Progress Report to the Council for Burley Tobacco (December 2016)

Title: **Sample Preparation for TSNA Analysis (2016 season)**

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Report type: Interim progress report

Lay Summary: This study was designed to test whether drying temperatures during sample preparation affect TSNAs (Tobacco Specific Nitrosamines). Some tobacco companies use drying temperatures which may be higher than optimal, and we are concerned that these drying temperatures may be too high for accurate and consistent TSNA analysis. The crop has been harvested, cured and will be taken down as soon as the tobacco is in order.

Introduction

When preparing samples for TSNA analysis, the normal procedure in our laboratory is to air-dry the tobacco at ambient indoor temperature (about 68°F). The sample preparation procedure used by some of the tobacco companies includes sample drying at considerably higher temperatures. Previous work has shown that subjecting cured tobacco to high temperatures can increase TSNAs. It is likely that this increase would not be uniform, so if tobacco is dried at these higher temperatures during sampling preparation, the TSNA analysis will not give a true reflection of the absolute TSNA levels at the time of sampling, and may not give a true reflection of the relative values.

A CORESTA protocol is being compiled to deal with sample handling of cured tobacco for TSNA analysis. A draft protocol was based on the sample handling procedures of several tobacco companies, which included drying the tobacco at 77°F, 86°F or 95°F. We are concerned that these drying temperatures may be too high for accurate and consistent TSNA analysis.

This work will give us a better understanding of the best way to prepare samples for TSNA analysis, in order to obtain reliable data. This is relevant for growers, because tobacco companies test growers' tobacco for TSNAs, and may refuse to contract a grower if his TSNAs are too high. If the method of sample preparation is causing an increase in TSNAs, the grower might be unfairly penalized. It is also relevant for tobacco companies, because if the method of sample preparation affects TSNA measurement, the company will be operating with inaccurate data.

The long-term objective of this study is to establish a universally acceptable protocol for sample preparation for TSNA analysis. The short term objective is to compare several sample drying methods and to measure the effect of these drying methods on TSNAs.

Summary of Progress

Procedure – Field Work

Design

The design was four randomized compete blocks of a split plot design with two main plots (variety), and five subplots (drying treatment) i.e. ten treatments and 40 plots.

Treatments

The varieties were commercial TN 90LC and TN 90H, a high converter selection of TN 90 which has high TSNA accumulation. The high converter was used because it is easier to detect small differences when TSNA levels are high.

1. TN 90LC
2. TN 90H

The drying treatments will be applied immediately after stripping. They will be freeze-drying, our normal procedure of air-drying at ambient indoor temperature, oven-drying at temperatures used by some tobacco companies, and the temperature used to dry samples for alkaloid analysis.

1. Freeze-dried; check
2. Air-dried at ambient temperature (about 20°C / 68°F); current lab procedure
3. Oven dried at 30°C (86°F); company protocol
4. Oven dried at 35°C (95°F); company protocol
5. Oven dried at 60°C (140°F); temperature used to dry samples for alkaloid analysis

60°C (140°F) is far too high a temperature to use for drying TSNA samples, but some researchers still do this. We have included this treatment to collect data supporting our assertion that this is an unacceptable practice.

Agronomic details

The tobacco was grown with all normal recommended practices, except that we used a higher rate of nitrogen than usual (300 lb/acre N as urea, instead of 200 lb/acre). We did this in an attempt to get higher levels of TSNAs, because in the last few years, TSNAs have been so low that most treatment differences were non-significant.

Lime was applied to the field at the rate of 3 tons/acre. Float trays were seeded March 28th, and the study was transplanted May 31st (Figure 1). Just before transplanting, we applied 300 lb/acre N as urea, and 270 lb/acre K_2O as potassium sulfate. The herbicides sulfentrazone (Spartan) and clomazone (Command) were applied pre-emergent immediately before transplanting. Planting water chemicals were mefenoxam (Ridomil), imidacloprid (Admire) and chlorantraniliprole (Coragen).

The rainfall in the early part of the season was ideal, but dried up during the grand growth stage. July was so dry that we applied drip irrigation on July 20th, almost two weeks before topping (Figure 2).

As in 2015, we had an unusual spectrum of pests and diseases, related largely to the wet weather. There was a heavy infestation of Japanese beetles (Figure 3); this is unusual as they are considered a minor pest in Kentucky. We sprayed to control them with thiamethoxam (Actara) two weeks before topping (July 19th). There was target spot at the bottom of the plant (Figure 4), which has been a

common occurrence for the last few years, necessitating spraying with azoxystrobin (Quadris) a week before topping, on July 27th.

The first flowers were counted (pink flowers, not open flowers) July 22nd (25%). The study was topped ten days later (August 1st), nine weeks after transplanting. Two days after topping, we applied fatty alcohol (Offshoot T), maleic hydrazide (MH) and butralin (Butralin).

The study was harvested 24 days after topping, on August 24th (Figure 5). This was a slightly shorter harvest interval than usual, because of labor constraints. Thirty plants were harvested for each plot; five sticks of six plants each. The tobacco was left sticked out in the field until the next day, when it was picked up and put onto a rail wagon which was parked in the barn (Figure 6) until housing (Figure 7) five days after harvest, on August 30th.

The tobacco is cured (Figure 8), and will be taken down as soon as it is in order.

Plans for Future Work

We do not yet have any results for 2016, and in view of the very unsatisfactory results from the first year of this study in 2015, we would like to repeat the study in 2017. TSNAs in all our 2015 studies were very low, and there were few differences between any treatments.

Figures and Tables

Figure 1: Transplanting

Figure 2: Drip irrigation

Figure 3: Japanese beetles

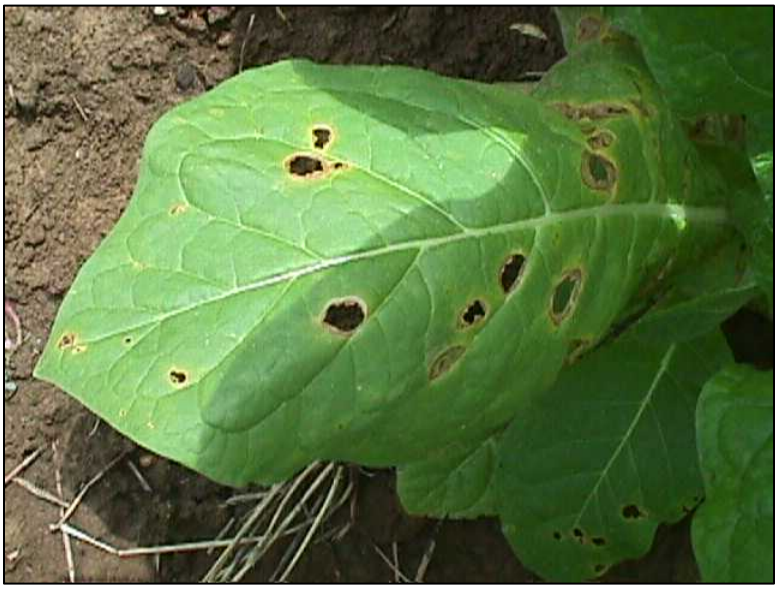

Figure 4: Target spot on lower leaves

Figure 5: Harvesting

Figure 6: Rail wagon in barn

Figure 7: Housing

Figure 7: Tobacco cured in barn