TSNA in Air-Cured and Fire-Cured Tobacco Sub-Group Report

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Objective 1: Data logger placement & maintenance

a. Determine proper placement of data loggers in curing barns to best represent the true curing conditions within the barn

b. Data logger calibration and maintenance
   - Reviewed by SG members
   - Minor formatting changes before submission to SC for approval before publication on website

Objective 2a: Sampling method of post-cure tobacco for TSNA

Draft protocol circulated Quebec, 2014

- Various aspects queried

Objective 2b: Sample preparation for TSNA analysis

Draft protocol was developed but some aspects queried

- Determine the optimal method for sample drying
  - University of Kentucky test:
    - Air dry
    - Freeze dry
    - Oven dry temperatures 30º & 60ºC
  - Data from 3rd year of test recently received
- To be included in update of CORESTA Guide #13

Objective 3: TSNA publications

Available TSNA publications being published on CORESTA website

- Initiated at University of Kentucky
- Suggested that a review should be written

Objective 2a: Sampling method of post-cure tobacco for TSNA

TSNA Sub-Group meeting, Quebec 2014

Aspects of protocol queried:

- Sample size = 1.4 kg (3 lb.)
  - Excessive?
- Number samples / bale
  - 2 grab samples of whole leaf
  - Requires opening bale
- Effect of inclusion of midrib, especially in cored samples
  - For Burley, lamina only of interest to industry
  - Cost of separating lamina from midrib

Objective 2a: Sampling method of post cure tobacco for TSNA (cont'd)

Study objectives:

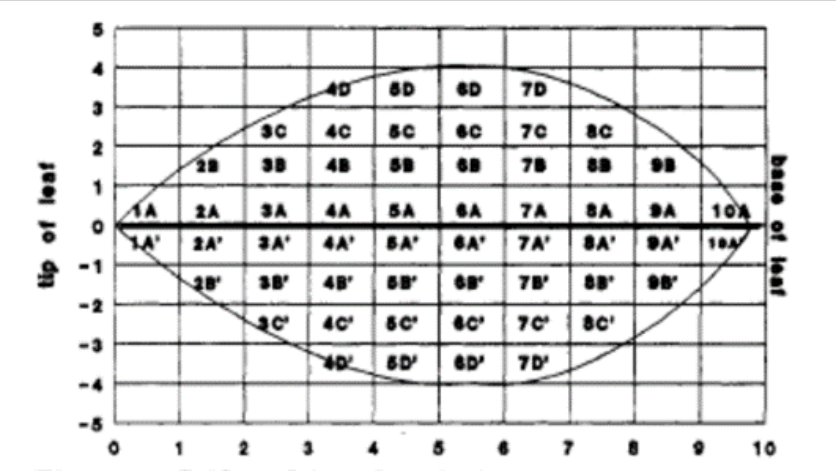
1. Clarify sampling protocol to optimize
   - sample size
   - number of samples

to best represent TSNA content of whole bale

2. Necessity of separating lamina from midrib for analysis

Objective 2a: Sampling method of post cure tobacco for TSNA (cont'd)

Burton, Dye, Bush. 1992. Distribution of tobacco constituents in tobacco leaf tissue. 1. Tobacco-specific nitrosamines, nitrate, nitrite, and alkaloids. J. Agric. Food Chem., 40 (6):1050–1055



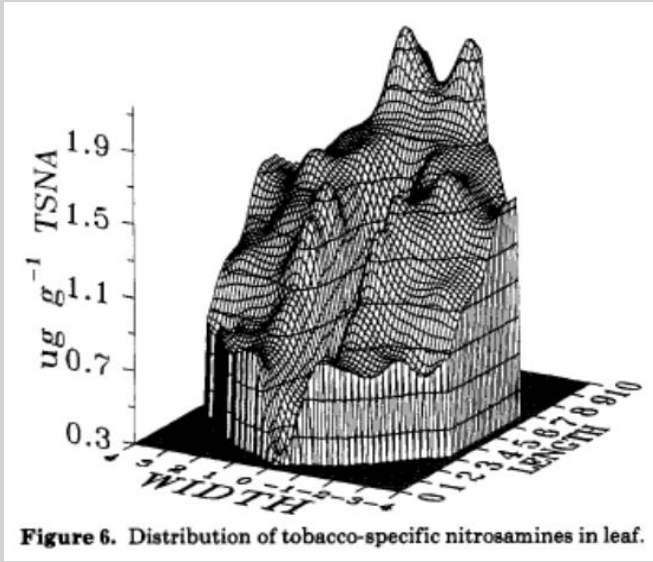


Figure 6. Distribution of tobacco-specific nitrosamines in leaf.

Objective 2a: Sampling method of post cure tobacco for TSNA (cont'd)

Study objectives:

1. Clarify sampling protocol to optimize
   - sample size
   - number of samples

to best represent TSNA content of whole bale

2. Necessity of separating lamina from midrib for analysis

i.e. can TSNA of whole leaf lamina be estimated by TSNA of lamina + midrib of cored sample at specific position along length of leaf ?

Objective 2a: Sampling method of post cure tobacco for TSNA (cont'd)

1. Core

Tip -
¾ leaf length

Mid-leaf

Midrib –
¼ leaf length

2. Grab Sample

30 – 40 leaves from each of 4 depths of each bale

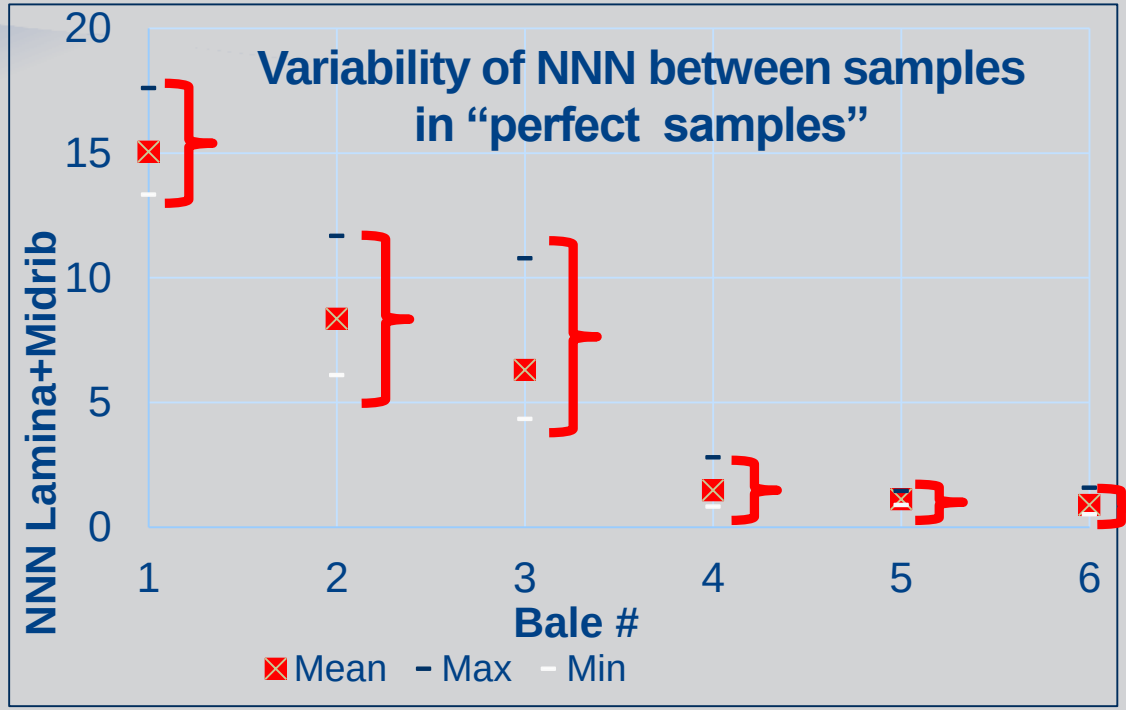
3. "Perfect Sample"

± 160 individual leaves randomly selected from throughout bale

….

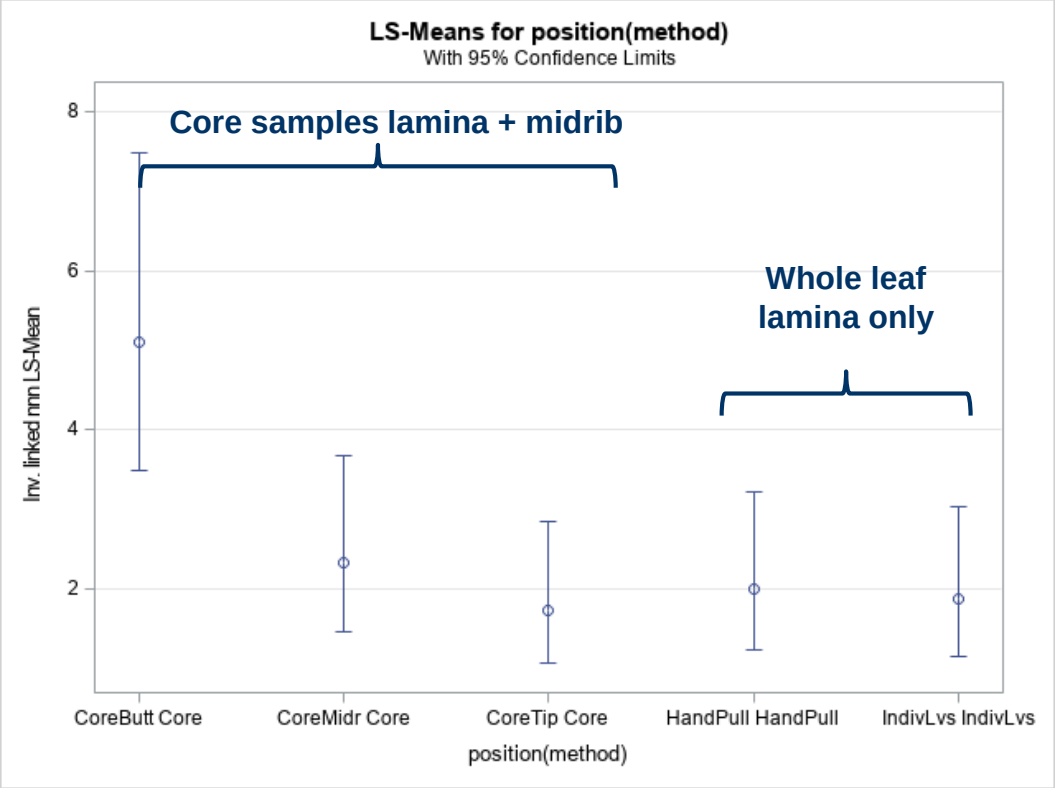
…… then randomly divided into 4 samples of 40 leaves each

Objective 2a: Sampling method of post cure tobacco for TSNA (cont'd)



Therefore: log transformed to normalize data for statistical analysis

Objective 2a: Sampling method of post cure tobacco for TSNA (cont'd)



Objective 2a: Sampling method of post cure tobacco for TSNA (cont'd)

Conclusion:

For burley, core samples and analysis of unseparated (lamina & midrib) could reasonably estimate NNN of whole leaf lamina

But:

What level of certainty for any single data point is required?

- Legal implications

Objective 2a: Sampling method of post cure tobacco for TSNA (cont'd)

CRM -

- Necessitate collaborative test
- Industry participation
  - 6 – 12 samples bales per origin
  - Ideally 4 core samples + 4 individual leaf samples per bale
  - Individually analyzed or bulked
  - Centralized analysis

- Draft protocol distributed to stake holders for review and comment

- ISO method
  - will require additional participation

Questions, comments or volunteers?