

**Title:** Varieties and Production Practices to Maximize Nicotine to be Utilized in Emerging Tobacco Products (2016 Season)

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**Report type:** Interim Progress Report

**Lay Summary:** Currently the nicotine used in e-cigarettes and other non-combustible products comes from foreign sources where there may be concerns about product quality and consistency. With FDA regulation of all tobacco products, there is an expectation that domestic production and purification may be desirable. The objective of this study is to develop a production system that growers can utilize to produce tobacco where the end product is pure nicotine for use in alternative nicotine delivery systems. Preliminary work identified 2 tobacco lines demonstrating high nicotine content (TI401 and Narrow Leaf Madole). We evaluated these two lines, along with a comparable check (TN90 LC), in a fully randomized, replicated study designed to define the optimal fertilization, topping height, and topping method to maximize nicotine content in whole leaves.

#### **Introduction:**

The market for electronic cigarettes continues to grow with most major US cigarette manufacturers now marketing an e-cigarette. In addition there are other innovative, non-combustible nicotine-containing products that are either under development or are already on the market. Such products create a potential new opportunity for Kentucky tobacco growers. However, these products are not likely to contain the typical burley tobacco that is currently produced in Kentucky. Other tobacco cultivars or other *Nicotiana* species may be better suited for these products and novel production methods from green or dried tobacco biomass will require economical purification. This project is designed to evaluate tobacco types for alkaloid production. Purification from harvested green tobacco biomass must be competitive with current suppliers and there is commercial interest in a domestic supply of nicotine.

#### **Summary of Progress:**

##### Objective

The goal of this research will be to evaluate two high nicotine tobacco lines (TI401 and Narrow Leaf Madole), which were selected from preliminary research conducted in 2014, in a fully randomized and replicated field study designed to define the optimal fertilization and topping height, as well as topping method, resulting in maximized nicotine content in the leaves. The 2016 study was designed in the same fashion as 2015, where unfavorable environmental conditions yielded inconclusive results.

##### Methods

Based on preliminary research conducted in 2014 the dark tobacco variety Narrow Leaf Madole (NL Madole) and tobacco introductory variety TI401 were selected to be evaluated again in 2016. In 2014 both lines demonstrated high nicotine content and desirable agronomic characteristics. In the same manner as 2015, we evaluated these two lines, along with a check (TN90 LC), in a fully randomized, replicated study designed to define the optimal fertilization and topping height required to maximize nicotine content in the leaves. It is known that both nitrogen fertilization and topping height influence

nicotine content. In a separate study, NL Madole was evaluated along with TN90 LC in a small, fully randomized and replicated study designed to assess the feasibility of mechanical topping compared to hand topping. Economically, it would be advantageous if topping for nicotine production could be done mechanically.

Study 1: Varieties x fertilization x topping

*Design*

4 replications of a split-split plot with 2 main plots (fertilization), 2 sub plots (topping), 3 sub-sub plots (variety)

*Treatments*

4 replications of 12 treatments; 48 experimental plots with appropriate border rows

1. Main plots – fertilization
  - a. Rate 1: 200 lb N/acre
  - b. Rate 2: 400 lb N/acre
2. Sub plots – topping
  - a. Topping 1: Standard (when 80% of plants were at the button stage, CORESTA growth stage 51)
  - b. Topping 2: Standard topping height minus 25%
3. Sub-sub plots – Variety
  - a. Variety 1: Check, TN 90LC
  - b. Variety 2: NL Madole
  - c. Variety 3: TI 401

Study 2: Mechanical vs. hand topping

*Design*

4 replications of a split-split plot with 2 main plots (topping), 2 sub plots (variety)

*Treatments*

4 replications of 4 treatments; 16 experimental plots with appropriate border rows

1. Main Plot - topping
  - a. hand topping
  - b. mechanical topping
2. Sub plot – variety
  - a. TN 90LC check
  - b. NL Madole

Agronomic details

The tobacco transplants were grown with all normal recommended practices. Float trays were seeded April 6<sup>th</sup>, and both studies were transplanted May 31<sup>st</sup>. Two days before transplanting, we applied the two fertilizer treatments of 200 lb/ac N and 400 lbs/ac N in the form of 46-0-0 urea. The pre-emergence herbicides Spartan and Command were applied the day before setting using recommended label rates. Chemicals added to the setter water included Orthene, Coragen, Admire (1/2 rate), and Ridomil using the respective labeled rates.

The plants demonstrated normal tobacco development and supplemental irrigation was not required. Intermittent mechanical weed control was conducted throughout the season as needed. The majority of the growing season was near average rainfall and temperature with minimal prolonged periods with no precipitation.

The plants in the fertilizer/topping height and the topping method study were topped when 80% of the plants in a plot were at the button stage (CORESTA growth stage 51), for the standard topping treatment. This is approximately a week earlier than conventional tobacco production.

The mechanical treatment in the topping study was conducted using a mowing machine attached to a high clearance sprayer (Figure 1) and was also done when 80% of the plants in the plots had reached the button stage. The sprayer was equipped with boom that could be adjusted up and down using hydraulics. This allowed topping height to be adjusted on the move to accommodate plants of differing heights (Figure 2). The system worked well in removing the flowers from both varieties in the test but caused more leaf damage to the TN90LC as the leaf morphology of the top leaves TN90LC (burley variety) is more upright than the top leaves of NL Madole (dark variety) which tend to droop and curve towards the ground (Figures 3, 4).

After topping the plants were treated with contact, local systemic and systemic sucker control and were monitored weekly to remove any suckers manually not controlled by the chemicals to ensure no suckers developed as it is known that the development of shoots can affect nicotine content. Five weeks after topping, the plants were hand harvested by removing the leaves from Approximately 30 plants in each plot. The leaves were harvested in two sub groups identified as “top” and “middle”, or leaved from the upper and lower halves of the plants excluding “bottom/trash” leaves. The bottom leaves, typically known as “trash” in conventional production were discarded as earlier work showed that the nicotine content in the bottom leaves is very low and would not be worth the time or cost to process the biomass for nicotine. The harvested leaves were bagged in cotton sacks and dried in a forced air heated drier at 60 C. When the leaves were dry they were weighed and packaged for processing. The samples are now in waiting for grinding to a particle size to fit through a 1mm screen. Laboratory analysis for nicotine content will be performed in the KTRDC analytical laboratory using GC/FID. The data will be analyzed using PROC MIXED function of SAS 9.1 (SAS Institute, Cary, NC, USA) for an analysis of variance to test for the main effects and interactions.

#### **Plans for Future Work:**

Upon determination of statistically significant differences in nicotine content between treatments we will plan to reapply for an additional year of funding to generate at least 2 years of scientifically sound data. We hope that in these more typical growing seasons we will see greater treatment differences in the expected direction along with higher nicotine concentrations.



**Figure 1:** Mowers mounted on high clearance sprayer



**Figure 2:** Mechanical topping in progress



**A**



**B**

**Figure 3: NL Madole A. Mechanical topping B. Hand topping**



**A**



**B**

**Figure 4: TN 90LC check A. Mechanical topping B. Hand topping**