Interim Report to
Council for Burley Tobacco

2016 Grant Funding for
Development of pale yellow dark burley:
An additional approach to reducing TSNAs in Kentucky burley

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Rationale

The 2014 proposal for this pale yellow dark burley project detailed the criticism expressed in the Surgeon General's 2014 Report (*The Health Consequences of Smoking—50 Years of Progress*), despite the fact that the data used predated the tremendous improvement in the reduction of TSNA in burley as a result of several factors, most notably the widespread use of LC seed (http://www.uky.edu/Ag/Tobacco/Pdf/LC-Protocol.pdf).

This project sets out to develop a burley style tobacco that has a high chlorophyll content, similar to Maryland tobacco, that requires less nitrogen than conventional burley tobacco. It is largely this high level of nitrogen that predisposes burley to higher levels of TSNA accumulation, and was demonstrated in tests at university of Kentucky in 2010.

This reduction in nitrogen requirement would also reduce the cost of production by approximately $80/acre because only one quarter to one third of the amount of nitrogen fertilizer need be applied to this green style of tobacco.

Comparison of the leaf chemistry between green tobacco types and burley demonstrate that these differences are the result of genetic diversity between varieties, regardless of the class of tobacco (burley or flue-cured), and not the result of the chlorophyll status of the plant, and there is often considerable overlap of the values between the analytes in flue-cured and burley. Selection of typical burley leaf chemistry in a green plant is therefore possible. This applies also to the manufactures' requirement that burley is able to absorb flavorings. This desirable burley characteristic is not necessarily linked to the chlorophyll status because Maryland (MD) tobacco is green but can be used as a burley substitute. There is, therefore, no evidence to suggest that a dark burley cannot have satisfactory burley manufacturing characteristics.

A potential risk during air curing of a green style of burley is that not all the chlorophyll will break down, resulting in green discoloration of the cured leaf. In the 2014 season, even the burley was afflicted by a considerable amount of this discoloration. Inserting the "pale yellow" gene into this dark burley line causes the tobacco plant to lose its chlorophyll very quickly after harvest (Fig. 1). This gene is totally independent of the genes that make higher levels of chlorophyll in the plant.

The opportunity, therefore, exists of developing a "dark" burley that has an increased level of chlorophyll and so requires less fertilizer, and also contains the pale yellow trait

that will ensure that the green tobacco will still cure like a conventional burley, even in a less than optimum curing environment.

This project started in 2012. The basis of the new tobacco is an elite burley line developed by the Kentucky Tennessee Tobacco Improvement Initiative (KTTII). This line is currently used as the parent for most of the newer commercial KT burley hybrids and already has resistance to blackshank race 0, PVY and TMV. This was crossed with Maryland tobacco to introduce the green color, and the pale yellow gene from the dark air-cured variety KY PY171 which has been tested for use in western Kentucky in recent years.

By early 2014, several breeding lines of the four-way cross that combined the green from Maryland with the pale yellow from the KY 171 in a background of the elite burley line were set for the next phase of development. Selection of breeding lines at this phase was to be done using markers to screen the seedlings for resistance to PVY, TMV, blackshank race 0 and the demethylase genes. Resistance to Race 1 of blackshank cannot be detected using markers, so this has to be done by growing these lines in a blackshank-infected field. Funding available was insufficient to do both the marker work and the field screening, and precedence was given to the field screening for blackshank. In late April of 2014, a contract with a grower to lease land in which a crop of tobacco had been seriously infected with blackshank in 2012. This field had been rotated into soybeans in 2013, and the level of blackshank was disappointing low for a disease screen: less than 25% of the most susceptible check, KY 14 x L8, were infected with blackshank by late September, and no plants died in the lines that included any resistance from the KTTII elite burley line. Selections of the plants from which seed was harvested for the next season of screening was therefore done based purely on agronomic type.

In this 2016 test, the most agronomically acceptable lines from those selected in 2014 were screened for both blackshank race 0 resistance and agronomic type, and the strongest survivors with the most acceptable type were intercrossed.

Procedures:

Five lines of the double cross between the burley elite line x Maryland and the burley elite line and KY PY171 were grown in the Fayette county blackshank-infected field. Two to three rows of the KY 907 which has a low level of resistance to blackshank was grown in between the breeding lines to encourage a buildup of disease pressure in the field. A stand count was done at 10 days after setting to establish a base line for the counts of infected plants later in the season. In mid-August, 53 Individual plants with good agronomic characteristics, including leaf number, leaf shape, internode length, flowering date, no sign of lodging, etc., were selected and flagged. In early September, pollen from two flowers from each of these selected plants was collected, bulked and dried. In mid-September two flowers from each of the flagged plants was then pollinated with the bulked pollen, and the seed from these capsules was collected in mid-October.

Reuslts:
Survival of the five breeding lines 80 days after setting were all greater than 96%. However, at this same time, the survival of KY 907, which is assigned a disease resistance rating of 2 on a scale from 0 to 10 in the Kentucky-Tennessee Production Guide, ranged from 33 to 52% but not considered very high for a breeding nursery.

The objective of producing a second generation of intercrossed progeny for continuing the selection for good agronomic type and blackshank resistance was achieved. A small block of this seed was grown in an uninfected field on Spindletop Farm in 2017 to assess the plant type (Fig. 1).

Fig. 1. Three rows of the segregating population of the recurrent cross population made in 2016 grown at the University's Spindletop farm in 2017