UK UNIVERSITY OF KENTUCKY
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Ongoing Studies of Barn, Field and Chamber Curing Environments on TSNA Formation in Burley Tobacco[1]

Abstract:

The study of the formation of tobacco specific nitrosamines (TSNAs) in various tobaccos continues as a topic of importance to the tobacco industry. 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 later stages of curing and post-curing conditions. Certain ongoing curing studies at the University of Kentucky Agricultural Experimental Station have focused on the relationships of curing environment and the resulting TSNAs of lamina. The environmental treatments have included whole plant harvest and natural air curing in a barn, in field curing structures, and in laboratory chambers with controlled temperature, relative humidity and air flow. Results show TSNA levels ranging from 1 ppm up to 46 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:

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. Freshly harvested burley has been shown to contain 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, especially high moisture, during the latter phase of curing and on through packaging and storage.

The investigation reported in this article (No. 02-05-185) is in connection with a project of the Kentucky Agricultural Experiment Station and is published with approval of the Director.

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Objectives:

These studies have investigated the effects of selected cultural practices, harvesting and curing methods, and curing environments on the resulting TSNA of stalk and leaf harvested burley while striving to obtain the best quality ('useable') burley possible.

Experimental Methods:

The 2000 and 2001 studies reported in this paper compared two levels of field fertility and three methods of curing on the resulting TSNA of stalk harvested tobacco. One method of curing was the use of field frames which held 50-52 sticks, six plants per stick, on each 4.3 m long frame (Fig. 1,2). One set of frames had plastic over the top to cover the tobacco and a second set had plastic over the top and down both sides to cover the tobacco. The second method of curing was in a typical conventional burley barn (Fig 3). A third method to provide more exact control of the curing environment involved two small laboratory chambers which held approximately 10 sticks of tobacco in each chamber with automatic controlled environmental conditions. The year 2000 controlled cure was set to represent a typical diurnal cycle of outdoor temperature of 9-28 deg. C and relative humidity of 55-90 percent. The year 2001 controlled cure was set to maintain an average of 75 percent relative humidity and 25 deg. C.

Burley variety TN 90 was grown in both years with one plot of year 2000 having a nitrogen 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. In 2001, one plot had 112 kg/ha of N fertilizer broadcast and side dressed with an additional 168 kg/ha of N fertilizer whereas the other plot had only the 112 kg/ha of broadcast N fertilizer. These rates comply with soil test results and general burley production practices, including application of small amounts of potash and phosphorus fertilizers. The lower rate for year 2001 was to study the effect of reduced N on TSNA levels and cured leaf yields.

Temperature and relative humidity data were recorded with HOBO® H8 Pro two-channel data loggers having an accuracy of +-0.2 °C in the range -40 °C to +75 °C and +- 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 environmental data was summarized for the first five weeks of curing which typically produces the desired brown color of the cured leaves with only midrib drying remaining

Joint Meeting of the Agronomy & Phytopathology Study groups held at the Fairmont Hotel, New Orleans, LA, September 23-27, 2002.

thereafter. The tobacco of these studies typically remained hanging in the curing structures or barn for another four to six weeks until stripping and sample taking were accomplished.

Results - 2000:

The results for the year 2000 study include TSNA data for lamina specimens from the cured leaves of the second grade position on the stalk (approximate leaves #5-10 from the bottom of the stalk) and USDA AMS grade quality shown by Table 1. The environmental data are shown by Table 2 .

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 had rainfall of over 380 mm. Irrigation was not needed during the growing season.

The TSNA data of Table 1 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 had essentially the same TSNA values (2.9 µg/g and 3.0 µg/g). The extra N fertilizer apparently resulted in additional nitrates and nitrites converted to TSNAs during the cure.

The stalk curing treatment in environmentally controlled chambers with cyclic temperature and relative humidity settings to approximate typical ambient conditions produced slightly higher TSNAs 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.for the first five weeks of curing.

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

Fig. 1. Plastic covered field curing structures.

Fig. 2. Filling the field curing frames.

Fig 3. Conventional air cure burley barn.

Fig. 4. Lab environmental chambers for controlled curing.

Table 1. TSNA of lamina for 2000 burley stalk curing treatments.

	Stalk	---------- µg/g ----------					USDA
Treatment	**Pos.**	**NNN**	**NAT**	**NAB**	**NNK**	**TSNA**	**Grade**
SPB	2nd	2.68	2.95	0.23	0.79	6.7	C3F
NPB	2nd	1.16	0.80	0.00	0.33	2.3	B4F
SPF2	2nd	2.84	2.71	0.00	0.69	6.2	B3F
NPF2	2nd	1.04	1.18	0.00	0.55	2.8	B4F
SPF0	2nd	0.96	1.35	0.00	0.60	2.9	C3F
NPF0	2nd	0.99	1.42	0.00	0.61	3.0	C3F
SPC	2nd	3.21	4.14	0.16	0.49	8.0	C4F
NPC	2nd	2.46	2.65	0.16	0.51	5.8	B3F

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All data an average of three samples per treatment.

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, Controlled environment cured
NPC = Not side dressed, Plant harvest, Controlled environment cured

Data:

2nd = Second grade of four from stalk, approximately leaves #5-10 from bottom of stalk

Table 2. Summary of environmental data for 2000 burley stalk curing study.

Treatment	MaxT	MinT	AvDyT	AvTMx	AvTMn	AvRH	AvHRH	AvLRH	HrRH>85	HrRH<60
Weather	28.9	-1.1	15.2	21.6	9.6	73	93	47	290	211
SB2*, NB2*										
SPF2	31.1	-1.1	15.3	23.1	9.1	85	94	52	522	64
NPF2	32.3	-1.5	15.1	22.5	9.1	85	96	49	497	83
SPF0	28.7	-2.0	14.7	22.2	8.9	80	95	48	427	152
NPF0	31.5	-1.5	14.8	21.4	9.3	78	92	47	393	142
SPC	27.5	8.6	20.1	24.4	16.8	72	88	56	175	127
NPC	27.9	8.6	20.0	24.5	16.4	70	89	57	181	383

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Treatments:

Weather = Data from Univ. of Ky. weather station about 1 km from tobacco site.
*No barn data due to equipment malfunction.
See Table 1 for remaining treatment descriptions

Data:

MaxT, MinT = Maximum and minimum temp. during first 35 days of curing period, °C
AvDyT = Average daily temperature, °C
AvTMx, AvTMn = Average of Maximum and minimum daily temperatures, °C
AvRH = Average daily relative humidity, %
AvHRh, AvLRh = Average of high (maximum) and low (minimum) daily R.H., %
HrRH>85 = Total hours R.H. above 85 percent (35 days x 24 hrs/day = 840 hrs possible)
HrRH<60 = Total hours R.H. below 60 percent (35 days x 24 hrs/day = 840 hrs possible)

Results, 2001:

The results for the 2001 study include TSNA data for lamina specimens from the cured leaves of four stalk positions and USDA AMS grade quality shown by Table 3. The environmental data are shown by Table 4.

The 2001 growing and curing season had low natural rainfall the first month after transplanting (52 mm rainfall) but adequate rainfall the next two months (234 mm). One half of the plots was irrigated once in the fourth week after transplanting with 43 mm but the data reported herein are for the non irrigated plots and treatments.

Overall, the TSNA data of Table 3 show generally higher total TSNA levels than for the previous year for comparable 2nd stalk position treatments even though the average relative humidities of the curing environments were lower and the total hours of RH>85% was lower. The 'normal' fertility treatment of 280 kg/ha produced much higher levels of TSNA than the lower N fertility for the barn cure (19.6 μg/g vs. 9.4 μg/g), field frame with two sides covered (38.8 μg/g vs. 7.1 μg/g) and controlled environment chambers (2.3 μg/g vs. 1.7 μg/g). Only the field curing frames with one side only covered had an opposite result on the TSNA levels (10.8 μg/g for side dress vs. 18.9 μg/g for the lower rate fertility). The averages for all four

grades of the total stalk followed these same trends. The stalk curing treatments in the environmentally controlled chambers again produced the lowest levels of TSNAs compared with the corresponding barn and outside frame curing treatments.

All USDA AMS grades for the stripped lamina were typical of marketable tobacco and very acceptable for the crop. Cure leaf yield was affected by wind damage during the latter stages of curing on the field frames and not considered reliable for presentation.

The environmental data shows comparable conditions for all the field treatments with average daily relative humidities of 75 to 79 percent. The controlled environment chambers had averages of 78 and 80 percent relative humidity, slightly higher than the programmed set points but within the variation of instrumentation controls and data loggers.

Two Year Data Comparison:

The data of the two years presented above are combined in Fig. 5 to show graphically the results of fertility and curing treatments on TSNAs.

Table 3. TSNA of burley lamina for 2001 curing treatments.

Treatment	Stalk Pos.	µg/g NNN	NAT	NAB	NNK	Trtmt. Total	USDA Avg.	Grade
SPB-1	1st	12.3	11.6	0.4	0.7	25.0		C4F
SPB-2	2nd	6.0	12.8	0.5	0.3	19.6		C2F
SPB-3	3rd	6.9	11.4	0.5	0.3	19.2		B3FR
SPB-4	4th	4.8	6.7	0.3	0.5	12.4	19.1	B4FR
NPB-1	1st	3.1	4.4	0.2	0.2	7.9		C4F
NPB-2	2nd	3.4	5.6	0.2	0.2	9.4		C3F
NPB-3	3rd	3.3	2.0	0.1	0.1	5.4		B3F
NPB-4	4th	0.9	0.8	0.0	0.1	1.8	6.1	B4FR
SPF2-1	1st	12.6	12.8	0.5	1.0	27.0		C4F
SPF2-2	2nd	16.9	19.8	0.8	1.2	38.8		B3F
SPF2-3	3rd	20.3	24.1	1.1	1.0	46.4		B3F
SPF2-4	4th	8.5	7.1	0.3	0.6	16.5	32.2	B4FR
NPF2-1	1st	8.2	9.5	0.4	0.9	19.0		C4F
NPF2-2	2nd	2.3	4.3	0.2	0.3	7.1		C3F
NPF2-3	3rd	3.4	4.2	0.2	0.2	7.9		B4F
NPF2-4	4th	6.0	2.0	0.1	0.2	8.3	10.6	B4FR
SPF1-1	1st	4.2	8.2	0.3	0.7	13.4		C4F
SPF1-2	2nd	6.0	4.4	0.2	0.2	10.8		C4F
SPF1-3	3rd	2.6	5.7	0.2	0.2	8.8		B4F
SPF1-4	4th	2.3	2.8	0.1	0.2	5.4	9.6	B4FR
NPF1-1	1st	5.5	11.2	0.4	0.6	17.8		X4F
NPF1-2	2nd	8.0	10.1	0.4	0.4	18.9		C3F
NPF1-3	3rd	3.3	2.3	0.1	0.1	5.7		B3F
NPF1-4	4th	4.5	3.6	0.2	0.2	8.5	12.7	B5FR
SPC-1	1st	3.7	1.9	0.1	0.2	6.0		- - - -
SPC-2	2nd	1.0	1.0	0.0	0.2	2.3		B2F
SPC-3	3rd	2.0	1.5	0.1	0.2	3.8		B2F
SPC-4	4th	1.4	1.1	0.0	0.1	2.6	3.7	B4FR
NPC-1	1st	1.7	1.0	0.1	0.1	2.9		- - - -
NPC-2	2nd	1.0	0.7	0.0	0.0	1.7		B2F
NPC-3	3rd	0.6	0.3	0.0	0.0	1.0		B2FR
NPC-4	4th	0.8	0.3	0.0	0.0	1.2	1.7	B3FR

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All data an average of three samples per treatment except SPC & NPC an avg. of 2 reps.

Treatments:

Same as Table 1 except following treatments used instead of SPF0, NPF0:

SPF1 = Side dressed with N, Plant harvest, Field Frame cured with one side covered

NPF1 = Not Side dressed, Plant harvest, Field Frame cured with one side covered

Data:

1st = First grade of four from stalk, approximately first 3-4 leaves from bottom of stalk

2nd = Second grade of four from stalk, approximately next 4-6 leaves of stalk

3rd = Third grade of four from stalk, approximately next 4-6 leaves of stalk

4th = Fourth grade of four from stalk, approximately top 3-4 leaves of stalk

Table 4. Summary- Environmental Data for Burley Stalk Curing Study, 2001

Treatment	MaxT	MinT	DyAvT	AvTMx	AvTMn	AvRH	AvHRH	AvLRH	HrRH>85	HrRH<60
Weather	28.9	0.6	14.9	20.9	9.4	68	91	48	183	277
SB2*										
NB2*										
SPF2	26.7	1.1	15.0	21.6	9.1	76	92	50	313	147
NPF2	28.3	1.1	15.3	21.7	9.2	75	92	50	288	176
SPF1	26.3	1.1	14.3	20.1	9.2	78	95	51	343	164
NPF1	26.3	1.1	14.2	19.8	9.1	79	94	52	366	133
SPC	24.8	24.0	24.5	--	--	80	--	--	0	0
NPC	24.8	23.6	24.4	--	--	78	--	--	0	0

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Treatments:

See Table 3.

Data:

See Table 2.

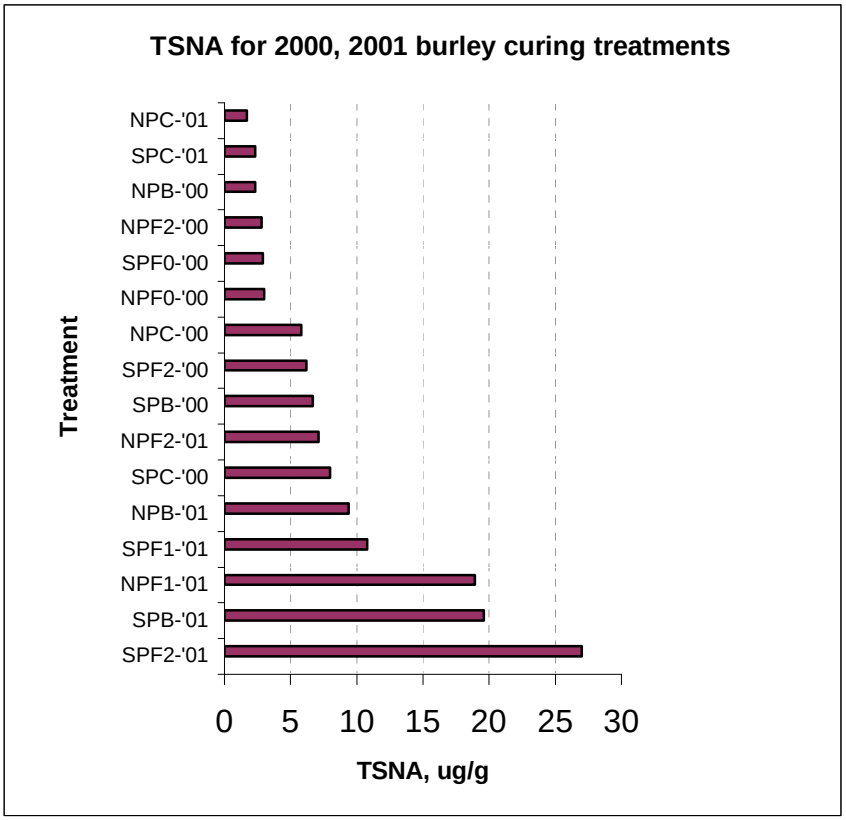

Fig. 5. Graph of TSNA results for 2000 and 2001 fertility and curing treatments.

Discussion and further results:

These data do not clearly reveal a phenomena that appears to be happening after the leaves have cured to the characteristically brown color and hang awaiting the stripping and market preparation time by tobacco producers. When the cured tobacco was being removed from the controlled environment chambers, several leaves were showing spots of 'white mold' which often occurs with tobacco hanging in a humid environment after the primary curing period and color changes have ended. Some of the tobacco in these chambers was retained intact until the TSNA test results from the first two replications of samples were obtained. The low TSNA results were quite favorable (generally 1.0 to 3.8 ug/g with one 6.0 value) After consultation with the tobacco company cooperators, a further test of the remaining stalk cured samples was subjected to an eight day period of two days (48 hours) of high relative humidity (approx. 90%) followed by two days (48 hours) of low relative humidity (approx. 40%) at a temperature of 22°C. The remaining leaf samples were taken at the end of eight days, or after two repeat cycles of these high-low humidity conditions. The TSNA results jumped to values ranging from 11 to 29 ug/g for the non side dressed treatment and 14 to 34 ug/g for the side dressed treatments. With these interesting results, a further investigation was quickly organized to explore this post curing effect of high humidity on TSNA. A quantity of cured tobacco that had been hanging in a barn for three months during the winter months was obtained and tested for initial TSNA levels which were around 2-3 ug/g - low values and a great specimen to use! A repeat of the above two day high humidity and two day low humidity cycle at 22°C was conducted with samples of this tobacco. Table 6 shows the results of the progressive increases of the TSNA due to these cyclic conditions. This trial gives strong evidence that the periods of high relative humidity during the post-curing and pre-market preparation have an important effect on the final TSNA levels. Thus, if samples are taken shortly after the completion of lamina browning and midrib dry-down, the TSNA values could be quite different from what will occur one to two months later at stripping and marketing time when typical periods of rain, fog and high humidities occur in the USA burley producing region.

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 μg/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.

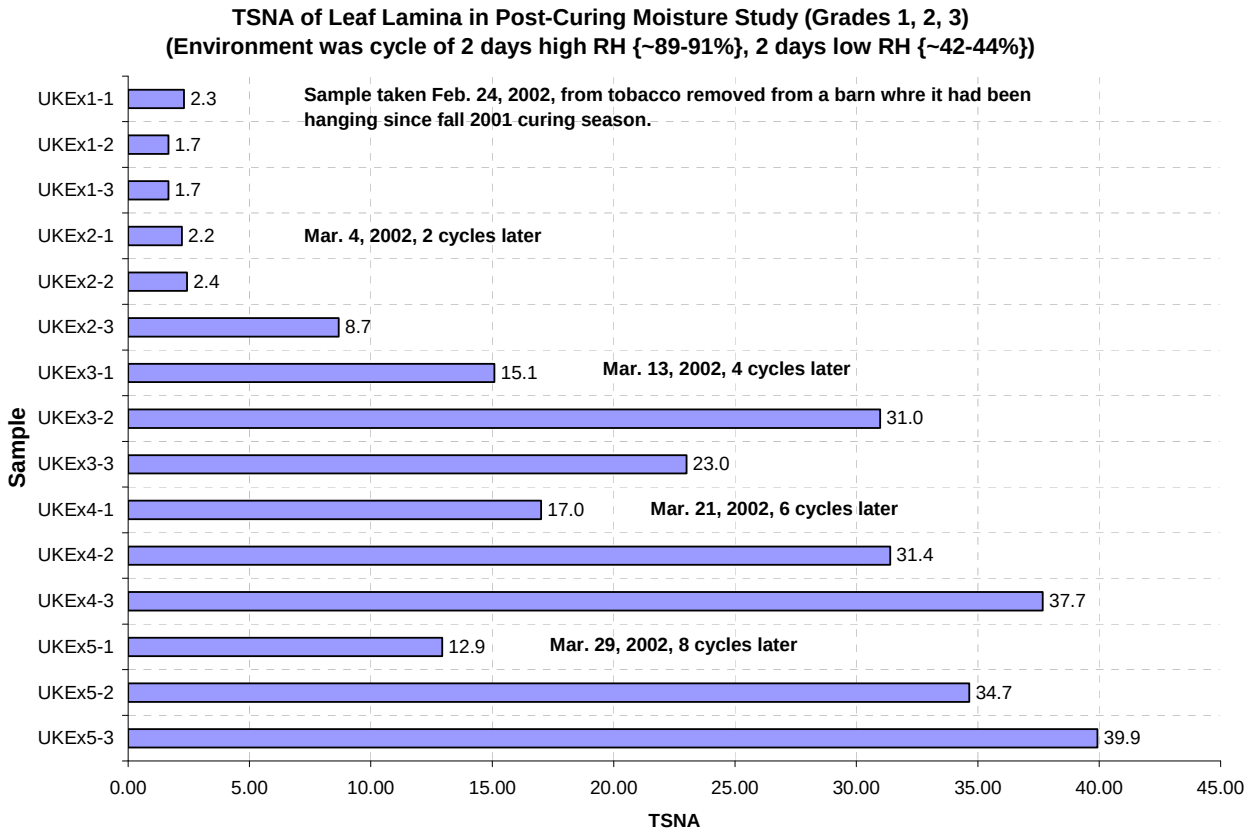

Fig. 6. Results of high humidity cycles on cured burley tobacco.

Acknowledgements:

We gratefully acknowledge the support and cooperative efforts of Lorillard Tobacco Co. in conducting these studies. We extend a hearty thanks and appreciation to the College of Agriculture Farm Managers and workers for the production, harvesting and stripping of the tobacco, and to the technicians of the Agronomy and Biosystems and Agricultural Engineering Depts. for the special assistance rendered.

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