Final Report to
Council for Burley Tobacco

2013 Grant Funding for
TSNA Accumulation in Controlled Curing Environments, 2013

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Rationale

There are several factors that are known to affect TSNA accumulation in burley tobacco, including nicotine to nornicotine conversion, absolute nicotine concentration, nitrogen fertilizer rate, spacing, and time of topping and harvest. Most of these factors can be controlled to some extent. Curing conditions, however, are also critical. Experiments have demonstrated that the curing conditions optimal for high quality leaf are also those that favor TSNAs, but the specific effect of temperature, relative humidity and airflow have not been defined. It is loosely assumed that the most conducive conditions are at temperatures >86^{o}F and relative humidity >80%. This previous work has been done by correlating TSNAs with the ambient environmental conditions in commercial barns on farms, with or without various configurations of fans and vents, or in smaller experimental structures covered with plastic. Fassino *et al*. (2012) developed a model based on the number of hours during the third to fifth week of the cure that the temperature and humidity were above predetermined limits. Limited data from a survey of curing conditions and corresponding TSNAs in Kentucky showed a good correlation with both humidity and temperature (Pearce, pers. com).

This approach is severely limited by the confounding of the three environmental factors, and by the range of varieties, fertilizer rates and growing conditions across the large volume of tobacco necessary to fill a commercial barn and furthermore, one barn provides a single data point with no replication. The effect of temperature, relative humidity and air flow, alone or in combination, on the accumulation of TSNAs can now be studied by curing the tobacco in small purpose-built curing chambers in which each of the three factors can be manipulated independently of each other and across a range of conditions to determine the critical levels of each environmental component.

The results of the 2012 test showed that relative humidity affected TSNA accumulation more than temperature. In both these tests, the samples were collected only when the midribs at all the temperature-humidity combinations had dried, about 10 weeks after loading the barns, despite the leaf being dry after about 3 weeks at the high temperature-low humidity combination.

Materials and Methods:

Twenty-four curing cabinets (Fig. 1A) were constructed and are housed in a purpose-built garage-style building. The temperature in each cabinet is controlled by a ducted air-conditioning unit. The humidity is regulated by a humidistat that controls the water flow through a nozzle which directs a fine mist of water into the air inflow stream behind a plenum separating the air-water mixing chamber from the front chamber holding the cut tobacco on sticks (Fig. 1B). The air flows up through the tobacco through a slatted floor. Dehumidifiers were installed on units that required low humidity. Temperature and humidity in each cabinet is monitored with a data logger.

The commercial variety TN 90 LC and the high converter selection TN 90H were grown in the field with normal agronomic practices. After wilting on a rail wagon for seven days, three six, each with six plants per stick, of each variety were loaded into each curing unit. The plants were cured one of nine predetermined combinations of temperature (61, 74 or 86^{o}F) and relative humidity (50, 65 or 80%). The curing was terminated after eight weeks. The sixth leaf on each plant was collected for chemical analysis and the mid-stalk leaf was collected for sample evaluation. The samples were air-dried, stemmed, ground and alkaloids and TSNAs analyzed. The temperature and humidity of the cure in each unit were summarized as the median value over the duration of the cure. The data was analyzed using regression analysis of temperature and relative humidity on the various leaf chemistry parameters.

Previous experiences with quality assessments based on grades have mostly not been discerning enough to separate treatment effects. Therefore, the method used for the quality evaluation of the cured leaf in this test, as well as several other tests of the 2013 crop, was a comparative method that has not previously been attempted as far as is known. The system was based on determining the relative quality of a set of three to five samples across which one factor was the same for all samples while the other factor was different for each. In this test, samples were evaluated in groups such that for each temperature, one sample from each of the humidities was compared, and conversely, for each humidity, one sample from each temperature was compared. In total, 48 groups of four samples each were evaluated. The evaluator was asked to rank the samples from the best quality, assigned a ranking of 1, to the worst quality which was assigned the highest rank of 3, 4 or 5, depending on the number of samples in the group. In the event that two or more samples could not be differentiated from each other, each was given the same ranking.

Results

The temperature and humidity for the duration of the cure in the conventional barn was ideal for ensuring a good quality leaf. The temperature decreased from near 70 to about 40^{o}F at the end of the cure (Fig 2). The temperatures and humidities in the curing chambers were much more stable (Fig. 3).

TSNA data for each of the two varieties, TN 90 LC and TN 90H, differ in magnitude but show the same trends. For TN 90H, the only regressions that were significant (p value <0.05 for the regression in Table 1) were those of temperature and humidity against NNN, total TSNA, NAT and nornicotine. It was similar for TN 90LC, except for nornicotine. Fig. 4 demonstrates this graphically: the relative humidity treatments are separated out when NNN is plotted against temperature, but there is little separation between the temperatures when NNN is plotted against humidity.

The quality of the leaf was very strongly influenced by the relative humidity (p = 0.000) although the effect of temperature was also discernible (p = 0.016), and this effect was different between the TN 90LC and TN 90H mainly because TN 90H produced, a higher proportion of better quality leaf at 65% humidity when the temperature was 62^{o}F than at either 74 or 86^{o}F (Fig. 5).

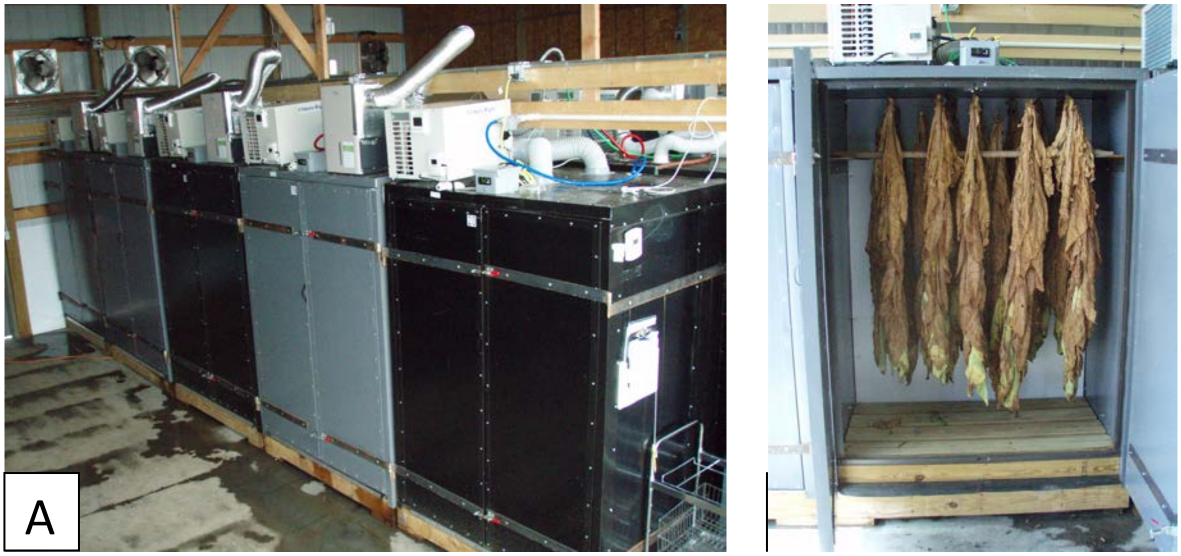

Fig. 1. A. Five of the twenty four curing chambers in purpose-built housing. The temperature and humidity in each curing chamber are controlled independently of each other. **B.** The airflow is directed down behind the plenum (white behind tobacco) and up through the slatted floor.

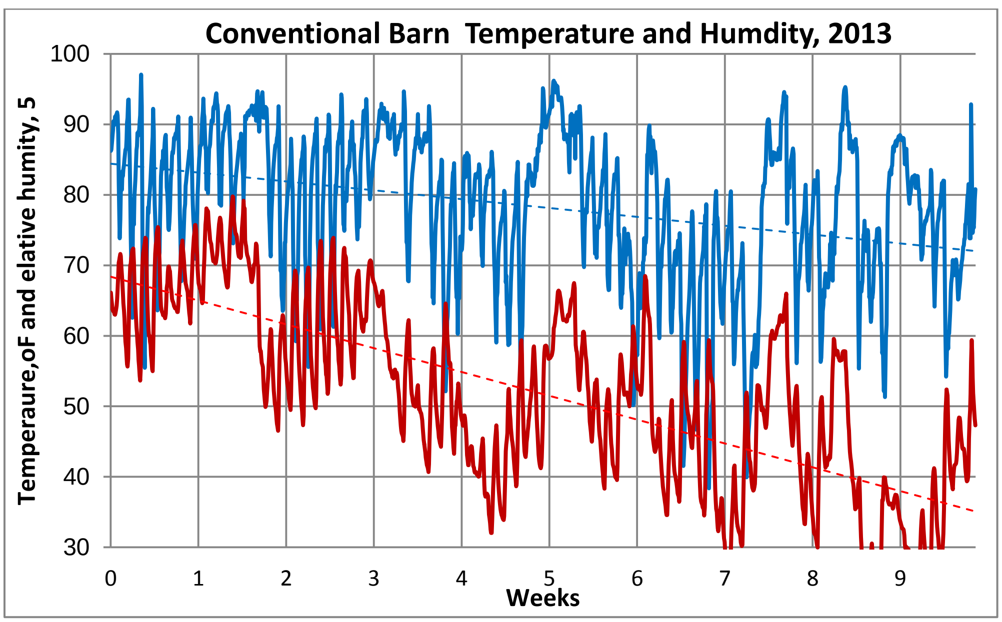

Fig. 2. Temperature and relative humidity in the conventional air-cured barn

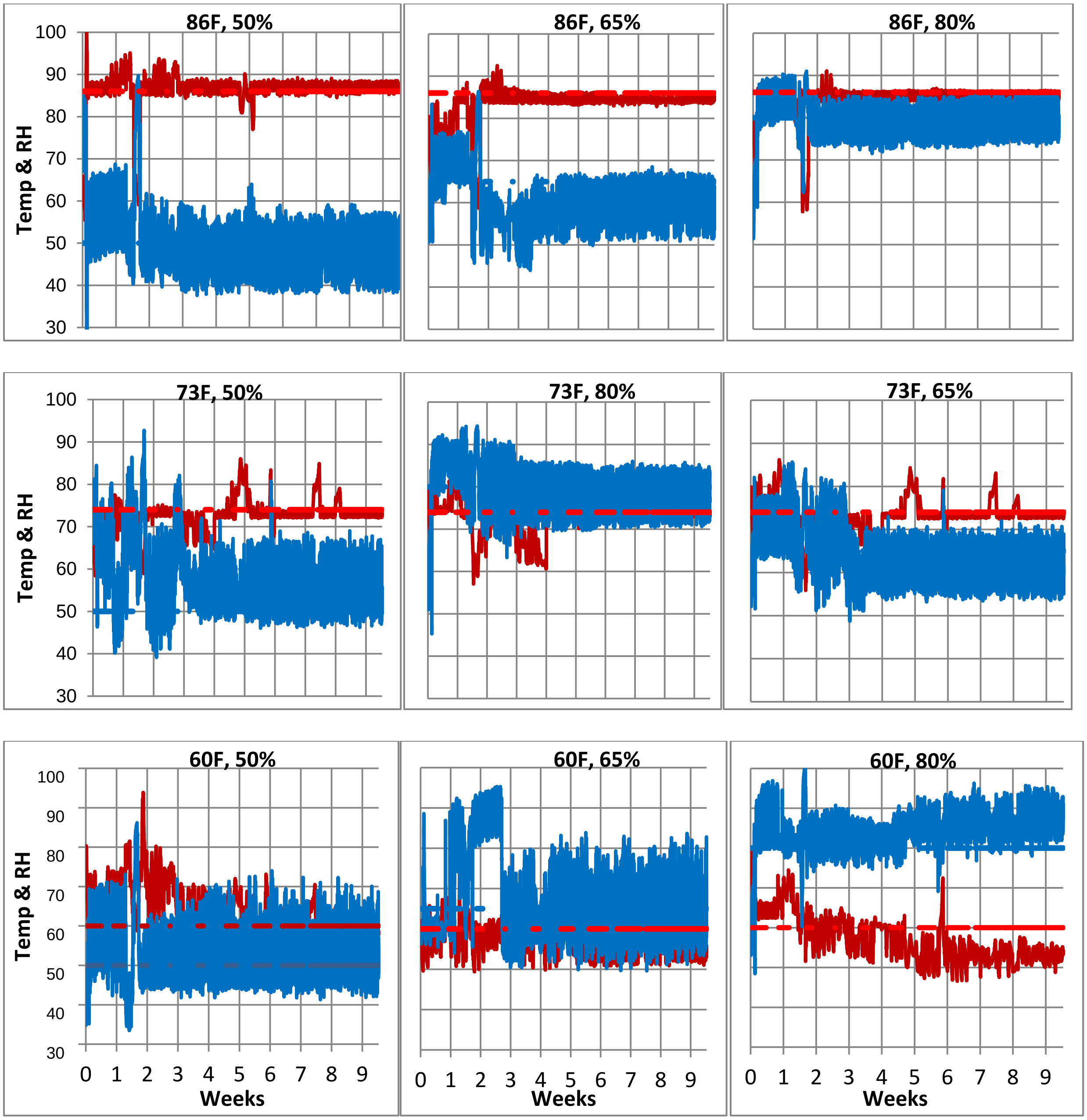

Fig. 3. The temperature and humidity in the curing chambers was maintained as close as possible to the target.

Table 1. Regression equations of median temperature and median relative humidity for the duration of the cure against alkaloid and TSNA components.

TN 90H							
	Regression Equation			p =			
	c	a * Temp	b * RH	Temp	RH	Regression	R-Sq
NNN	-13.1	0.0967	0.164	0.009	0.000	0.000	45%
Total	-15.1	0.111	0.188	0.009	0.000	0.000	45%
NAT	-2.03	0.0137	0.0239	0.008	0.000	0.000	47%
Nornicotine	3.58	0.00274	-0.0113	0.451	0.004	0.006	29%
Conversion	54.8	0.192	-0.01	0.074	0.925	0.166	11%
Nic + Norn	6.26	-0.009	-0.0166	0.308	0.073	0.169	11%
Nicotine	2.68	-0.0118	-0.00535	0.113	0.475	0.269	8%
Anabasine	0.0361	0.00002	-0.000002	0.739	0.971	0.937	0%
Anatabine	0.386	0.000214	-0.000029	0.729	0.963	0.931	1%

TN 90LC							
	Regression Equation			p =			
	c	a * Temp	b * RH	Temp	RH	Regression	R-Sq
NNN	-2.14	0.0142	0.0292	0.058	0.000	0.001	36%
Total	-6.05	0.0464	0.0695	0.002	0.000	0.000	48%
NAT	-3.68	0.0304	0.0377	0.000	0.000	0.000	52%
Nornicotine	0.477	-0.00056	-0.00227	0.635	0.067	0.181	11%
Conversion	7.41	-0.005	-0.0323	0.806	0.129	0.305	8%
Nic + Nornic	7.06	-0.010	-0.0071	0.381	0.543	0.629	3%
Nicotine	6.58	-0.0095	-0.0048	0.399	0.674	0.683	3%
Anabasine	0.0331	0.000004	0.000039	0.949	0.556	0.834	1%
Anatabine	0.393	-0.000261	-0.000299	0.695	0.659	0.870	1%

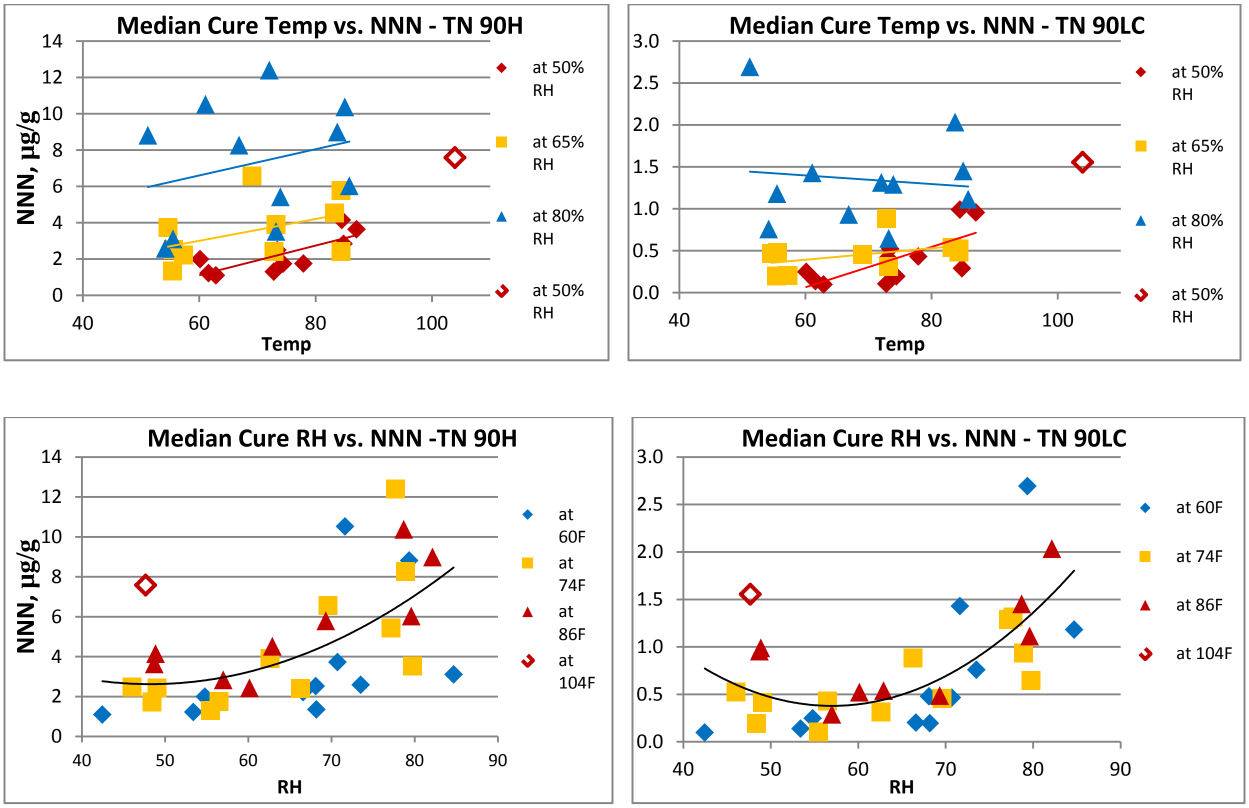

Fig. 4. NNN accumulation was affected more by relative humidity than by temperature of the cure.

Table 2. Chi-square probabilities of differences in quality between curing conditions

Source	DF	Chi-Square	Pr > ChiSq
Rep	3	2.47	0.482
Variety	1	1.39	0.238
Target Temp	2	8.3	0.016
Target RH	2	28.9	<0.000
Variety * Target Temp	2	3.8	0.149
Variety * Target RH	2	3.95	0.139
Variety * Target Temp * Target RH	8	25.6	0.001

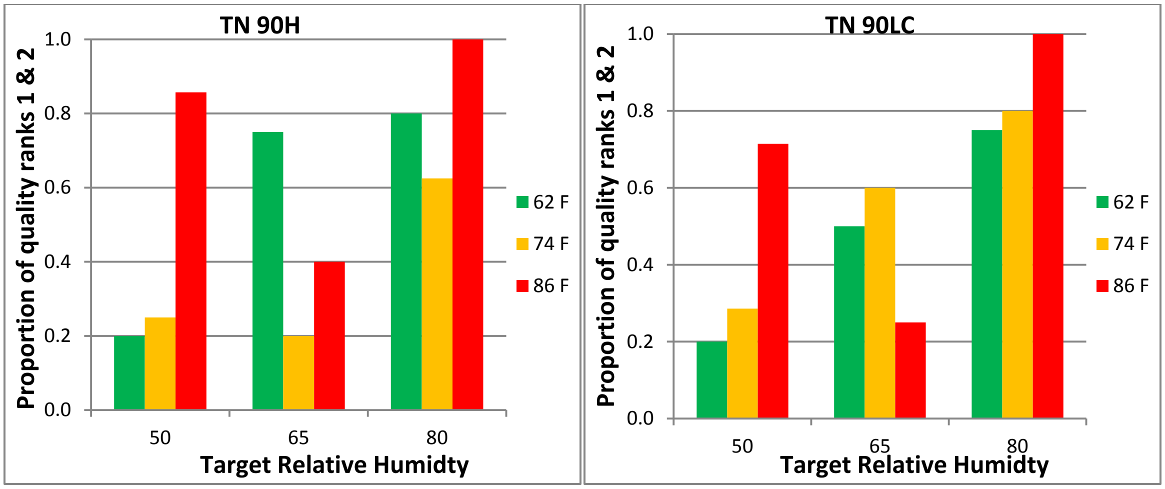

Fig. 5. Proportion of quality assessments of cured leaf of TN90H and TN 90LC at each combination of temperature and relative humidity