Nicotine Variability of Low Nicotine Cultivars versus Normal Nicotine Cultivars

Kenny Lion, Andrew Adams, Whit Morris, Emily Brown, Brittany Irving, Marcos Lusso, Dongmei Xu

Altria Client Services LLC, Richmond, VA 23219

73rd TSRC - Tobacco Science Research Conference
September 15-18, 2019, Leesburg, VA, USA

WWW.ALTRIA.COM/ALCS-SCIENCE

Altria

ABSTRACT

Nicotine is the most abundant alkaloid in cultivated tobacco (Nicotiana tabacum), typically constituting more than 90% of total alkaloids. Recently, the U.S. Food and Drug Administration (FDA) issued an Advance Notice of Proposed Rulemaking (ANPRM) to obtain information for consideration in developing a tobacco product standard to set the maximum nicotine level in cigarette filler to "minimally addictive or non-addictive" levels. Nicotine levels are highly variable across different years and locations as data collected over decades in the Minimum Standards Programs show. Analysis of the Burley check varieties in the Minimum Standards Program showed relative standard deviations ranging from 20% to 28% for individual varieties across locations from 2012 to 2018. Though there are decades of nicotine analysis from the Minimum Standards Program, there is not much data collected and publicly available on nicotine variability in low nicotine cultivars. The purpose of this study is to determine variability in nicotine content across multiple years and locations from low nicotine cultivars and compare it to normal nicotine cultivars. To initiate this long term study we analyzed low nicotine cultivars alongside normal nicotine cultivars at the same locations from 2012 to 2018. Assuming the variability in nicotine content for low alkaloid cultivars is similar to or greater than the variability in normal nicotine cultivars, it will have major impacts on meeting any regulatory standard. This could suggest that to meet a standard of 0.3-0.5 mg/g nicotine content the cultivars themselves may need to have much lower nicotine levels to consistently meet the proposed standard year after year. This will have major implications on the technical achievability of such a standard.

DESCRIPTION

- Since 2012, ALCS has been regularly assessing the performance of low nicotine cultivars against normal nicotine cultivars for both burley and flue-cured tobacco types in field research trials. In each trial, a low nicotine cultivar was grown alongside at least one normal nicotine cultivar with a minimum of 3 plot replicates per cultivar configured in a randomized complete block design. The nicotine averages across plot replicates within a trial for each cultivar are shown in Tables 1 and 2 in the Results section. The nicotine averages were then used to assess trial-to-trial variability of each cultivar as shown in Charts 1 and 2 in the Results section.
- To assess whether the trial-to-trial nicotine variability observed for ALCS research trials was comparable to that observed for similar trials conducted by others, data was gathered from the Burley and Flue-Cured Variety Evaluation Minimum Standards Programs which evaluate many different normal nicotine level cultivars at multiple locations on an annual basis.
  - For the Burley Minimum Standards Program, new cultivars are entered into their Regional Quality Test (7-8 locations across 4 states) and Regional Preliminary Test (4 locations across 4 states) each year to be assessed against 4 established check cultivars. The trial-to-trial variability of the nicotine data for the 4 check cultivars across the 2012-2018 time period is compared with the trial-to-trial nicotine variability of the normal nicotine level burley cultivars from ALCS research trials across the same time period in Chart 3 in the Results section.
  - As part of the Flue-Cured Minimum Standards Program, the top commercial cultivars on the market are evaluated at 3 locations within the state of North Carolina each year. Across the 2012-2017 time period, there were 20 cultivars that were evaluated in each of the years for total alkaloid content. The trial-to-trial variability of the total alkaloid data for these 20 cultivars is compared with the trial-to-trial total alkaloid variability of the normal nicotine level flue-cured cultivars from ALCS research trials across the same time period in Chart 4 in the Results section.

CONCLUSIONS

As data collected over decades through the Minimum Standards Programs have shown, there is considerable variability in nicotine levels in normal nicotine cultivars from year-to-year and location-to-location. Results from this study indicate that nicotine variability may be even greater for low nicotine cultivars from year-to-year and location-to-location. High levels of nicotine variability in low nicotine cultivars will have a significant impact in the technical achievability of any potential nicotine standard.

REFERENCES

- *Regional Burley Variety Evaluation Committee
  - Personal Correspondence-Committee Chair: Dr. Carol Wilkinson, Virginia Polytechnic Institute and State University
- **Flue-Cured Tobacco Variety Evaluation Committee
  - Personal Correspondence-Committee Chair: Dr. Loren Fisher, North Carolina State University

METHODS & RESULTS

DATA SET 1 – ALCS RESEARCH TRIALS

Chart 1. Trial-to-trial Nicotine Variability of Burley Cultivars

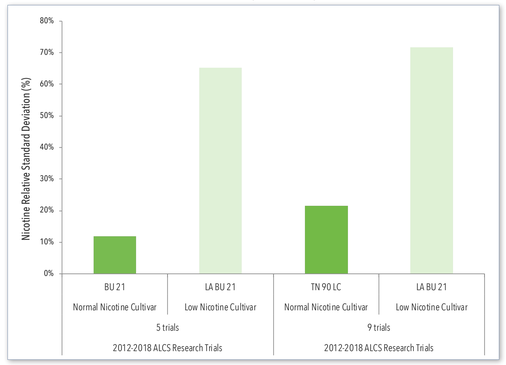
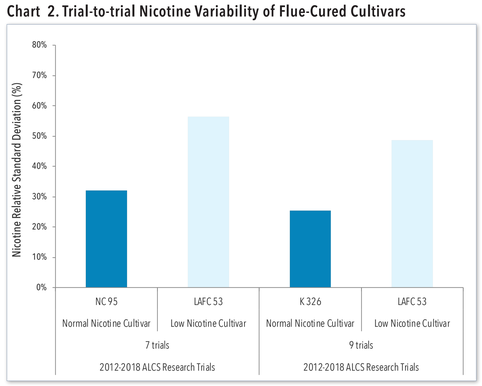


Chart 2. Trial-to-trial Nicotine Variability of Flue-Cured Cultivars



- Randomized complete block design
- 3-8 plot replicates per cultivar per trial (each cultivar within given trial has same number of plot replicates)

- Sample description:
  - Sampling stage: end of cure
  - Tissue type: lamina only (midrib removed from leaf)
  - Stalk position: weighted composite of all stalk positions (500 g sample) or "leaf" grade position only (10-15 leaves)
- Chemistry analysis: nicotine/total alkaloids

Average Nicotine Value (mg/g dwb)

Table 1. Nicotine Averages for Burley Cultivars – ALCS Research Trials

Field Trial	Crop Year	Location	BU 21	TN 90 LC	LA BU 21
1	2012	Blackstone, VA	61.62	59.92	4.16
2	2013	Blackstone, VA	51.10	47.97	1.38†
3	2015	Blackstone, VA	61.63	69.58	3.35
4	2015	Blackstone, VA	71.45	77.28	1.20†
5	2016	Blackstone, VA	60.72	71.03	0.98†
6	2016	Rancagua, Chile		52.90	1.33†
7	2017	Blackstone, VA		55.25	1.34†
8	2017	Rancagua, Chile		41.88	1.51
9	2018	Blackstone, VA††		45.70	0.22

Table 2. Nicotine Averages for Flue-Cured Cultivars – ALCS Research Trials

Field Trial	Crop Year	Location	NC 95	K 326	LAFC 53
1	2013	Blackstone, VA		29.73	1.72†
2	2014	Blackstone, VA	48.85	39.59	3.72
3	2015	Blackstone, VA	51.90	46.13	4.35
4	2016	Blackstone, VA		45.80	7.38
5	2016	Rancagua, Chile		33.50	4.27
6	2016	Blackstone, VA	44.90	37.35	5.00
7	2017	Tifton, GA	29.76		1.21†
8	2018	Blackstone, VA		24.15	2.29
9	2018	Kinston, NC	18.87	22.47	1.84
10	2018	Rocky Mount, NC	29.57	29.19	2.86
11	2018	Tifton, GA	38.45		0.87

† Nicotine average contains at least one data point that was below the limit of quantitation (LOQ) but above the limit of detection (LOD) of the analytical method. Such data points were treated as extrapolated values determined using the standard calibration regression.

†† Environmental conditions in Blackstone, VA in 2018 produced unusable tobacco with an average grade index of 6 (out of 100) for LA BU 21 and 35 for TN 90.

DATA SET 2 – BURLEY AND FLUE-CURED VARIETY EVALUATION MINIMUM STANDARDS PROGRAM

- Randomized complete block design
- 3 plot replicates per cultivar per trial
- Sample description:
  - Sampling stage: end of cure
  - Tissue type: lamina only (midrib removed from leaf)
  - Stalk position: weighted composite of all stalk positions (500 g sample)
- Chemistry analysis:
  - Burley – nicotine/total alkaloids
  - Flue-Cured – total alkaloids only

Chart 3. Trial-to-Trial Nicotine Variability of Burley Normal Nicotine Cultivars*

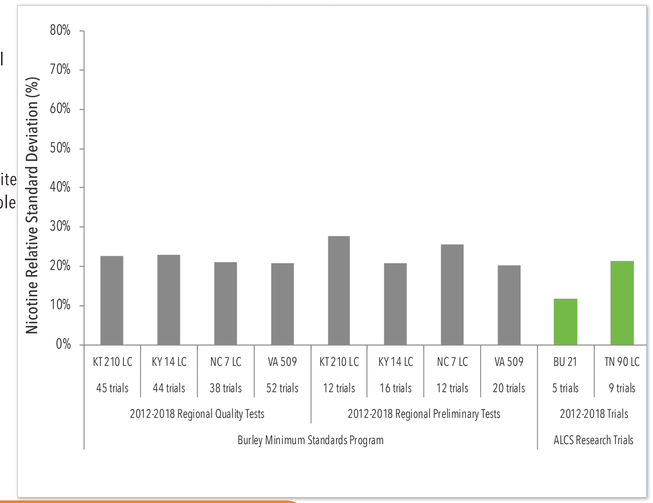
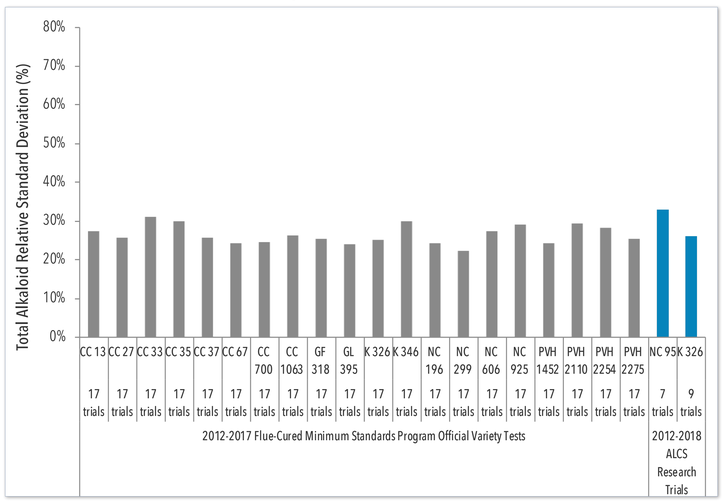


Chart 4. Trial-to-Trial Total Alkaloid Variability of Flue-Cured Normal Nicotine Cultivars**



STRENGTHS AND LIMITATIONS

Strengths

- Six crop years' worth of research trials across multiple geographic regions represents a substantial data set with which to analyze nicotine variability
- Nicotine variability for normal nicotine cultivars within ALCS research trials falls right in line with nicotine variability shown in both the Burley and Flue-Cured Minimum Standards Programs over the same timeframe

Limitations

- ALCS research trials were not designed with the purpose of evaluating year-to-year and location-to-location nicotine variability. The research trials would have incorporated greater geographic diversity and more consistency in sampling plans from one trial to the next if they would have been designed for this purpose from the beginning.
- Nicotine levels of the publicly available low nicotine cultivars used in this analysis were considerably greater than the levels proposed in the ANPRM.