

**Further Studies of Fertility levels and Barn and Chamber Curing Environments on TSNA Formation in Burley Tobacco<sup>1</sup>**

**Abstract:**

This paper reports on the third year of a series of studies involving burley production practices and barn and chamber curing methods on the formation of tobacco specific nitrosamines (TSNAs). The TSNA formation 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 and moisture during the later stages of curing and post-curing conditions. The 2002 field production included three levels of nitrogen fertility: 112, 224, and 336 kg/ha in a drought growing season with three applications of irrigation. The environmental treatments included whole plant harvest and natural air curing in a barn, in a fan ventilated chamber in the barn and in laboratory chambers with controlled temperature, relative humidity and air flow. Results show TSNA levels ranged from less than 1 ppm up to 7.2 ppm for an individual replication of the various treatments. A three-way ANOVA of the results showed significance at the 95% level for the curing and grade treatments and interactions of cure x fertility, fertility x grade and cure x fertility x grades. The fertility treatment was not significant at the 95% level as the P value of 0.055 was just 0.005 beyond the 0.05 level taken for significance. The controlled environment laboratory chamber cure produced the lowest overall TSNA result with a mean of 1.67 ppm. The barn cure with natural weather conditions produced the highest overall TSNA value with a mean of 2.44 ppm. The barn chamber with forced air curing produced an overall TSNA mean of 2.26 ppm. The effects of the fertility levels were inconclusive from this study.

**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. 04-05-149) is in connection with a project of the Kentucky Agricultural Experiment Station and is published with approval of the Director.

<sup>1</sup> G. A. Duncan, J. Calvert, Extension Professor and Research Specialist, respectively, Univ. of KY College of Agriculture, D. Smith, and D.C. Middleton, Leaf Supervisor and Senior Research Chemist, respectively, Lorillard Tobacco Company, Greensboro, NC, USA. For presentation at the 2004 CORESTA CONGRESS, Kyoto, Japan, 3-7 October 2004.

## Objectives:

This study was a continuation of studies on cultural practices and curing environments on the TSNA formation in burley tobacco (Duncan et. al. 2001, Duncan et. al. 2002) and focused on the effects of various levels of fertility and curing methods on the resulting TSNA of burley leaf. The treatments were:

1. 3 fertility levels: "normal" nitrogen fertilizer levels, approximately 75% of normal ("mid") and 50% of normal ('low') nitrogen,
2. 3 curing environments:
  - natural air cure in conventional barn
  - forced air ventilation curing in enclosed chamber in barn, and
  - laboratory chamber with controlled temperature, relative humidity and air circulation

## Experimental Methods:

Normal nitrogen fertility for burley is around 280-336 kg/ha (250-300 lbs N/ac), per University of Kentucky recommendations, depending on soil types. Other fertility and pH levels are based on soil tests. For this study, nitrogen rates of 336 kg/ha ('Norm.'), 224 kg/ha ('Mid.') and 112 kg/ha ('Low') were used.

The fertilizer was broadcast 3 June 2002. Variety TN 90 plants were transplanted the same afternoon. The plots were non-replicated and assigned as shown by Fig. 1.

Another Plot	<b>224 kg/ha N,</b> 202 kg/ha K, Phos. test High, pH=6.3	<b>112 kg/ha N,</b> 202 kg/ha K, Phos. test high, pH=6.3	<b>336 kg/ha N,</b> 202 kg/ha K, Phos. test High, pH=6.3
--------------	---	---	---

Fig. 1. Layout of plots for 2002 study.

Normal cultural practices for insect, disease and sucker control were followed throughout the production season. Plant topping was performed on 21 August and harvest was done on 17 September with placement in the barn and curing chambers on 19 September.

Temperature and relative humidity data for the curing environments were recorded with HOBO® 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 for these instruments were made before and after use. The curing methods are shown by Fig. 2, a conventional burley barn, Fig.

3, a fan ventilated plastic enclosed chamber within the same barn and Fig. 4, one of two laboratory environmental chambers.

The fan ventilated chamber in the barn was 5.2 m by 3.9 m horizontal dimension and had controls set to activate the top fan to pull fresh air in from under the roof and force through the tobacco, exiting at the bottom near the ground, during daytime hours of 9:00 am to 6:00 pm. The fan was sized to provide an air exchange in the chamber approximately once every two minutes. The recirculation fan had similar capacity and was set to operate



Fig. 2. Conventional barn curing

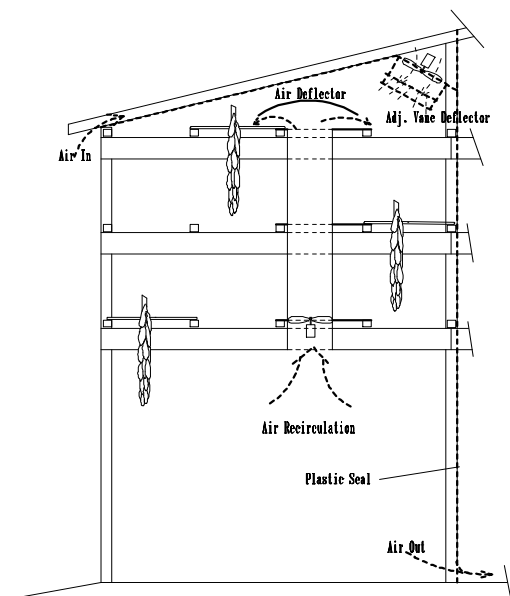


Fig. 3. Plastic enclosed fan ventilated chamber within barn.

Fig. 4. Tobacco cured in laboratory environmental chamber.

during the night time when the relative humidity within the tobacco was above 80 percent. The controls of the lab chambers were set for a daily cycle of 10 to 21°C (50 to 70 °F) temperature and 40 to 90% relative humidity for the first week, then set to 15.6 to 26.7 °C (60 to 80 °F) and 55 to 90% relative humidity for the remainder of the seven week curing period. These cyclic conditions represented typical fall curing weather cycles for the location and a goal of approximately 72-75 percent daily average relative humidity for good burley curing which has been observed by the author to produce good burley curing for stalk harvested plants. After the eight week curing period, 24 plant samples were randomly taken from



each curing and fertility treatment and the leaves stripped into four grades. Each grade was approximately one fourth of the leaves from each plant. Grade 1 represented the lower leaves, grade 4 the top leaves. The midribs were removed from the leaf and the remaining leaf lamina oven dried at 70 °C for 24 hours. The dried lamina was shipped to the Lorillard Tobacco Company Research Department for analysis.

The 2002 season in Central Ky. was noted for being a very dry growing season, especially from mid June through mid Sept. at the Spindletop Farm where no rain over 16.5 mm /day (0.65 inch/day ) fell during the main growing period. Due to the dry weather of the growing season, irrigation was necessary and applied on dates shown by Fig. 5.

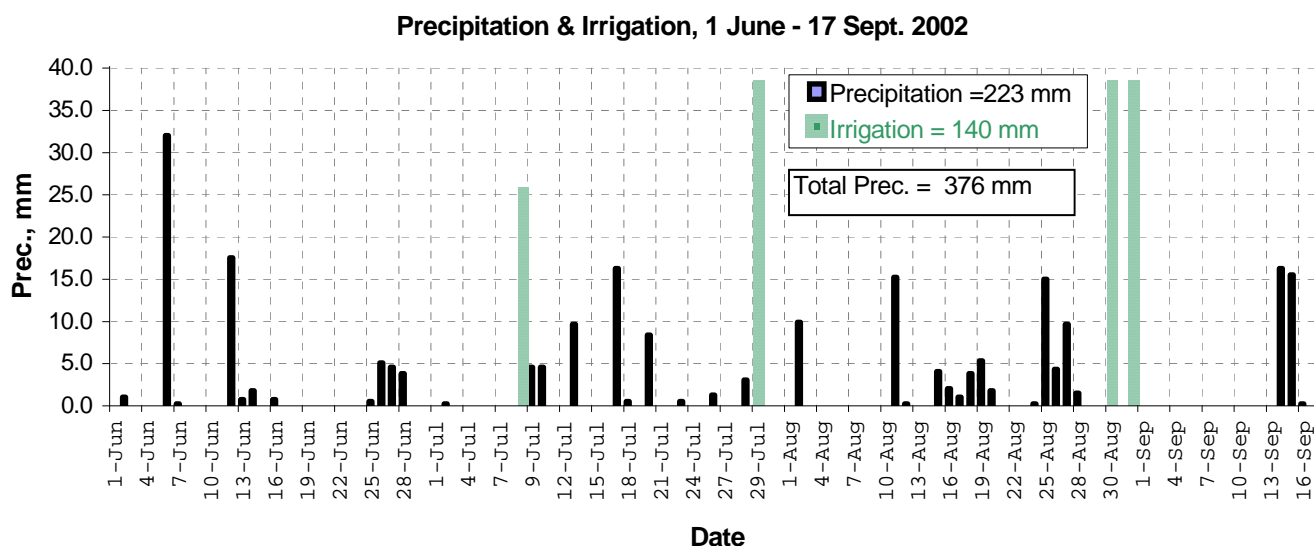


Fig. 5. Graphical representation of precipitation and irrigation data.

The curing period beginning 19 Sept. was generally warm and rather humid for two weeks as fall showers came, then cooler and normal humidity. September and October were above average in rainfall, a relief from the hot dry summer, and gave very favorable recovery from the drought and provided good natural curing weather. A summary of the environmental data for the various curing methods is shown by Table 1.

The "Weather Station" and precipitation data were from a certified NOAA weather station<sup>2</sup> approximately 3/4 km west of the curing barn. The "Air at west side of barn" was a HOBO recorder placed at the inside surface of a barn siding board and adjacent to a ventilator opening, thus near fresh air exchange but protected from rain. HOBO recorders were placed among the tobacco plants for the other curing locations .

<sup>2</sup>Ag Weather Center, University of Kentucky College of Agriculture, Mr. Tom Priddy, Manager. [www.agwx.ca.uky.edu](http://www.agwx.ca.uky.edu)

Table 1. Summary of environmental data for ambient weather and three curing locations.

<u>Location &amp; Date of Cure</u>	<u>Days</u>	<u>MaxT</u>	<u>MinT</u>	<u>AvT</u>	<u>AvTMx</u>	<u>AvTMn</u>	<u>AvRH</u>	<u>ARHMx</u>	<u>ARHMn</u>	<u>HRH&gt;85</u>	<u>HRH&lt;60</u>
Weather Station											
20 Sept.-13 Nov.	55	30	-3	13	17	9	81	95	51	304	72
Air at west side of Barn											
20 Sept.-13 Nov.	55	32	-2	13	18	9	76	89	51	202	89
Barn Cure											
20 Sept.-13 Nov.	55	31	-1	13	17	10	83	92	50	348	43
Barn Cham. Cure											
20 Sept.-13 Nov.	55	31	-1	14	18	11	76	91	53	194	87
Lab Cham. Cure											
21 Sept.-13 Nov.	55	27	10	19	26	14	74	90	55	117	81

Legend: Days = Days of cure

MaxT = Maximum temperature during cure, °C

MinT = Minimum temperature during cure, °C

AvT = Average daily temperature for cure, °C

AvTMx = Average of daily maximum temperatures during cure, °C

AvTMn = Average of daily minimum temperatures during cure, °C

AvRh = Average relative humidity during cure, percent

ARHMx = Average of daily maximum relative humidities during cure, percent

ARHMn = Average of daily minimum relative humidities during cure, percent

HrRH>85 = Total hours relative humidity was above 85% during cure

HrRH<60 = Total hours relative humidity was below 60% during cure

### **TSNA data by curing, fertility and grades:**

The TSNA analytical tests were performed in the Research Laboratory and by personnel of Lorillard Tobacco Company. Bar graphs of the TSNA results by fertility treatments, curing methods and grades (stalk positions) are illustrated by Fig. 6-8. Each datum shown by a bar represents the average of three independent laboratory analyses on each sample of prepared leaf tobacco and the average of three replications. The data are for leaf lamina only; leaf midribs were removed from the cured leaves and not included in these tests and analyses.

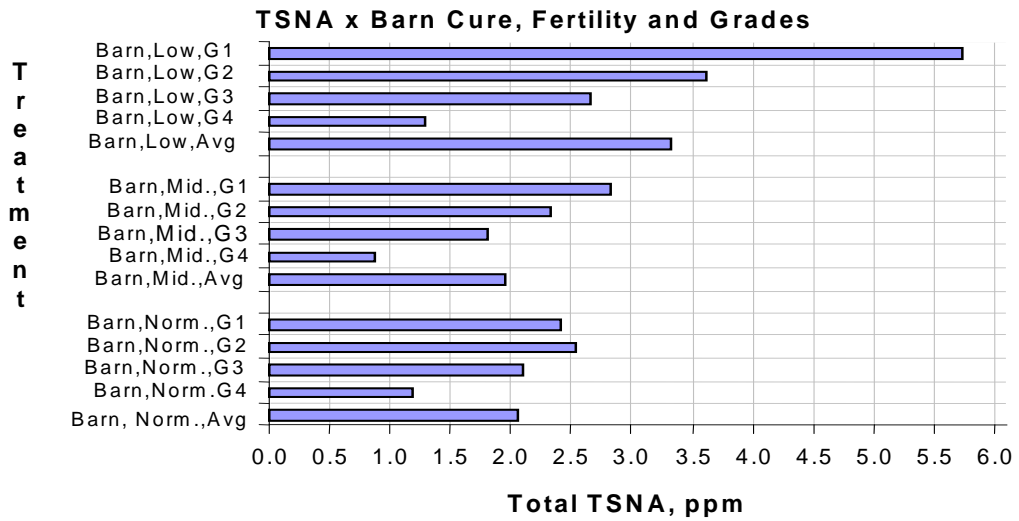


Fig. 6. Graph of TSNA data for barn cure, fertility treatments and grades.

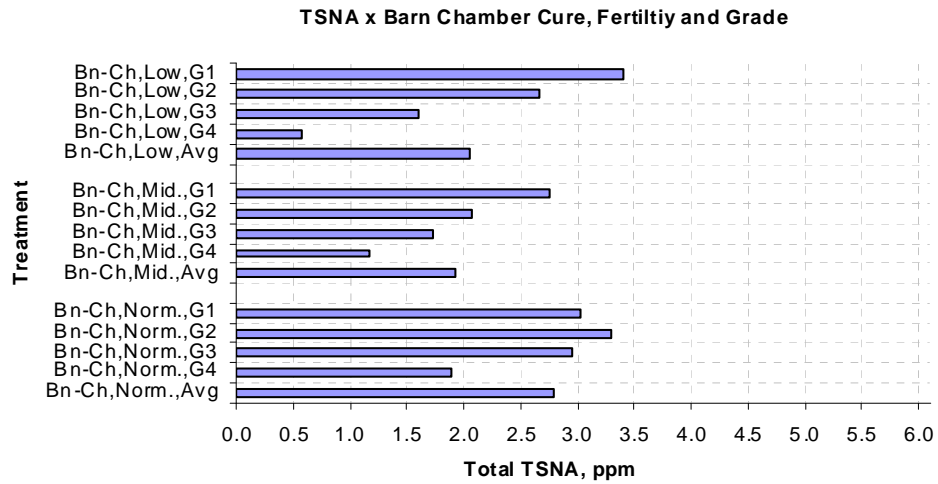


Fig. 7. Graph of TSNA data for barn chamber cure, fertility treatments and grades.

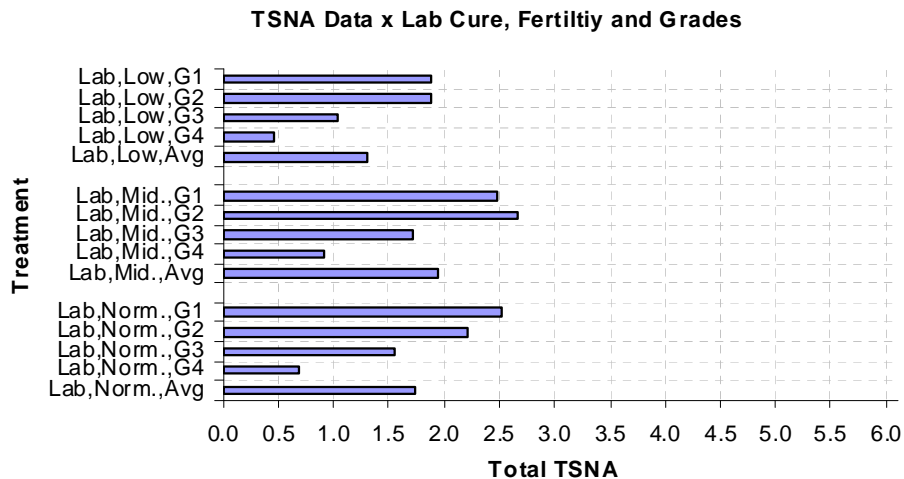


Fig. 8. Graph of TSNA data for laboratory chamber cure, fertility treatments and grades.

## Statistical analyses

A statistical analysis of TSNA data using a three way ANOVA<sup>3</sup> gave significance at the 95% level for the curing and grade treatments and interactions of cure x fertility, fertility x grade and cure x fertility x grades (Table 3). The fertility treatment was not significant at the 95% probability level as the P value was 0.055, which was just 0.005 beyond the 0.05 level taken for significance. Thus, we consider the fertility effects as inconclusive for this study. The cure x grade interaction was not significant.

Table 3. Three way ANOVA analysis of TSNA data.

<u>Source of Variation</u>	<u>DF</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>P</u>	<u>Signif.</u>
Cure	2	11.815	5.907	20.073	<0.001	<b>S</b>
Fertility	2	1.778	0.889	3.021	0.055	NS?
Grade	3	61.852	20.617	70.060	<0.001	<b>S</b>
Cure x Fertility	4	19.836	4.959	16.851	<0.001	<b>S</b>
Cure x Grade	6	2.502	0.417	1.417	0.220	NS
Fertility x Grade	6	7.123	1.187	4.034	0.002	<b>S</b>
Cure x Fert. x Grade	12	6.687	0.557	1.893	0.049	<b>S</b>
Residual	72	21.188	0.294			
Total	107	132.781	1.241			

The TSNA means and standard deviation data for fertility and curing treatments with the grades combined are summarized in Table 4.

Table 4. TSNA Means and Standard Deviation for Cure x Fertility treatments using combined Grade data (TSNA in ppm).

	Barn Cure, Mn., StDev	Barn Cham., Mn., StDev	Lab. Cham., Mn., StDev	All Cure, Mn., StDev
Low Fert.	3.32, 1.86	2.06, 0.16	1.32, 0.57	2.23, 1.54
Mid. Fert.	1.96, 0.11	1.93, 0.29	1.95, 0.11	1.94, 0.74
Norm. Fert.	2.06, 0.14	2.79, 0.27	1.75, 0.11	2.20, 0.90
All Fert.	2.44, 1.32	2.26, 1.03	1.67, 0.81	-----

The laboratory chamber cure produced the lowest TSNA mean overall (2.20 ppm) and lower values for the low and normal fertility levels (1.32 ppm, 1.75 ppm, respectively) with the mid level (1.95 ppm) essentially the same as the other curing treatments, thus indicating the lower relative humidity of this environment as shown by Table 1 had an effect of curtailing TSNA formation (fewest hours above 85 percent (117) and among greatest hours (81) below 60 percent).

<sup>3</sup> Statistical analysis performed using SigmaStat software and consultation by Dr. Dwayne Edwards, BAE Dept., Univ. of Ky., Lexington.

The barn cure had the highest TSNA mean for the grouped fertility data and the highest for low fertility treatment. The mean for the mid fertility level (1.96 ppm) was essentially the same as the other curing treatments. The mean for the normal fertility treatment (2.06 ppm) was in between the other curing treatment values. The barn cure had the highest relative humidity hours shown by Table 1 (348 hours above 85 percent and only 43 hours below 60 percent).

The barn chamber curing mean (2.06 ppm) was between the other two curing treatment means for low fertility and all fertility grouped means but the highest for the normal fertility treatment (2.79 ppm). The relative humidity environment was likewise between the other two for hours above 80 percent (194) but had the largest number of hours below 60 percent (87).

Curing treatments had more impact on TSNA accumulation than did fertility levels.

The mean TSNA data and standard deviations for all fertility and curing data grouped by grades are shown by Table 5. As cited above, the grade TSNA were significant at the 95% level.

Table 5. TSNA Means and Standard Deviations for Grades using combined Cure and Fertility treatments.

Grade	TSNA, ppm Mn., StDev
1 (bottom of plant)	3.00, 1.22
2	2.59, 0.69
3	1.90, 0.67
4 (top of plant)	1.00, 0.56

The significance of grades in the ANOVA is clearly shown by the consistent relationship of TSNA with stalk position - highest TSNA for the lowest stalk position grade and lowest TSNA for the highest stalk position grade.

All cured leaf tobacco was inspected by a Leaf Supervisor and considered representative quality and usability for manufacturing purposes.

## Summary and Conclusions

This study focused on the relationship of nitrogen fertility and curing methods on the resulting TSNA of stalk cured burley tobacco lamina. Three levels of nitrogen fertility (336, 224, and 112 kg/ha) and three air curing methods were used (conventional barn cure, forced air plastic enclosed chamber in the barn and a laboratory environmental chamber). The growing season was unusually deficient of natural rainfall with only 223 mm of rainfall. Four irrigations applied an additional 140 mm of water. After eleven weeks of air curing, replicated samples of leaf lamina were taken and TSNA analyses performed. A three-way ANOVA of the results showed significance at the 95% level for the curing and grade treatments and interactions of cure x fertility, fertility x grade and cure x fertility x grades. The fertility treatment was not significant at the 95% level as the P value of 0.055 was just 0.005 beyond the 0.05 level taken for significance. The cure x grade interaction was not significant. The controlled environment laboratory chamber cure produced the lowest overall TSNA result with a mean of 1.67ppm. The barn cure with natural weather conditions produced the highest overall TSNA result with a mean

of 2.44 ppm. The barn chamber with forced air curing produced an overall TSNA mean of 2.26 ppm.

Conclusions reached from this study include:

1. curing stalk harvested burley with a lower average relative humidity curing environment (74%) still provided good quality leaf and produced the lowest TSNA levels, and
2. the effects of nitrogen fertility on TSNA were inconclusive for this study, likely due to the effects of a dry growing season.

### **Acknowledgements**

We gratefully acknowledge the financial and administrative support of Lorillard Tobacco Company for this study and the College of Agriculture Farm Management Supervisors (Mr. Dave Smith and Mr. Steve Nichols) and workers for the production and handling of the tobacco.

### **References**

Duncan, G. A., L. P. Bush, H. R. Burton, M. Montross and J. Calvert 2001. Studies of Barn and Field Curing Environments on TSNA Formation in Burley Tobacco. Paper presented at the CORESTA Joint Meeting of the Agronomy & Phytopathology Study groups, Spier Conference Centre, Stellenbosch, South Africa, 30 September - 4 October, 2001.

Duncan, G.A., M. Montross, J. Calvert, Don Smith and D. Mereand, 2002 Ongoing Studies of Barn, Field and Chamber Curing Environments on TSNA Formation in Burley Tobacco. Paper presented at the 2002 CORESTA Congress, 22-27 September 2002, New Orleans, LA, USA