the center of three vertical tiers levels of tobacco.

### **Results - 1999:**

The results for the 1999 study include TSNA data for lamina specimens and an industry representative evaluations of quality shown by Table 1. The environmental data are shown by Table 2. Figure 1 shows a conventional air cure burley barn. Figure 2 shows workers filling the field curing frames with harvested tobacco. Figure 3 shows plastic covered field curing frames at

the 'upland' (hill) location. Figure 4 shows two frames in the 'lowland' (bottom) location with one structure in the 'open' and one 'under the shade of trees.'

TSNA data are reported for the lamina portion of sample leaves. TSNA data were determined for the midribs of these curing treatments but are not reported here. The midrib data were very similar to the lamina data for this curing season.

A rainfall gauge located at the 'upland' location recorded only about 38 mm of rain during the eight week curing period - a very dry period for this region. The very dry weather caused too rapid drying of the tobacco and resulted in most of the specimens being graded as 'variegated.'

The dry curing season kept the TSNA components at a very low level due to the rapid drying of the tobacco in the latter stages of the cure. The TSNA for the lamina from the three stalk positions (top, middle, and bottom) ranged from 0.87 to 4.14  $\mu\text{g/g}$  (Table 1).

Three samples were judged 'useable' by a company representative. These had TSNA values of 3.60, 2.72 and 3.28  $\mu\text{g/g}$ . All were the top leaves of the stalks which hung nearest the sod surface during the cure, hence possibly had a higher relative humidity than recorded at the mid-stalk level by the data loggers. The 'best' sample was from the curing frames in the 'low' terrain and under the afternoon shade of trees. The environmental data show this cure had 441 hours of relative humidity above 80% in the 42 days of data monitoring whereas other 'upland' locations had 212 to 280 hours of RH above 80% (Table 2).

The TSNA's of the other treatments were quite varied with stick spacing and method of covering having no clear distinction on the resulting TSNA's. In general, the drier the curing environment, the lower the TSNA's and less desirable the cured tobacco by an industry evaluation.



Fig. 1. Conventional air cure burley barn.



Fig. 2. Filling the field curing frames.



Fig. 3. Plastic covered field curing structure in 'upland' location showing 'open side' and 'closed side' sections.



Fig. 4. Plastic covered field curing structure in 'lowland' location with one in the 'open' and one under the 'shade' of trees.

**Table 1. TSNA of burley lamina for curing treatments shown by Table 2.**

Treatment	Stalk ----- µg/g -----						Avg.	Co.Eval.
	Pos.	NNN	NAT	NAB	NNK	TSNA		
Barn Cure	Top	1.12	0.78	ND	0.19	2.09		*
	Mid.	1.80	1.44	ND	0.25	3.48		*
	Bott.	1.20	1.08	ND	0.24	2.52	2.70	*
0s, Tob., 48	Top	1.73	1.13	ND	0.42	3.28		'Useable'
	Mid.	1.65	1.53	ND	0.44	3.63		*
	Bott.	1.26	1.33	ND	0.36	2.96	3.29	*
0s, Tob, 64	Top	0.91	0.63	ND	0.14	1.68		*
	Mid.	2.04	0.96	ND	0.20	3.21		*
	Bott.	1.85	1.09	ND	0.16	3.10	2.66	*
1s, Tob, 48	Top	0.80	0.54	ND	0.15	1.49		*
	Mid.	1.20	1.00	ND	0.20	2.40		*
	Bott.	0.97	1.01	ND	0.23	2.21	2.03	*
1s, Tob, 64	Top	0.53	0.30	ND	0.07	0.91		*
	Mid.	1.64	1.10	ND	0.18	2.92		*
	Bott.	1.48	1.09	ND	0.21	2.77	2.20	*
2s, Tob, 48	Top	1.29	0.92	ND	0.16	2.38		*
	Mid.	1.58	1.34	ND	0.19	3.11		*
	Bott.	1.81	1.38	ND	0.21	3.39	2.96	*
2s, Tob, 64	Top	0.62	0.17	ND	0.07	0.87		*
	Mid.	0.95	0.91	ND	0.17	2.03		*
	Bott.	1.25	0.99	ND	0.17	2.42	1.77	*
0s, Tob, 48, O	Top	1.37	1.06	ND	0.29	2.72		2 <sup>nd</sup> best
	Mid.	1.37	1.40	ND	0.27	3.05		*
	Bott.	1.61	1.59	0.07	0.92	4.14	3.30	*
0s, Tob, 48, S	Top	1.78	1.37	0.05	0.43	3.60		Best
	Mid.	1.62	1.79	ND	0.36	3.77		*
	Bott.	1.08	1.15	ND	0.15	2.38	3.25	*

Treatments explained in Table 2.

\* = Evaluated by Industry Buyer as 'variegated' and undesirable due to 'fast curing' in dry weather.

**Table 2. Summary- Environmental Data for Burley Curing Study, 1999**

<u>Treatment</u>	<u>MaxT</u>	<u>MinT</u>	<u>DyAvT</u>	<u>AvTMx</u>	<u>AvTMn</u>	<u>AvRH</u>	<u>AvHRh</u>	<u>AvLRh</u>	<u>HrRH&gt;80</u>	<u>HrRH&lt;50</u>
Amb.,Hill	36.6	2.9	20.7	30.1	12.4	59	93	32	281	420
Barn Cure	32.3	7.0	21.4	27.1	16.3	59	88	38	162	371
0s,Tob,48	37.0	4.2	20.9	29.5	13.3	60	88	33	276	359
0s,Tob,64	35.7	4.2	20.8	29.3	13.3	60	88	33	280	355
1s,Tob,48	37.4	3.7	21.3	30.7	13.3	59	88	32	258	384
1s,Tob,64	37.0	4.2	21.3	30.4	13.4	59	87	33	254	374
2s,Tob,48	41.1	2.9	22.1	34.4	12.9	57	87	31	234	393
2s,Tob,64	42.9	3.3	22.0	33.2	13.5	58	87	32	212	365
Amb.,Bott.O	34.9	-2.0	19.2	29.3	9.4	63	92	32	364	365
Amb.,Bott.S	35.7	0.7	19.9	29.6	11.4	64	91	32	382	335
0s,Tob,48,O	34.0	-0.6	19.7	28.3	10.9	62	90	35	311	355
0s,Tob,48,S	34.9	-2.0	19.1	28.7	9.7	67	92	34	441	315

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**Treatments:**

Ambient, Hill = Ambient temp. and relative humidity at upland (hill) location

Barn Cure = Temp. and R.H. within tobacco in conventional air cure barn

0s = Side plastic not covering tobacco of field structure (top cover only)

1s = Side plastic covering tobacco on one side of field structure (toward wind)

2s = Side plastic covering tobacco on both sides of field structure

Tob = Temperature and relative humidity within tobacco, about mid-stalk level

48, 64 = Number of sticks of tobacco per 4.3 m curing frame length

Amb.,Bott.O = Ambient Temp. and R.H. in low terrain (bottom) location, open area

Amb.,Bott.S = Amb.Temp. and R.H. in low terrain (bottom) location, in shade of trees

**Data:**

MaxT, MinT = Maximum and minimum temp. during 42 day curing period, °C

DyAvT = Daily average temperature, °C

AvTMx, AvTMn = Average of Maximum and minimum daily temperatures, °C

AvRH = Average daily relative humidity, %

AvHRh, AvLRh = Average of maximum and minimum daily R.H., %

HrRH>80 = Total hours R.H. above 80 percent (42 days x 24 hrs/day = 1008 hrs possible)

HrRH<50 = Total hours R.H. below 50 percent

## Results - 2000:

The results for the 2000 study include TSNA data for lamina specimens from stalk and leaf curing trials, and USDA AMS grade of quality shown by Table 3. The environmental data are shown by Table 4. Figure 5 shows the plastic covered field curing frames used for this study. Figure 6 shows a laboratory chamber and metal containers for the leaf curing trials.

TSNA data were determined for the midribs of these curing treatments but are not reported here. The midrib data ranged below and above the lamina data for this curing season.

The 2000 growing and curing season was more normal with rainfall of over 380 mm. Irrigation was not needed during the growing season.

The TSNA data of Table 3 show a general trend of the side dressed treatments (60 kg/ha N above the broadcast rate of 250 kg/ha) having higher total TSNA levels than the non side dressed for the stalk harvested and cured treatments, ranging from 6.2 to 8.0 µg/g vs. 2.3 to 5.8 µg/g for three treatments. A fourth treatment was essentially the same (2.9 µg/g and 3.0 µg/g). The extra N fertilizer apparently resulted in additional nitrates and nitrites converted to TSNA's during the cure.

The open side field curing frame treatment (SPF0 and NPF0) had the lowest set of TSNA levels (2.9 and 3.0 µg/g respectively) but were comparable to the non side dressed barn and two sides covered frame treatment cures (2.3 and 2.8 µg/g, respectively).

The stalk curing treatment in an environmentally controlled chamber with cyclic temperature and relative humidity settings to approximate typical ambient conditions produced slightly higher TSNA's than the corresponding outside frame and barn curing treatments, even though the total hours of relative humidity above 80% were considerably less than the other environments. This leads to speculation that the cycles of lower humidity in the natural environment might interrupt the growth of microbial organisms and retard the chemical conversions better than a more even humidity level used in the chamber curing environment.

The leaf harvested and eight day cured treatments were higher than the stalk harvested and air cured, being 9.3 µg/g and 11.0 µg/g, respectively, for the side dressed and non side dressed treatments. The quality of this cured leaf was typical of experimental 'bulk cured burley' with variegated, 'slick' surface and yellowish colored leaves. Other results in this series of leaf curing treatments had TSNA's as high as 22 µg/g.

The environmental data of Table 4 show considerably more hours of higher humidities (RH>80%) for the 2000 curing season compared with the unusually dry 1999 season. The higher relative humidity produced a better quality cured leaf but also resulted in higher TSNA levels (generally 2.3 to 8.0 µg/g vs. 2 to 4 µg/g).

Yield data (kg/ha) were not determined for these past studies but the tobacco size and general weight was comparable to normal tobacco. Yield data will be determined for ongoing and future studies.



Fig. 5. Plastic covered field curing frames for the 2000 study.



Fig. 6. View of metal boxes with plexiglass front in laboratory environmental chamber used for leaf curing trials.

**Table 3. TSNA of burley lamina for 2000 curing treatments shown by Table 4 and Fig. 7.**

<u>Treatment</u>	Stalk <u>Pos.</u>	----- $\mu\text{g/g}$ -----					<u>USDA Grade</u>
		<u>NNN</u>	<u>NAT</u>	<u>NAB</u>	<u>NNK</u>	<u>TSNA</u>	
SPB	2 <sup>nd</sup>	2.68	2.95	0.23	0.79	6.7	C3F
NPB	2 <sup>nd</sup>	1.16	0.8	0.33	0.00	2.3	B4F
SPF2	2 <sup>nd</sup>	2.84	2.71	0.00	0.69	6.2	B3F
NPF2	2 <sup>nd</sup>	1.04	1.18	0.00	0.55	2.8	B4F
SPF0	2 <sup>nd</sup>	0.96	1.35	0.00	0.60	2.9	C3F
NPF0	2 <sup>nd</sup>	0.99	1.42	0.00	0.61	3.0	C3F
SPC	2 <sup>nd</sup>	3.21	4.14	0.16	0.49	8.0	C4F
NPC	2 <sup>nd</sup>	2.46	2.65	0.16	0.51	5.8	B3F
SLC	2 <sup>nd</sup>	4.21	4.67	0.25	0.19	9.3	C4K
NLC	2 <sup>nd</sup>	5.31	5.13	0.27	0.33	11.0	C4K

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 All data an average of three samples per treatment.

**Table 4. Summary- Environmental Data for Burley Stalk Curing Study, 2000**

<u>Treatment</u>	<u>MaxT</u>	<u>MinT</u>	<u>DyAvT</u>	<u>AvTMx</u>	<u>AvTMn</u>	<u>AvRH</u>	<u>AvHRh</u>	<u>AvLRh</u>	<u>HrRH&gt;80</u>	<u>HrRH&lt;50</u>
SPB	(No environmental data for barn cured)									
NPB	(No environmental data for barn cured)									
SPF2	29.5	-1.1	14.8	22.7	8.5	88	95	44	750	30
NPF2	27.5	-1.5	14.7	22.3	8.5	84	94	41	664	59
SPF0	28.3	-2.0	14.3	21.9	8.3	78	93	39	551	108
NPF0	27.5	-1.6	14.4	21.0	8.8	82	94	40	627	67
SPC	27.6	8.6	20.3	24.4	17.0	72	85	43	211	1
NPC	27.9	8.6	20.3	24.5	16.8	69	85	48	212	11

(Note: these data for SPC and NPC represent only 37 days of curing time vs. 40 for the others)

SLC See Fig. 7

NLC See Fig. 7

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**Treatments:**

SPB = Side dressed with N, Plant harvest, Barn cured

NPB = Not Side dressed, Plant harvest, Barn cured

SPF2 = Side dressed with N, Plant harvest, Field Frame cured with 2 sides covered

NPF2 = Not Side dressed, Plant harvest, Field Frame cured with 2 sides covered

SPF0 = Side dressed with N, Plant harvest, Field Frame cured with no sides covered

NPF0 = Not Side dressed, Plant harvest, Field Frame cured with no sides covered

SPC = Side dressed with N, Plant harvest and chamber cure with simulated natural air schedule

NPC = Not Side dressed, Leaf harvest and chamber cure with simulated natural air schedule

SLC = Side dressed with N, Leaf harvest and chamber cure with 8 day schedule

NLC = Not Side dressed, Leaf harvest and chamber cure with 8 day schedule

(No environmental data for barn cure)

**Data:**

2<sup>nd</sup> = Second grade of four from stalk, approximately leaves #6-10 from bottom of stalk

MaxT, MinT = Maximum and minimum temp. during 42 day curing period, °C

DyAvT = Daily average temperature, °C

AvTMx, AvTMn = Average of Maximum and minimum daily temperatures, °C

AvRH = Average daily relative humidity, %

AvHRh, AvLRh = Average of maximum and minimum daily R.H., %

HrRH>80 = Total hours R.H. above 80 percent (42 days x 24 hrs/day = 1008 hrs possible)

HrRH<50 = Total hours R.H. below 50 percent

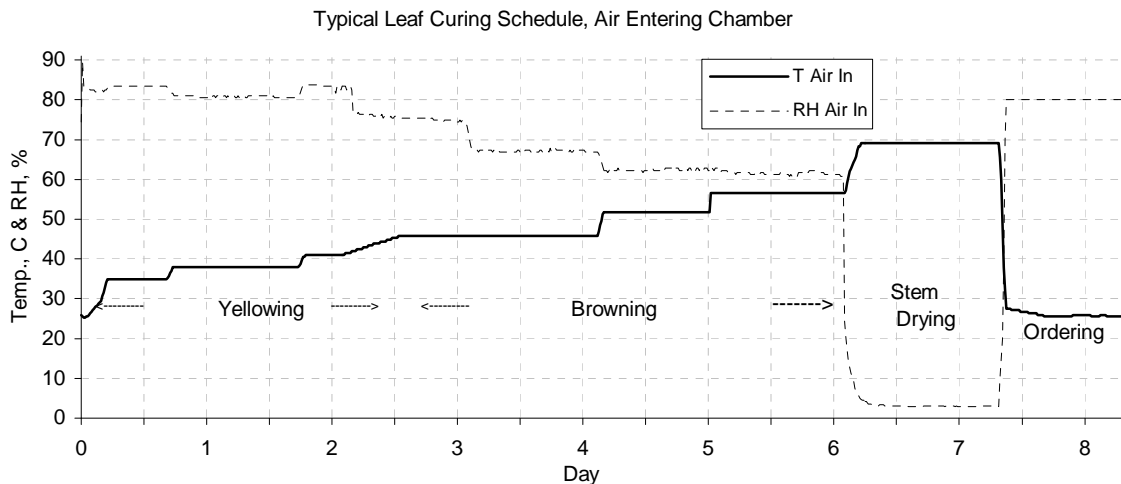


Fig. 7. Typical curing schedule used for leaf curing treatments.

### Summary:

The studies and data reported herein are part of ongoing studies aimed at seeking the best management, cultural and curing practices to reduce the TSNAs in burley tobacco while obtaining the best quality possible to meet the industry's needs. These studies collaborate the work of others in that judicious use of N fertilizer and ensuring a good drying environment during the latter stages of curing can minimize the TSNAs in burley leaf and produce total TSNA in the range of 2-4  $\mu\text{g/g}$  and provide useable burley quality. Studies are continuing on improving the methods and management of the curing environment, the effects of reduced fertility levels on yield and TSNAs and other innovative methods to reduce TSNAs and provide the best possible quality to meet the needs of burley industry customers.

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