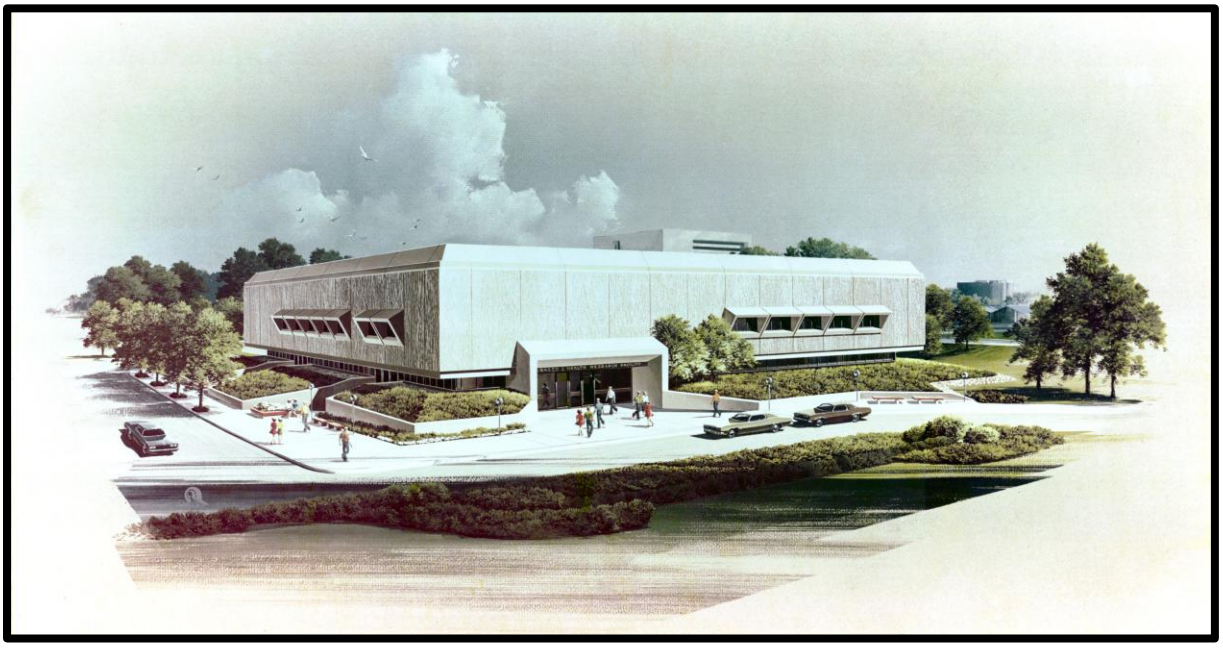
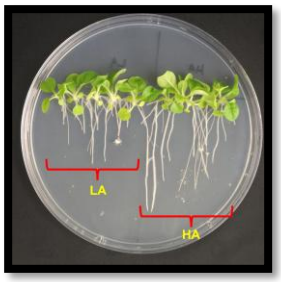
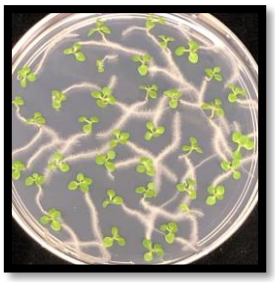
Kentucky Tobacco Research & Development Center



ANNUAL REPORT

July 1, 2024 – June 30, 2025





Martin-Gatton College of Agriculture, Food and Environment | Kentucky Tobacco Research & Development Center

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December 17, 2025

The Honorable Andy Beshear
Governor of Kentucky
Frankfort, KY 40601

Dear Governor Beshear,

With reference to the requirements of KRS 248.520, KRS 248.560, and KRS 248.570, the Annual Report of the Kentucky Tobacco Research & Development Center (KTRDC) is herewith submitted. This report details the research and financial activities of KTRDC from July 1, 2024 to June 30, 2025. It identifies the need for continued tobacco research and development, for continuation of the Kentucky Tobacco Research Board (KTRB), and the supporting tax funds provided for in the Act.

Favorable reviews of the Center's research program have been received from outside scientists. The financial status is sound and financial statements are consistent with acceptable accounting principles as evidenced by audits of books and records.

I cordially invite you to visit the KTRDC Building at the University of Kentucky at your convenience and look forward to discussing KTRDC's program with you.

Sincerely yours,

Todd C. Clark

Todd Clark
KTRB Chairman

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EXECUTIVE SUMMARY

EXECUTIVE SUMMARY 2024-25

Cover illustration:

Photographs provided by David Zaitlin, Barun Patra, Sitakanta Pattanaik, and Ruth McNees.

Top: Kentucky Tobacco Research and Development Center (KTRDC), formerly Tobacco Health Research Institute (THRI), original architecture sketch recreated by Dave Zaitlin.

Bottom left: Burley 21 tobacco seedlings with low alkaloid (LA) and high alkaloid (HA) showing differences in root development with HA plants having longer roots with more hairy roots.

Bottom center: Examples of filtered cigars, cigarillos, and large cigars tested in the KTRDC analytical laboratory for development of production parameters for the newly produced certified reference cigars in collaboration with the FDA.

Bottom right: *Nicotiana tabacum (SNN)* seedlings on MS medium.

The Kentucky statutes which define the KTRDC research mission state that KTRDC-supported research should be directed towards:

- preserving and strengthening tobacco agriculture in Kentucky
- facilitating the progress of commercial endeavors in crop agriculture which have potential to provide entirely new market opportunities for tobacco growers
- applying, when appropriate, previously authorized research, initially conducted with tobacco, to other plants which might be grown commercially in Kentucky

The KTRDC research programs and projects represented in this annual report are directed toward meeting the mission goals above. This is accomplished through research efforts focused on modifying conventional tobacco to meet evolving FDA (Food and Drug Administration) standards and new industry requirements, by helping to build the science base for tobacco regulation and emerging reduced-risk tobacco products, and by helping to establish new crop opportunities, such as *Artemisia annua*, as commercial crops for Kentucky agricultural producers. KTRDC scientists utilize technology (conventional and molecular marker-based breeding, biochemical analyses, genome editing, genomics, etc.) to do research that benefits Kentucky's agricultural economy and producers, and to meet the KTRDC mission goals.

This annual report includes descriptions and data from specific programs or research projects done at, or funded by, the Kentucky Tobacco Research and Development Center (KTRDC) during the 2024-2025 fiscal year. These research projects range from basic scientific exploration to applied field tests. The main focus of the work is on harm reduction and crop performance.

FIELD WORK

KTRDC has had a large field program dedicated to research that is directly applicable to growers, but with the approaching retirement of the two field researchers, the program has been much reduced. For several years, the KTRDC field group collected data to support recommendations in the Burley and Dark Tobacco Production Guide for minimizing the levels of tobacco specific nitrosamines (TSNAs). There is still some work being done on TSNAs, most notably a variety breeding project that aims to reduce nitrogen use as well as TSNA levels.

A novel low alkaloid gene has been stacked with the LA nic1nic2 mutants to produce a line with ultra-low nicotine levels, considerably lower than reported for any other conventionally bred burley line: this work is now complete.

The KTRDC Field Research Program has continued work on crops that are alternative or complementary to tobacco. The research supporting the development of scalable production practices for the medicinal herb Artemisia annua (sweet wormwood) completed its seventh year in 2024. This work is now complete.

A new sponsored project was initiated this year, for a company working on a metabolite produced in the seed. It involved testing varieties and agronomic practices to maximize seed production.

Seed Screening and Low Nicotine

An important part of our work involves helping growers meet impending FDA standards. KTRDC scientists have studied the genetic regulation of nicotine biosynthesis and TSNA formation in tobacco. The Family Smoking Prevention and Tobacco Control Act, passed by the US Government in 2009, and the WHO (World Health Organization) Framework Convention on Tobacco Control demand the development of tobacco varieties with low nicotine and tobacco-specific nitrosamines (TSNAs). KTRDC personnel were, for many years, responsible for the development and implementation of the LC (low converter) tobacco seed screening program which results in substantial reductions in nornicotine levels in raw tobacco, and thus reduces levels of NNN in the final commercial tobacco product. KTRDC scientists worked closely with University of Kentucky tobacco breeders and Foundation Seed personnel to screen Foundation seed of both burley and dark tobacco, eliminating plants that convert nicotine to nornicotine. Through this process, LC-screened tobacco has much lower levels of NNN compared to tobacco produced prior to the establishment of the LC protocol. With the closure of the tobacco breeding program and the Foundation Seed Project, this work has come to an end after more than 20 years, but all tobacco seed on the market in Kentucky is LC screened. Although the screening of Kentucky Foundation seed has ended, KTRDC scientists still assist other organizations with seed screening and analysis. We undertook two such seed screening projects this year. Recently, the Food and Drug Administration (FDA) is considering lowering the nicotine content in tobacco for combustible products to no more than 0.7 mg of nicotine per gram of tobacco. The normal nicotine concentration in cured leaves of commercial tobacco varieties is about 50 mg/g, approximately 100 times greater than the FDA proposal. In addition to nicotine, tobacco plants also accumulate three other alkaloids, namely nornicotine, anabasine, and anatabine. Nicotine can be converted to nornicotine during senescence and curing, a process known as nicotine conversion. Nornicotine is a precursor for N'-nitrosonornicotine (NNN), a carcinogenic TSNA. We are employing molecular genetic methods to identify the factors (genes) involved in nicotine biosynthesis, transport, and conversion. Using the available genomic resources, we have been successful in identifying genes responsible for nicotine biosynthesis, transport, and conversion. These candidate gene(s) can be manipulated to generate low TSNA tobacco varieties for harm reduction. In recent years, KTRDC has been awarded a US and multiple international patents for using a tobacco gene to reduce the nicotine to reduce the nicotine to nornicotine conversion KTRDC scientists have developed new intellectual property related to alkaloids, and have initiated several projects to develop lower alkaloid tobacco varieties, in an effort to both meet the potential FDA standards and to provide varieties that are acceptable to Kentucky tobacco producers. It is noteworthy that the original work which made this possible came out of a KTRDC summit grant, as a collaboration between agronomists and molecular scientists. It is well known in the industry that there are negative agronomic impacts on tobacco yields and quality in low alkaloid (LA) lines, so KTRDC has projects to address these problems.

The Center for Tobacco Reference Products (CTRP), housed in and operated by KTRDC, provides reference tobacco products and operates a proficiency testing program that benefits the entire worldwide tobacco industry. Grants from the FDA have enabled the CTRP to produce the 1R6F certified reference cigarette, four certified smokeless tobacco products (Swedish style snus, snus, loose leaf chewing tobacco, and moist snuff), and three certified reference cigars products (large, cigarillo, and filtered cigars). Non-certified products include 11 ground tobaccos (six straight grade and five ground filler) that cover all major tobacco types, an ultra-low yield cigarette (2R5F), and four non-certified reference cigars (filtered cigars, cigarillos, large cigars, and large cigars with natural wrapper). Since the inception of the program in 2016, the CTRP has managed 33 rounds of proficiency testing using the certified reference products, 27 rounds for the 1R6F reference cigarette, and six rounds for the certified smokeless tobacco products. The CTRP currently runs an average of four rounds of proficiency testing per year. With the completion of the manufacturing, characterization, and certification of the three certified reference products, the documentation for adding these research materials to the proficiency testing program will be submitted to A2LA with the intent to start testing cigars in 2027.

To date, the University of Kentucky, Center for Tobacco Reference Products (CTRP) has been awarded three Cooperative Agreements from the U.S. Food and Drug Administration (FDA) Center for Tobacco Products (CTP), to produce and distribute reference tobacco products. The first was in 2014, to develop a cigarette tobacco reference products program and produce the 1R6F certified reference cigarette. This first grant, to produce, characterize, and certify 50,000,000 reference cigarettes (1R6F) is complete. In 2016, the CTRP was awarded a second Cooperative Agreement, to develop four certified smokeless reference products. The second grant, to produce, characterize, and certify four smokeless reference products is complete. In 2019, the CTRP was awarded a third Cooperative Agreement, to develop three certified cigar reference products is complete. All of the products were machine-made with an HTL (homogenized tobacco leaf) wrapper. The large cigar (1RLC) was manufactured by ITG Brands (Imperial Tobacco Group) with a production run of 200,000 cigars and completed on May 4, 2022. The cigarillo reference cigar (1RSC) was manufactured by Swedish Match with a production run of 712,800 cigarillos and produced on December 13, 2022. The filtered cigar reference product was manufactured by Scandinavian Tobacco Group (STG) with a production run of 702,000 filtered cigars and completed on March 9, 2023. All three certified reference cigars were added to the CTRP web-based ordering system by early 2025. Research projects focusing on the toxicological effects of cigar tobacco products and how their HPHCs play a role in the etiology of lung and oral cancers is ongoing. In addition, projects on analytical method development for constituent measurement in cigars and long-term storage impacts on reference cigars are ongoing. CTRP presented a poster at the 2025 Tobacco Science Research Conference (TSRC) in Knoxville to share updated information with stakeholders regarding the reference cigar project and to plan for future cigar research projects and proficiency testing studies moving forward. The information was also presented at the 2025 CORESTA Smoke Techno meeting in Annecy, France. KTRDC and CTRP continue to speak with stakeholders in the tobacco research environment to provide additional reference products as needed for research efforts. In addition, the CTRP collaborated with CORESTA to produce two nicotine pouch products: NP1 Dry Nicotine Pouches and NP2 High Moisture Nicotine Pouches. These products are available for purchase on the CTRP website and are planned to be included in a CORESTA collaborative study in early 2026.

The KTRDC annual report comprises four types of reports: (1) in-house projects supported by KTRDC research funding, (2) faculty research, (3) KTRDC Tobacco Summit grants, and (4) externally funded projects. Each report contains the title of the report, the associated investigators, the report type, a lay summary, and a description of the study. In some cases, these reports are intentionally brief or omitted to limit the disclosure of specific data that may be used in publications or patent applications.

For our in-house research (section 1A.i), investigators submit proposals describing their intended research projects for the upcoming year. All KTRDC scientists collaborate on research projects and debate new research topics. Through this process, there is continual oversight and fine-tuning of KTRDC's research to help focus our efforts to benefit Kentucky agriculture.

Section 1A.iii of the annual report comprises KTRDC Tobacco Summit reports. No summit grants have been awarded since 2021, but some of the projects are continuing. The program emphasized collaboration and favored projects that paired basic researchers with applied researchers. Through this program, numerous research projects and collaborations have been supported. Many of these projects have advanced and received significant additional funding to continue the research.

Section 1A.iv of the annual report comprises the externally funded projects. There are significantly fewer externally funded projects than in the past, as industry funding has declined, and increasingly, those projects funded are confidential contracts which cannot be reported here.

At the end of the report (section 1B), there is a list of KTRDC publications and presentations given at conferences and meetings. Our peer-reviewed publication output continues to grow and illustrates the high-quality of the KTRDC research program: this report references 24 publications and 41 presentations from KTRDC scientists. As in previous years, KTRDC scientists continued to actively participate in conferences, workshops, and other events worldwide as a way of engaging with the international tobacco research community. Nine scientists employed by or supported by KTRDC attended the September Tobacco Science Research Conference (TSRC) in Knoxville, Tennessee, and presented six presentation and five posters. The CORESTA Smoke Techno conference was held in October in Annecy, France, and the Agro-Phyto conference was held in-person in Surabaya, Indonesia. Eight scientists employed by or supported by KTRDC attended the conference, presenting five presentations and one poster.

KTRDC employs around 30 full-time and part-time scientists and staff. Our program is a unique entity, recognized worldwide for its integrated expertise in tobacco breeding, analytics, genetics, biotechnology, and field testing. The Center's work would not be possible without our dedicated research staff whose productivity in terms of extramural funding and scientific publications is impressive for a unit of our size. This work would also not have been possible without the assistance that has been generously provided by the UK Martin-Gatton College of Agriculture, Food and Environment, UK farm personnel, and the Kentucky Tobacco Research Board. I would like to thank the research and administrative personnel at KTRDC for their dedicated effort to our research mission.

Ruth McNees, Ph.D.

Research Scientist, KTRDC and Quality Manager, CTRP

SECTION 1: RESEARCH

SECTION 1: RESEARCH

PART A: PROJECT REPORTS 2024-2025

SECTION 1: RESEARCH

PART A: PROJECT REPORTS 2024-2025

i. IN-HOUSE RESEARCH PROJECT REPORTS

AGRONOMIC TRIAL OF LOW NICOTINE LINES COMBINING A NOVEL LOW ALKALOID GENE WITH THE LA *NIC1NIC2* MUTANTS (2024)

Investigator(s): Anne Fisher (KTRDC), Colin Fisher (KTRDC), Jeff Kinney (KTRDC), Huihua Ji (KTRDC)

Report type: KTRDC in-house research project – final report

Lay Summary: We have found a spontaneous low alkaloid mutation in some of our material. These lines carry the *nic2* mutation, but the *Nic1* wild type, suggesting that this is a novel gene, which is not allelic to *nic1.* Alkaloids in these lines are not as low as in the low alkaloid (LA) *nic1nic2* mutants: they are about a quarter of the levels in the wild type, whereas the LA lines are about a tenth of the level of the wild type. We have made F_5 lines stacking this novel gene with the LA *nic1* and *nic2* in an attempt to lower alkaloids further. Preliminary data indicate that the addition of this novel gene to the LA mutants lowers alkaloids very significantly. However, the quality of these lines is extremely poor, even worse than the LA lines. These lines are also very late-flowering, so they are relatively more immature than the checks when the whole trial is topped and harvested at the same time. This may contribute to the green grades responsible for the poor quality. To accommodate the different flowering times, we split the trial, topping one treatment at the normal stage (25% of all plants in that treatment with at least one pink flower) and the other when the F_5 line was ready (25% of that line only with at least one pink flower). Both treatments were harvested as close to 28 days after topping as possible. The two objectives of this trial were (1) to measure cured leaf nicotine levels in F_5 lines stacking the novel gene with the LA *nic1* and *nic2* and (2) to establish if the quality of the F_5 line is improved by topping at the stage appropriate for that line. Stacking the novel gene with the LA *nic1* and *nic2* lowered overall nicotine in the LA line very considerably, but not low enough to meet the possible regulatory limit of 0.3-0.5 mg/g. Later topping improved the quality of all the low alkaloid lines very considerably, eliminating all green grades. Topping time had no effect on nicotine or on yield. Although we have stabilized a very low nicotine genotype, this LALN line will not be suitable for commercial production without further work: it has poor quality, lower yield and most importantly, no disease resistance.

Introduction

In 2017, the U.S. Food & Drug Administration (FDA) first announced its intention to regulate nicotine in combustible cigarettes to "non-addictive levels" (U.S. Food & Drug Administration, 2017). Benowitz and Hennigfield (2013) put "non-addictive levels" in the region of 0.5 mg nicotine per cigarette. Most manufactured cigarettes contain 10-15 mg nicotine per cigarette, so this target nicotine level would be 3-5% of current levels in cigarettes.

No threshold level has been stated by the FDA, but comments were requested "*about the merits of nicotine levels like 0.3, 0.4, and 0.5 mg nicotine/g of tobacco filler, as well as other levels of nicotine"*

(U.S. Food & Drug Administration, 2018). We are therefore testing the technical feasibility of achieving nicotine levels of 0.3-0.5 mg/g of nicotine in tobacco leaf, which is about 1% of the levels found in commercial tobacco leaf.

Nicotine levels in the low alkaloid (LA) *nic1nic2* mutant varieties currently available are about 10% of those in commercial varieties (Legg and Collins, 1971), and they are of notoriously poor quality (Figure 1). Clearly, these varieties would not enable manufacturers to meet the 0.3-0.5 mg/g nicotine levels. It may be possible to lower nicotine further with cigarette design, by using more stems and reconstituted sheet, which have lower nicotine. However, there is a limit to how much of these can be used in cigarette manufacturing. The FDA Advance Notice of Proposed Rule Making (ANPRM) specifies these levels in the filler (U.S. Food & Drug Administration, 2018), ruling out cigarette ventilation modification, so the best solution is almost certainly leaf with much lower nicotine.

There are multiple research projects focused on producing ultra-low nicotine varieties, through the use of various technologies such as gene editing. However, these technologies are not universally accepted in the tobacco industry. A solution might be a conventionally bred (and therefore acceptable) line with nicotine levels lower than the existing LA lines. We have found a spontaneous low alkaloid mutation in some of our material. These lines carry the *nic2* mutation, but the *Nic1* wild type, suggesting that this is a novel gene, which is not allelic to *nic1.* Alkaloids are not as low as in the low alkaloid (LA) *nic1nic2* mutants: they are about a quarter of the levels in the wild type, whereas the LA lines have about a tenth of the level of the wild type. We have made F_5 lines stacking this novel gene with the LA *nic1* and *nic2* in an attempt to lower alkaloids further. Preliminary data indicate that stacking this novel gene with the LA *nic1nic2* genes lowers nicotine very considerably. Although some grades in some trials did meet the 0.3-0.5 mg/g of nicotine limit, it could not be met consistently. However, the quality of these lines is extremely poor, even worse than the LA lines. These lines are also very late-flowering, so they are relatively more immature than the checks when the whole trial is topped and harvested at the same time. This may contribute to the green grades responsible for the poor quality (Figure 2). To accommodate the different flowering times, we split the trial, topping one treatment at the normal stage (25% of all plants in that treatment with at least one pink flower) and the other when the F_5 line was ready (25% of that line only with at least one pink flower). Both treatments were harvested as close to 28 days after topping as possible. The two objectives of this trial were (1) to measure cured leaf nicotine levels in F_5 lines stacking the novel gene with the LA *nic1* and *nic2* and (2) to establish if the quality of the F_5 line is improved by topping at the stage appropriate for that line.

Summary of Progress

Procedure – Field Work

Design

Three randomized complete blocks of a split plot design, with two main plots (topping time) and four subplots (varieties). Thirty plants per plot were harvested. *There were originally four blocks, but shortly after transplanting, we had significant mole damage in the fourth block and had to abandon it.*

Topping time

1. Standard topped when 25% of all plants in this main plot had at least one pink flower
2. Late topped when 25% of plants in the F_5 subplot had at least one pink flower

Varieties

1. Burley 21LC HA, high alkaloid, *Nic1Nic2* (AABB), commercial variety
2. LA Burley 21LC LA, low alkaloid, *nic1nic2* (aabb), about 10% of HA
3. F_5(LALN)-06-01-21 ULA, ultra-low alkaloid line, LA + novel gene, *nic1nic2* (aabbcc), about 2% of HA
4. J14 ULA, ultra-low alkaloid Burley 21 CRISPR line, 1-2% of HA

Agronomic Details

The seedlings were grown with all normal recommended practices, except that that we did not sow pelleted seed into float trays. Because we were using raw seed, seed was sown into trays using a vacuum raw seeder on March 19. Seedlings were transferred into float trays in randomized field order on May 7, at CORESTA growth stage 1006 (CORESTA, 2009), 15 days before transplanting.

For the first time, soil tests showed no requirement for lime. Two days before transplanting, on May 20, we applied 300 lb/ac K_2O as potassium sulfate and 200 lb/ac N as urea. The herbicides sulfentrazone (Spartan, 15.2 oz/ac) and clomazone (Command, 2.67 pt/ac) were applied pre-emergent the same day, May 20.

The study was transplanted on May 22 (Figure 3). Planting water chemicals were mefenoxam (Ridomil, 1 pt/ac), acephate (Orthene, 0.77 lb/ac), chlorantraniliprole (Coragen, 7.5 oz/ac) and imidacloprid (Nuprid, 10.5 oz/ac). On June 29, having observed some plants with black shank, we applied mefenoxam (Ridomil, 1 pt/ac) as a spray, incorporated into the top 2-4 inches of soil.

Shortly after transplanting, we had significant mole damage in the fourth block. The plants were so uneven and so far behind the other three blocks that we decided to abandon it and use only three replications.

There were a few good rains shortly after transplanting, and then it was very dry for weeks. Shortly before 6 weeks after transplanting, on July 1, we applied drip irrigation.

We have not used imidacloprid in the planting water for many years, because of environmental concerns. However, because of the insect problems experienced in previous years with the LA lines' susceptibility to both leaf eaters and sucking insects (Figure 4), we used it this year, and control of insects was significantly improved. Only two insecticide sprays were necessary.

Insecticide sprays applied were:

July 12, 7½ weeks after transplanting: chlorantraniliprole (Coragen), thiamethoxam (Actara), lambda-cyhalothrin (Warrior)

July 26, 9½ weeks after transplanting, 1 day after topping the standard treatment: chlorantraniliprole (Coragen), thiamethoxam (Actara), lambda-cyhalothrin (Warrior)

Rates were: chlorantraniliprole (Coragen) 6 oz/ac
thiamethoxam (Actara) 3 oz/ac
lambda-cyhalothrin (Warrior) 2 oz/ac

Flowers were counted in the standard main plot (Figure 5A) on July 10 (1%), July 12 (2%), July 15 (6%), July 17 (8%), July 19 (13%) and July 22 (25%), at which point this treatment was topped. Flowers were counted in the late main plot (Figure 5B) on July 10 (0%), July 12 (1%), July 15 (2%), July 17 (10%), July 19 (16%), July 22 (29%), July 24 (37%), July 26 (49%), July 29 (55%), July 31 (62%) and August 2 (68%), at which point this treatment was topped.

The standard main plot was topped July 22 and July 25, just under 9 weeks after transplanting (61 days), when the mean flower count for this main plot was 25%. The late main plot was topped August 2 and August 5, about 10½ weeks after transplanting (72 days), when the flower count for the F_5LALN line was 25%. Most of the plants in the standard main plot were topped in early flower or extended bud (Figure 6), at CORESTA growth stage 55-60 (CORESTA, 2009), but the F_5 line was later flowering than the other entries, and almost all plants were bud-topped. In the late main plot, the F_5 line was topped in early flower or extended bud, but almost all other entries were in full flower.

Three days before the first topping, on July 19, we applied the full rate of fatty alcohol (Sucker Plucker, 2.5 gal/ac) by backpack sprayer (12 ml/plant), below the bud and young leaves. On July 22, the first topping of the standard treatment, we applied the full rate of fatty alcohol by backpack sprayer only to the plants topped that day. On July 25 (the second topping of the standard treatment), August 2 (the first topping of the late treatment) and August 5 (the second topping of the late treatment) this was repeated for all topped plants. On August 6, the day after the last topping, we sprayed butralin (Butralin 2 qt/ac) over the top of all plants. From then until harvest, hand suckering was done regularly.

We applied azoxystrobin (Quadris) on August 14, because frogeye (*Cercospora nicotianae*) was starting to develop on the lower leaves. By the time we harvested, there was a heavy infection of frogeye.

The standard main plot was harvested on August 21, 29 days after topping, and picked up the next day. The late main plot was harvested 8 days later, on August 29, 26 days after topping, and picked up the next day. All harvested tobacco was put onto rail wagons (Figure 7) which were parked in the barn until housing. The tobacco was taken down on November 14, and stripped November 14-15.

Sampling for chemical analysis

Samples for chemical analysis were taken at stripping, from all four stalk positions plus the 4th leaf from the top. We stripped all 30 plants in the plot into four stalk positions, thoroughly mixed the leaves in each stalk position, and randomly drew 30 leaves from each pile. These 30 random leaves comprised the sample for each stalk position. The 4th leaf from the top of the stalk was taken from each stalk by the person pulling the B (leaf) grade.

The leaves were stemmed, then oven-dried at 50°C. They were then ground in a Wiley mill to pass through a 1 mm screen.

Procedure – Analytical Laboratory

Constituents to be analyzed

The ground lamina was analyzed for alkaloids in the cured leaf samples taken at stripping.

Laboratory analysis

Alkaloid analyses were done on a GC (gas chromatograph) with FID (flame ionization detection). We made the same adjustments as done previously to the sample weight, because of the expected low nicotine

values of some samples. Depending on the sample, we increased the weight 2- or 3-fold (200 mg or 300 mg in 5 ml solvent), correcting the data accordingly.

For Bu 21LC, we used the normal 100 mg in 5 ml solvent. For LA Bu 21LC, we increased that 2-fold (200 mg in 5 ml solvent). For the ultra-low J14 and F_5 LALN lines, we increased the normal sample weight 3-fold (300 mg), in a solvent volume of 5 ml.

Procedure – Statistical Analysis

We used a generalized linear effects model, with treatment (topping) as a random effect.

For all the nicotine-related variables, there was considerable heteroscedasticity, because of the large difference between the HA lines, compared with the LA and ULA lines: alkaloids in the HA line are typically 10x the level in the LA lines, and 50-100x the level in ULA lines. The HA line is included as a reference point, but the important comparisons for nicotine are between the LA and the two ULA lines. For these reasons, the statistical analysis was done only on the three low alkaloid lines, adjusting for the HA line. With this adjustment, there was no heteroscedasticity, and no need for transformation.

For all other variables, there was no heteroscedasticity, so all four lines were included in the statistical analysis.

Results and Discussion

Field Observations

There were a few good rains shortly after transplanting, and then it was very dry for weeks. Shortly before 6 weeks after transplanting, on July 1, we applied drip irrigation.

Generally, the crop grew fairly well; it was even and growth was similar in all varieties (Figure 8), although the LA Bu21 and J14 were slightly later flowering, and the F_5 LALN line was much later flowering.

Aphid pressure (Figures 4A, 4B) was not as high as in previous years, probably because of the effectiveness of the imidacloprid. A few plants were damaged by leaf-eaters, mainly hornworm (Figure 4D) and a few budworm (Figure 4C). We did not have the heavy Japanese beetle pressure that we have had previously (Figure 4E).

Yield and Quality

The ANOVA for grade index (Table 1) showed highly significant differences between varieties and treatments, as well as a significant interaction between variety and treatment; but for yield, there were significant differences only between varieties.

Grade Index: As has been found in all previous studies, all three low alkaloid lines had lower grade indices than the HA check in the standard topping treatment (Figure 9A). However, later topping considerably improved the quality of all low alkaloid lines, but had no effect on the quality of the HA check. In the late topping treatment, the LA Bu21 was no different from the HA check: to our knowledge, it has always had poorer quality. The two ULA lines still had lower grade indices than the HA check, but the difference was much less than in the standard topping. In the standard topping, the low alkaloid lines had grade indices of 32-42; such tobacco is mostly unusable. In the late topping, the two ULA lines had grade indices of 63-68: while not of top quality, this tobacco is still usable.

In figure 9B, the same data are arranged by variety, showing the magnitude of quality improvement with later topping in the low alkaloid lines.

Data are not presented for main plot means, because there was a variety x treatment interaction (Table 1).

Percentage Green Grades: In the calculation of grade index, no other factor decreases grade index as much as green grades. The preponderance of green grades is a major contributor to the poor quality of all low alkaloid lines, but particularly the LALN line. For this reason, we calculated the percentage of green grades for each plot (Figure 10). The HA check had no green grades in either topping treatment: the low alkaloid lines had 62-76% in the standard topping. In the late topping, there were no green grades at all in any of the varieties. The absence of green grades is largely responsible for the marked improvement in the quality of the low alkaloid lines in the later topping treatment.

Yield: The LA *nic1nic2* mutants have been reported to be lower yielding than the HA checks, but we have never found this to be the case. The LA line was no different from the HA check, but the two ULA lines were lower yielding than the check (Figure 11A). Topping treatment had no effect on the yield of any of the varieties (Figure 11B). This is also shown in Figure 12, where there is no difference between the topping main plot means. This was surprising, as later topping would be expected to result in a yield reduction).

Chemistry

The ANOVA for nicotine + nornicotine and for nicotine (Table 2) showed highly significant differences only between varieties; but for conversion, there were no significant differences at all.

Nicotine Conversion: We often present our data as nicotine + nornicotine, to account for nicotine levels being affected by different levels of conversion in the different varieties. However, there were no differences in conversion levels between varieties (Table 2), and conversion was very low (2.5-3.8%), so we have presented the data as nicotine (mg/g).

Nicotine: Overall nicotine (mg/g) was calculated as a weighted average of all stalk positions (Figure 13). The nicotine levels relative to the check were within the expected range (Figure 13A). Topping treatment had no effect on the nicotine levels of any of the varieties (Figure 13B), and there was no difference between the two main plots (Figure 14). This is because, regardless of topping time, the topping-harvest interval remained the same in both topping treatments, and this is what affects nicotine accumulation. It should be noted that none of the varieties have overall nicotine low enough to meet the possible regulatory limit of 0.3-0.5 mg/g (Figure 13A). The same is true of the individual grades (Figure 15).

Conclusions

Stacking the novel gene with the LA *nic1* and *nic2* lowered overall nicotine in the LA line very considerably, from 3.4 mg/g to 0.84 mg/g, almost as low as the gene-edited line J14. The same pattern was observed in the individual grades. However, nicotine was not low enough in any of the grades or overall to meet the possible regulatory limit of 0.3-0.5 mg/g.

Later topping improved the quality of all the low alkaloid lines very considerably. There was a high percentage of green grades in all the standard topped low alkaloid lines, but there were no green grades in any varieties in the later topping.

Topping time had no effect on nicotine levels, because the topping-harvest interval was the same for both topping treatments.

Topping time had no effect on yield.

Plans for Future Work

This work is now complete, as we are confident that both *nic1* and the novel low nicotine gene are homozygous: the *nic2* gene was not segregating. This line will need a lot of work if it is to be used commercially: it has a poor type, very poor quality, slightly lower yield and probably most importantly, no disease resistance. Resistance to TMV, PVY/etch and black shank are essential for most farms in Kentucky.

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U.S. Food & Drug Administration. 2018. Advance Notice of Proposed Rulemaking – Tobacco product standard for nicotine level of combusted cigarettes. Federal Register, 03/16/2018. https://www.federalregister.gov/documents/2018/03/16/2018-05345/tobacco-product-standard-for-nicotine-level-of-combusted-cigarettes

Tables and Figures

Table 1: ANOVA for yield and quality

	Grade Index	Yield
Variety	***	***
Treatment	**	NS
Var x Trmt	**	NS

NS $p>0.05$ ** $p<0.01$ *** $p<0.001$

Table 2: ANOVA for overall nicotine + nornicotine, conversion and nicotine

	Nic + Nornic (Wtd)	Conversion (Wtd)	Nicotine (Wtd)
Variety	***	NS	***
Treatment	NS	NS	NS
Var x Trmt	NS	NS	NS
	Grades similar, but C & T significant interaction	Grades similar, but C significant interaction	Grades similar, but C & T significant interaction

NS $p>0.05$ *** $p<0.001$

Figure 1: Poor quality of the LA line vs HA

Figure 2: Green grades in the F_5 LALN

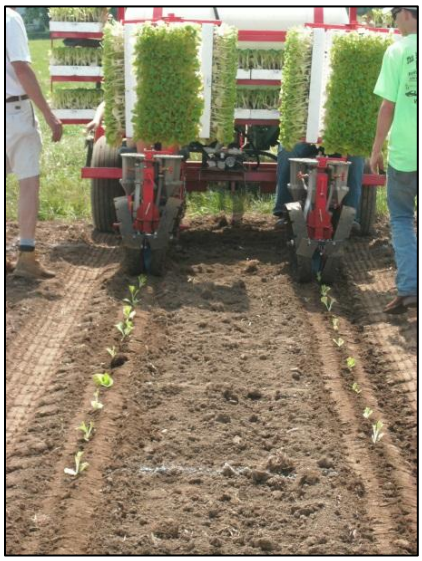


Figure 3: Transplanting

A

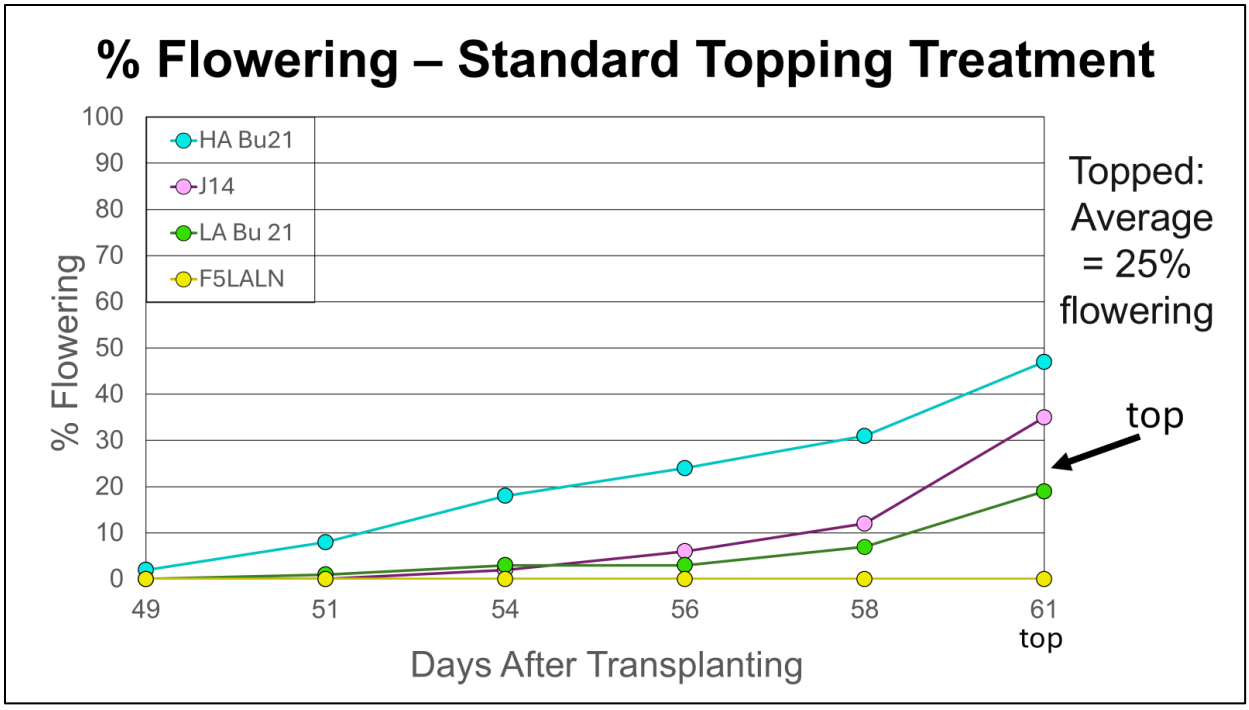
B

C

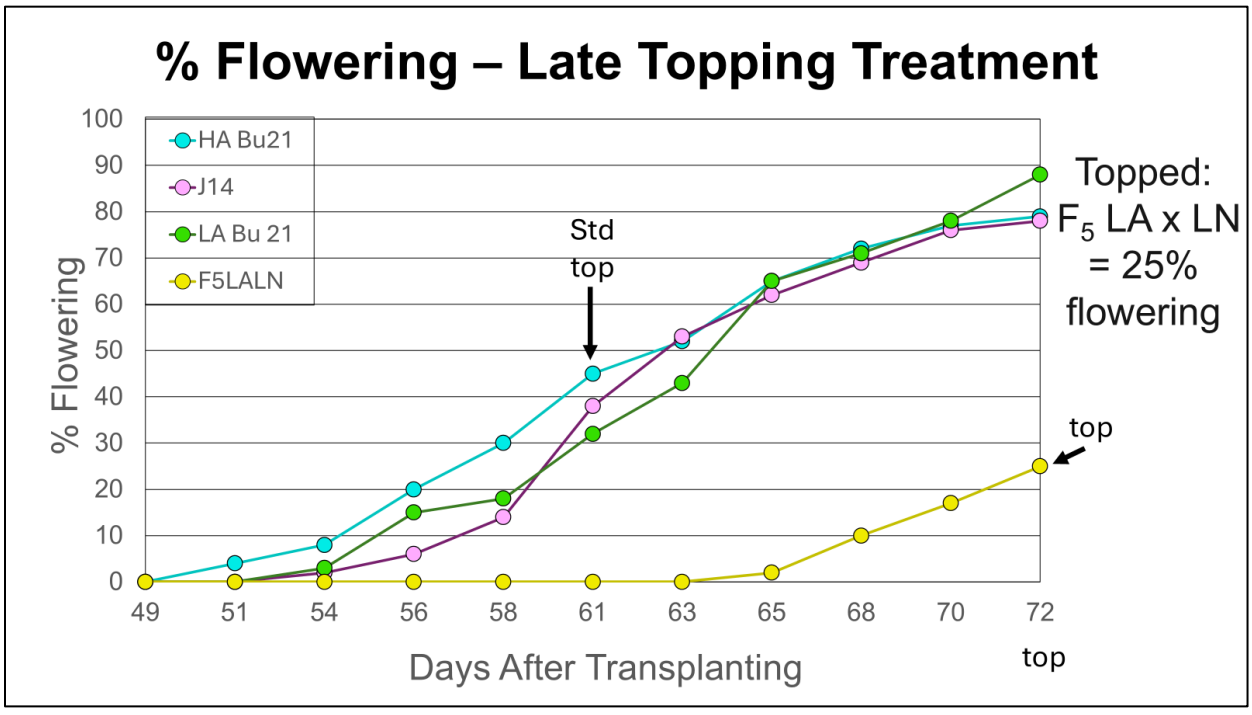
D

E

Figure 4: Sucking and chewing insect pests. Sucking insects: **A, B.** Aphids Chewing insects: **C.** budworm **D.** hornworm **E.** Japanese beetles



A



B

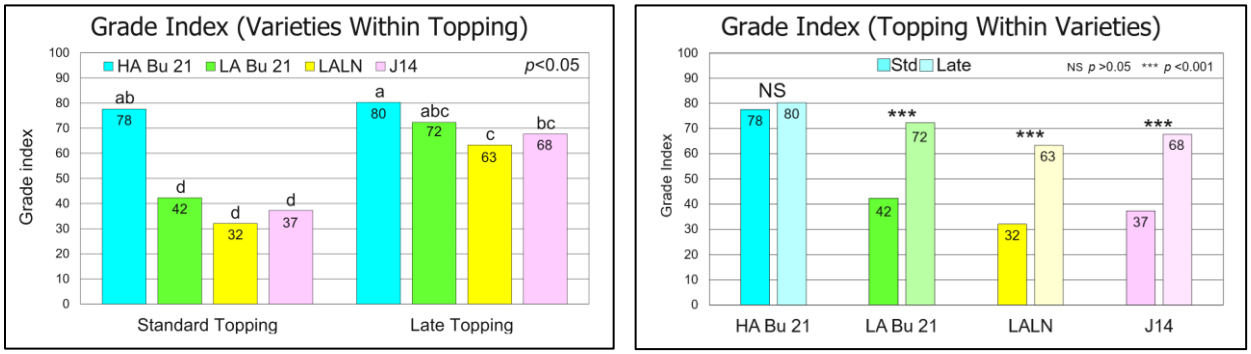
Figure 5: Flowering percentage for each variety. **A.** Standard topping treatment, topped when the average flowering over the standard main plot is 25% (61 days after transplanting)

B. Late topping treatment, topped when the F_5LALN line is 25% in flower (72 days after transplanting)

Figure 6: Most plants topped in early flower or extended bud

Figure 7: Tobacco on railwagons

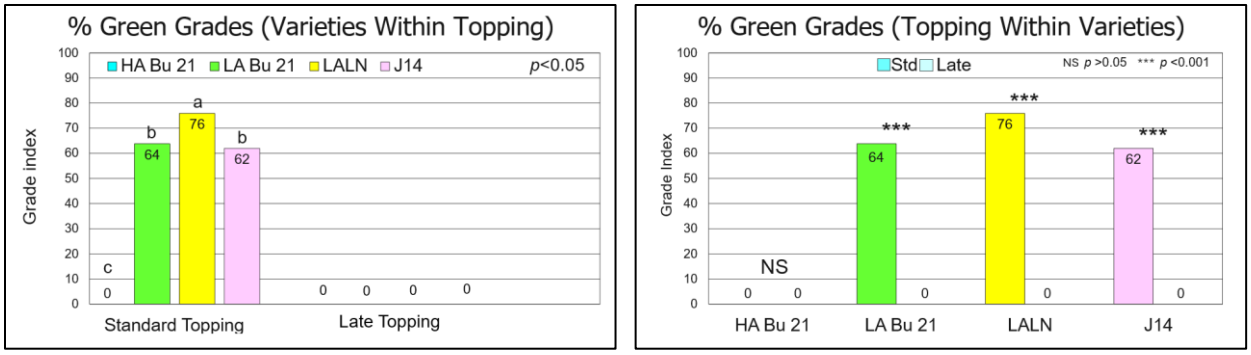
Figure 8: Even, well-grown field



A **B**

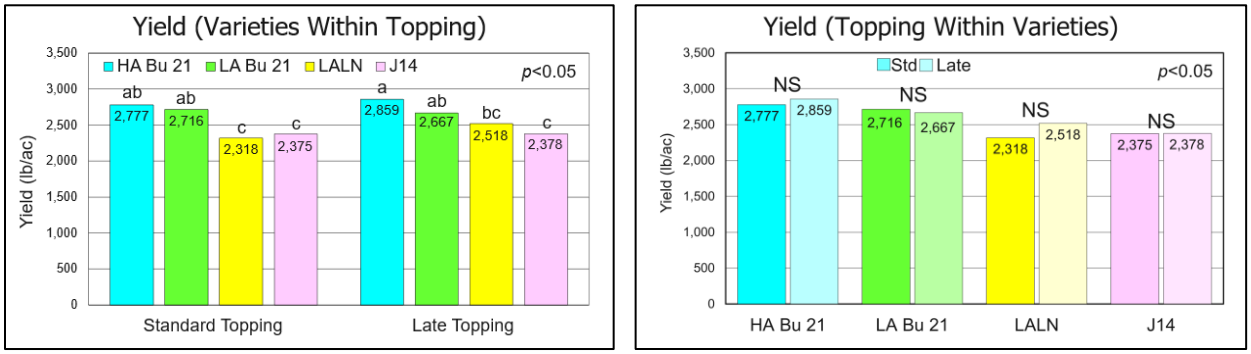
Figure 9: Grade index **A.** Varieties within topping **B.** Topping within varieties

Bars with a common letter are not significantly different (p>0.05)



A **B**

Figure 10: Percentage green grades **A.** Varieties within topping **B.** Topping within varieties
Bars with a common letter are not significantly different (p>0.05)



A **B**

Figure 11: Yield (lb/ac) **A.** Varieties within topping **B.** Topping within varieties
Bars with a common letter are not significantly different (p>0.05)

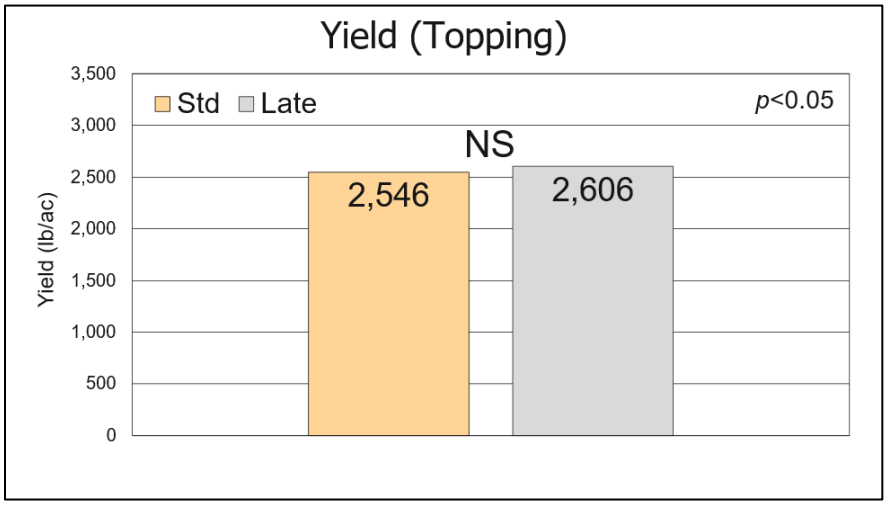
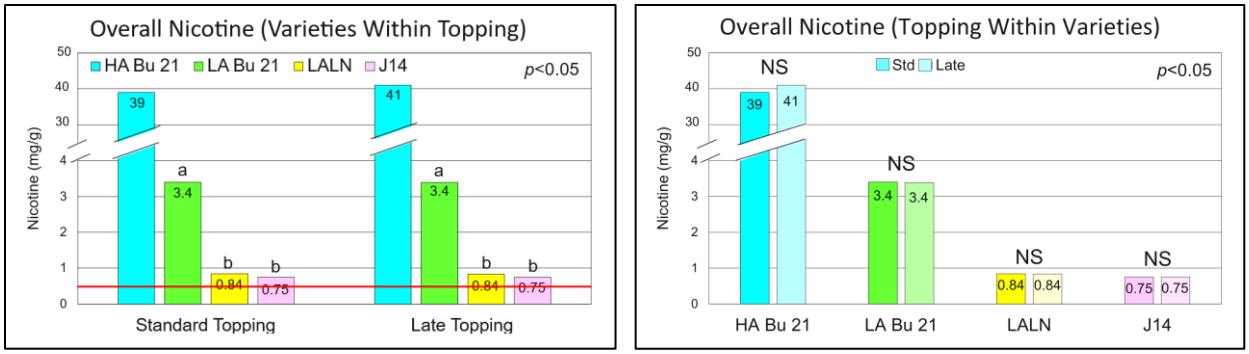


Figure 12: Yield (lb/ac) for topping main plots, averaged over varieties



A **B**

Figure 13: Overall nicotine (mg/g) **A.** Varieties within topping **B.** Topping within varieties. Red line represents 0.5 mg/g possible regulatory limit.

Bars with a common letter are not significantly different ($p>0.05$)

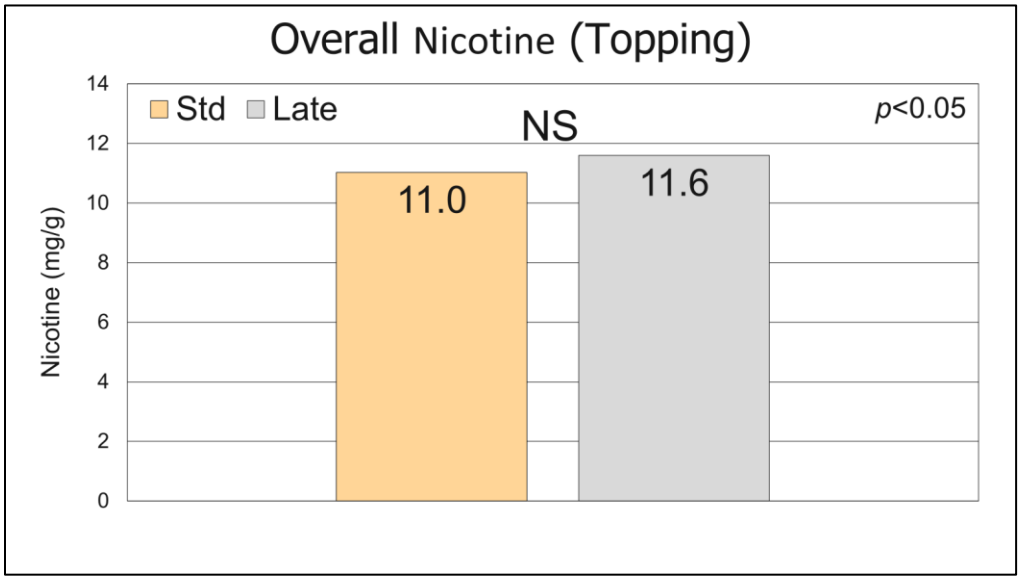


Figure 14: Overall nicotine (mg/g) for topping main plots, averaged over varieties

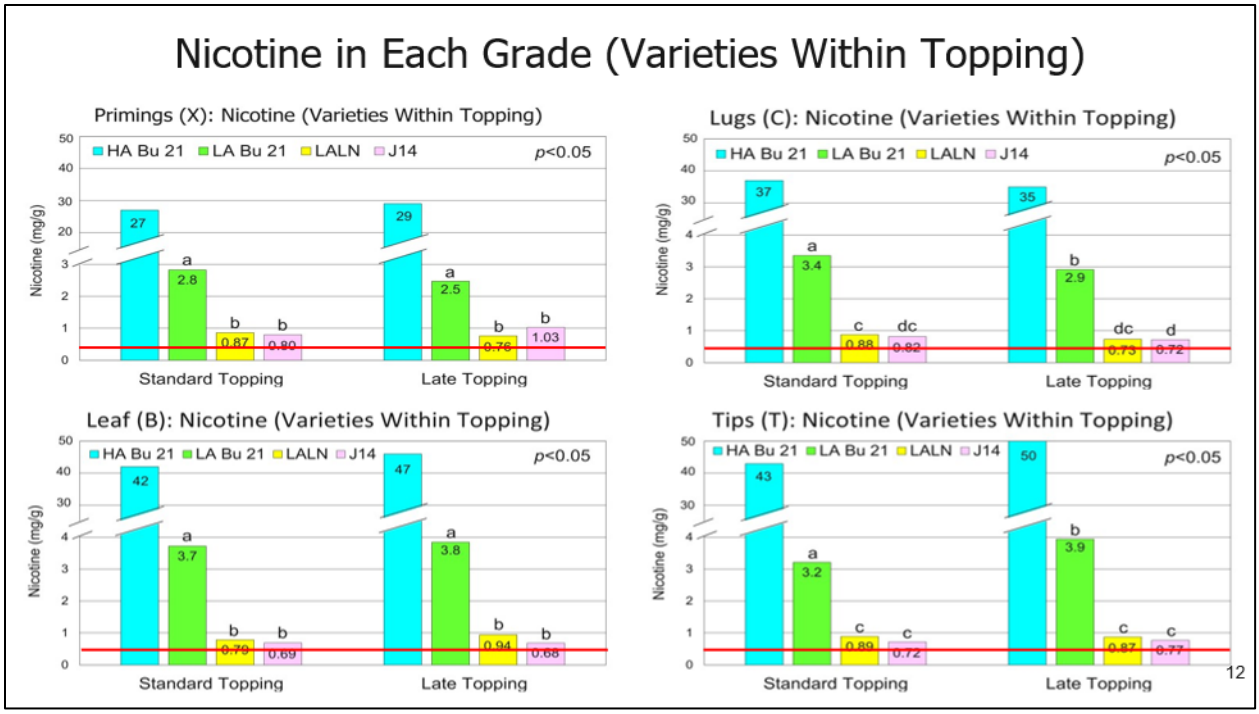


Figure 15: Nicotine (mg/g) in each grade, (varieties within topping). Red line represents 0.5 mg/g possible regulatory limit.

Bars with a common letter are not significantly different (p>0.05)

KTRDC ANALYTICAL LABORATORY (2024)

Investigator(s): Huihua Ji (KTRDC)

Report type: KTRDC in-house research project – final report

Lay Summary: The Kentucky Tobacco Research and Development Center (KTRDC) analytical laboratory provides analytical methods development and validation and sample analyses for tobacco, hemp, artemisia, and forage research at the University of Kentucky and our collaborators at other institutes and companies.

Introduction

Kentucky Tobacco Research and Development Center (KTRDC) analytical laboratory in the University of Kentucky's Martin-Gatton College of Agriculture, Food, and Environment provides analytical methods development and validation and quantitative sample analyses for tobacco, hemp, artemisia, and forage research at the University of Kentucky and our collaborators at other institutes and companies. We work collaboratively with other academic institutions, commercial tobacco companies, private research institutes, and tobacco growers. Our analytical services include (1) alkaloids, nitrite and nitrate, benzo[a]pyrene (B[a]P), pseudo-oxynicotine (PON), ammonia, tobacco-specific N-nitrosamines (TSNAs), fatty acid analysis, and carbonyls in tobacco and tobacco products; (2) smoke analysis for the cigarettes, cigars, e-vapors, and heated tobacco products; (3) alkaloid analysis in forage; (4) cannabinoid analysis in hemp; (5) smoke condensate of cigarettes and cigars; (6) artemisinin and its precursors' analysis in artemisia and (7) preparation of extraction solutions for inorganic analyses. In addition, on-demand analytical method developments and validation are provided when possible.

Summary of Progress

Routine analysis

Tobacco analysis

KTRDC analytical laboratory provided the tobacco and tobacco products analysis and mainstream smoke analysis.

Tobacco and tobacco product analyses include

- Alkaloids (nicotine, nornicotine, cotinine, myosmine, anabasine, and anatabine)
- TSNAs (N'-nitrosonornicotine (NNN), N'-nitrosoanatabine (NAT), N'-nitroso-anabasine (NAB), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK))
- B[a]P
- PON
- Fatty acid (Oleic acid (OA), trans-Vaccenic acid (VA), Linoleic Acid (LA), Linolenic Acid (LN), Palmitic Acid (PA), and Stearic Acid (SA))
- Selected Carbonyls (formaldehyde, acetaldehyde, and crotonaldehyde)
- Nitrite and nitrate
- Ammonia

- Reduced sugar (fructose, glucose, and sucrose)
- Protein
- Water activity
- PH
- Oven volatiles

Mainstream smoke analyses include

- Alkaloids
- TSNAs
- B[a]P
- Selected aromatic amines (1-aminonaphthalene (1-AN), 2-aminonaphthalene (2-AN), 3-aminobiphenyl (3-ABP), 4-aminobiphenyl (4-ABP), o-toluidine (o-TOL), and o-anisidine (o-ANI))
- Selected volatile substances (1,3-butadiene, isoprene, acrylonitrile, benzene, and toluene)
- Carbon monoxide
- Water
- Total particulate matter (TPM)
- Nicotine-free dry particulate matter (NFDPM)
- Puff counts

physical parameters measurements

- Ventilation
- Hardness
- Resistance to draw (pressure drop)
- Length
- Diameter
- Weight

Our data supported the projects listed below:

1. Cigar Reference Products Program
2. Service - Regional Quality Trials
3. Potential ultra-low nicotine limit in tobacco
4. The roots of tobacco plants probably dictate the leaf quality
5. Stacking a novel low nicotine gene with the LA nic1nic2 mutants lowers nicotine to ultra-low levels
6. Pearce fertility trial
7. CORESTA low nicotine collaborative study
8. Pale yellow tobacco
9. Sulfur and chloride fertilization impact on burley tobacco growth, yield and leaf chemistry

Fescue analysis

KTRDC analytical laboratory also analyzed tall fescue forage and seed, including

- Lysergic acid
- Ergovaline, ergovalinine
- Ergotamine, ergotaminine
- Peramine
- N-acetylloline, N-formylloline, and N-acetylnorloline

Our work supported the Forage-Animal Production Research Unit of the US Department of Agriculture (FAPRU) and research groups at the University of Kentucky, including the groups of PIs Rebecca McCulley, James Klotz, Michael Flythe, Randy Dinkins, Kyle McLeod, and Ray Smith. The projects we supported include

1. Serotonin can stimulate vasorelaxation in ovine lateral saphenous veins precontracted with ergovaline
2. Isoflavone supplementation via red clover hay in endophyte-infected tall fescue grazing systems improves growth and postgraze physiological recovery of beef steers
3. Association of serotonin and ergot alkaloids on tissue partitioning and contractile response of bovine blood vessels

Artemisia analysis

KTRDC analytical laboratory provides the analyses of artemisinin, dihydroartemisinic acid (DHAA), and artemisinic acid (AA) in Artemisia *annua L.* Our work supported Artemisia *annua L.* farm research for the KTRDC in the University of Kentucky's College of Agriculture, Food, and Environment.

Hemp analysis

KTRDC analytical laboratory also provided cannabinoid analyses in hemp samples. It includes cannabidiol (CBD), cannabinol (CBN), delta 9- and delta 8- tetrahydrocannabinol (delta9- and delta8- THC), cannabidiolic acid (CBDA), and tetrahydrocannabibolic acid (THCA). Our work supported hemp research work at the University of Kentucky.

In 2025, we analyzed a total of 1,303 tobacco samples (Table 1 shows the details of tobacco analysis) and 886 fescue forage and seed samples. Usually, several different constituents are analyzed for each sample. In addition to these sample analyses, we also provided some smoke condensates to companies or institutes from all over the world.

Hemp analysis Proficiency testing

Industrial hemp is a versatile Cannabis plant grown for its fiber, seed, or oil. Hemp has thousands of applications such as food, clothes, paper, textile, and personal care products, etc. Growing hemp crops is attracting more and more investors and farmers. Hemp contains many kinds of cannabinoids including psychoactive component tetrahydrocannabinol (THC). THC is present in two different isomers, Delta-9 THC and Delta-8 THC. According to the literate, Delta-8 THC has a similar euphoric effort as Delta-9 THC. The KTRDC analytical laboratory developed a UPLC/MS/MS method to determine Delta-8 and Delta-9 THC

in hemp products. This method successfully separates Delta-8 and Delta-9 THC in samples. The Regulatory Service Laboratory of the University of Kentucky continued to conduct hemp proficiency testing in Sept. and Nov. 2025. Hemp samples and CBD oil were included in the 2025 proficiency testing. In order to evaluate our in-house UPLC/MS/MS methods for cannabinoid analysis, we continued to participate in the proficiency study. The results (shown in Table 2) were submitted to the Regulatory Service Laboratory of the University of Kentucky. The Sept. 2025 hemp PT report demonstrated that our in-house UPLC/MS/MS method is accurate and precise. It is suitable for Delta-8 and Delta-9 THC, CBD, CBN, THCA, and CBDA analysis in hemp samples. We are waiting for the Nov. 2025 PT reports.

Determination of the storage stability of the certified reference cigars

This is an in-progress project. In 2019, the Center for Tobacco Reference Products in KTRDC, Martin-Gatton College of Agriculture, Food and Environment at the University of Kentucky obtained a cooperative agreement with the Center for Tobacco Products (CTP) of the U.S. Food and Drug Administration (FDA) to produce new well-characterized cigars reference products that include the filtered cigar, cigarillos, and large cigars. These reference cigars are the first certified cigar reference products. There are no data to demonstrate the storage stability of reference cigars. An assessment of long-term and short-term stability needs to be conducted for the three reference cigars. The long-term stability assessment of these materials will reflect the behavior of the cigars on the storage shelves while the short-term stability will reflect extra effects due to the handling and transport of the reference materials. These stability assessments are done in terms of both physico-chemical as well as biological parameters.

The new reference cigars, including the large cigar (1RLC), cigarillo (1RSC), and filtered cigar (1RFC) (Figure 1), were manufactured in May 2022, December 2022, and March 2023, respectively. To systematically evaluate the stability of the newly certified reference cigars, the long-term storage studies for 1RLC, 1RSC, and 1RFC were initiated in September 2022, May and June 2023, respectively.

A total of 24 boxes of 1RLC, 22 boxes of 1RSC, and 16 cartons of 1RFC from different cases were sampled and stored in their original packaging within a sealed plastic bag at room temperature, 4°C, and -20°C for long-term storage study. After each storage period (0, 1, 2, 3, 6, 9, 12,18, 24, 30, 36, 42, 48, 54, and 60 months), some cigar samples from each storage condition were and will be used for unburned tobacco and smoke analysis. Initial analyses of new reference cigars included moisture content (oven volatiles), alkaloids, and TSNAs in unburned cigar tobacco; alkaloids, CO, TSNAs, total particulate matter (TPM), and puff counts in cigar mainstream smoke. Chemical constituents were determined in five replications from each storage condition. TPM, CO, pressure drop, and puff counts were measured in ten replicates from each storage condition.

Prior to analysis, the cigar samples that were stored in the freezer (-20°C) were first transferred to a refrigerated environment for 24 hours and then moved to room temperature to equilibrate the temperature and moisture. The cigar samples stored at 4°C were directly moved to room temperature to reach equilibrium. The cigars were conditioned for at least 72 hours at 22°C and 60% relative humidity prior to smoking. The cigars were smoked under the cigar-smoking regime using a linear cigar-smoking machine. The smoking procedures followed were CRM 46, CRM 64, and CRM 65. TSNAs in smoke were determined by a validated in-house GC/MS/MS method. The moisture and TSNAs in unburned cigar tobacco were measured using CRM 76 and CRM 72, respectively. Alkaloids were measured with GC-FID

using the validated in-house method adapted from CRM 62. Until now, three years of data for 1RLC, and two years of data for 1RSC and 1RFC have been collected. The data are shown in Tables 3-20.

Determination of cannabinoids in infused beverages

Prior to June 1, 2025, THC (Tetrahydrocannabinol)-infused beverages made from cannabis were legal and could be purchased in Kentucky by individuals aged 21 and over, under certain regulatory conditions. However, the market for THC-infused beverages was disorganized and lacked consistent regulation. THC levels varied widely, ranging from 2 mg to as much as 100 mg per 12-ounce can, and in many cases, the actual THC content did not match what was stated on the label.

Based on the request from a member of the Kentucky State Legislature, the KTRDC analytical lab has determined the THC level in some cannabinoid-infused beverages available on the Lexington market. After reviewing the literature, the KTRDC analytical lab selected and modified the method based on the QuEChERS extraction kit, following the ultra-performance liquid chromatography with tandem mass spectrometry (UPLC/MS/MS) measurement. The beverage sample was first degassed for 20 minutes, then extracted with acetonitrile, followed by the addition of a QuEChERS extraction pouch. The resulting supernatant was transferred to a Captiva EMR–Lipid cleanup tube. The eluate from the EMR–Lipid tube was then diluted with an 80:20 (v/v) acetonitrile/water solution and prepared for UPLC-MS/MS analysis.

Cannabinoid analysis was performed using a Waters ACQUITY UPLC H-Class system coupled with an Xevo TQD Triple Quadrupole Mass Spectrometer (Figure 2). Separation of the cannabinoids was achieved using an ACQUITY UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm particle size).

The KTRDC analytical lab has determined the THC contents in 10 different brands of cannabinoid-infused beverages (Figure 3), ranging from 2 to 100 mg per 12-ounce can based on labeling. These beverages were purchased in Lexington, KY, from the end of February to March 2025. The test results are shown in Table 21. Based on our preliminary data, the actual THC content did not match what was stated on the label for most products.

Determination of terpenes in orange peels

Terpenes are a large and diverse class of naturally occurring organic compounds produced by many plants. They are responsible for the distinctive aromas and flavors of plants. Based on the USDA researchers' request, the KTRDC analytical lab set up a method to determine the terpenes in orange peel using gas chromatography-tandem mass spectrometry (GC/MS/MS), including α-pinene, β-pinene, β-myrcene, limonene, terpinolene, linalool, and β-caryophyllene.

The freeze-dried orange peels were ground to pass through a 1 mm screen. 500 mg of ground orange peel were weighed into a glass vial, and the tridecane solution, serving as an internal standard, and 10 mL of hexane were also added to the vial. The mixture was sonicated for 15 minutes, then filtered through a 0.45µm PTFE filter to remove the orange powder. The filtrate might need further dilution before it is injected into GC/MS/MS, depending on the analyte levels in the samples.

Terpenes analysis was performed using an Agilent 7890B gas chromatograph equipped with a 7000C Triple Quad mass spectrometer system (GC/MS/MS). The full separation of terpenes was achieved using an Agilent DB-17 GC capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness) and the following GC gradient program: the column temperature program was started at 40 °C for 2 minutes, and then increased to 200 ˚C with the rate of 5 ˚C/min, at last held at 280˚C for 5 minutes. The tandem mass

spectrometer was operated in an electron ionization source with the multiple reaction monitoring (MRM) mode. The temperatures of the transfer line, ion source, and quadrupoles were set at 290, 230, and 150 °C, respectively.

The KTRDC analytical lab is also working on the terpenes analysis for the hemp sample using the same methodology.

CORESTA collaborative studies

Please see our annual report for 2025 CORESTA collaborative studies.

Plans for Future Work

We plan to continue providing analytical services to our academic colleagues and the tobacco industry. We will also continue to develop new analytical methods as required, as well as undertake new investigative works.

References

CORESTA Recommended Method N° 69: Determination of pH in smokeless tobacco products

CORESTA Recommended Method N° 72: Determination of tobacco-specific nitrosamines in smokeless tobacco products by LC-MS/MS

CORESTA Recommended Method N° 76: Determination of moisture content (oven volatiles) of smokeless tobacco products

CORESTA Recommended Method N° 81: Routine analytical machine for E-cigarette aerosol generation and collection - definitions and standard conditions

CORESTA Recommended Method N° 82: Determination of benzo[a]pyrene in tobacco products by GC-MS

CORESTA Recommended Method N° 62: Determination of nicotine in tobacco and tobacco products by gas chromatographic analysis

CORESTA Recommended Method N° 87: Determination of nicotine in tobacco products by GC/MS

CORESTA Recommended Method N° 46: Atmosphere for Conditioning and Testing Cigars of all Sizes and Shapes

CORESTA Recommended Method N° 64: Routine Analytical Cigar-Smoking Machine - Specifications, Definitions and Standard Conditions

CORESTA Recommended Method N° 65: Determination of Total and Nicotine-Free Dry Particulate Matter using a Routine Analytical Cigar-Smoking Machine – Determination of Total Particulate Matter and Preparation for Water and Nicotine Measurements

CORESTA Recommended Method N° 66: Determination of Nicotine in the Mainstream Smoke of Cigars by Gas Chromatographic Analysis

CORESTA Recommended Method N° 67: Determination of Water in the Mainstream Smoke of Cigars by Gas Chromatographic Analysis

CORESTA Recommended Method N° 68: Determination of Carbon Monoxide in the Mainstream Smoke of Cigars by Non-Dispersive Infrared Analysis

Figures and Tables

Table 1: Details of tobacco samples analyses in 2025

Analyte	**Number of samples**
Alkaloids	556
TSNAs	127
Nitrates	
NH4	
sugar	
Total 2024-25 cured	**683**
2025 early samples	
Alkaloids	620
Total run 2024-25	**1,303**

Table 2: Hemp proficiency testing data

Sample	CBD	CBN	delta-8-THC	delta-9-THC	THCA	CBDA	Total THC	Total CBD
	%	%	%	%	%	%	%	%
HM25 Sep1-1	4.867	0.057	ND	0.183	0.025	2.932	0.205	7.438
HM25 Sep1-2	4.432	0.057	ND	0.170	0.024	2.490	0.191	6.616
HM25 Sep1-3	4.732	0.057	ND	0.186	0.039	2.940	0.221	7.311
HM25 Sep2-1	4.534	0.093	ND	0.097	0.009	1.985	0.104	6.275
HM25 Sep2-2	4.337	0.082	ND	0.088	0.008	1.879	0.096	5.986
HM25 Sep2-3	4.244	0.090	ND	0.081	0.005	1.786	0.085	5.811
HM25 sep OIL-1	0.517	0.017	0.282	0.032	ND	ND	0.031	0.517
HM25 sep OIL-2	0.501	0.016	0.275	0.026	ND	ND	0.030	0.501
HM25 sep OIL-3	0.488	0.012	0.261	0.027	ND	ND	0.029	0.488
HM25 Nov1-1	5.084	0.057	ND	0.152	0.012	2.109	0.163	6.934
HM25 Nov1-2	5.761	0.068	ND	0.179	0.016	2.461	0.193	7.919
HM25 Nov1-3	4.700	0.056	ND	0.143	0.008	1.872	0.149	6.341
HM25 Nov2-1	6.428	0.079	ND	0.182	0.068	3.977	0.242	9.916
HM25 Nov2-2	6.553	0.073	ND	0.192	0.066	4.001	0.250	10.062
HM25 Nov2-3	5.023	0.056	ND	0.141	0.048	2.987	0.184	7.643
HM25 Nov OIL-1	0.986	0.010	0.324	0.047	ND	ND	0.371	0.099
HM25 Nov OIL-2	0.906	0.010	0.312	0.045	ND	ND	0.356	0.091
HM25 Nov OIL-3	0.963	0.011	0.318	0.045	ND	ND	0.363	0.096

Table 3: Unburned tobacco data of the reference cigar (1RLC) stored at -20°C (n=5)

Months	weight	Pre-conditioning	Nicotine	NNN	NNK
	g	Moisture (%)	mg/g	ug/g	ug/g
0	7.782 ± 0.458	13.39 ± 0.48	14.47 ± 0.98	12.84 ± 2.15	1.73 ± 0.33
1	7.797 ± 0.374	13.94 ± 0.17	14.47 ± 0.85	13.17 ± 0.57	2.42 ± 0.47
2	7.763 ± 0.334	13.78 ± 0.36	15.27 ± 0.65	12.72 ± 1.08	1.63 ± 0.31
3	8.012 ± 0.329	14.01 ± 0.55	15.58 ± 0.88	12.18 ± 1.09	1.81 ± 0.34
6	7.778 ± 0.385	14.00 ± 0.33	14.61 ± 1.29	12.47 ± 0.43	1.75 ± 0.43
9	7.957 ± 0.257	13.96 ± 0.49	15.53 ± 0.52	13.40 ± 1.41	2.66 ± 0.74
12	7.662 ± 0.272	12.37 ± 0.67	14.11 ± 1.08	9.61 ± 1.47	1.61 ± 0.24
18	7.793 ± 0.232	13.07 ± 0.66	15.97 ± 0.95	11.79 ± 1.08	1.74 ± 0.04
24	7.724 ± 0.335	14.28 ± 0.36	14.36 ± 0.85	12.19 ± 1.69	1.90 ± 0.42
36	7.662 ± 0.303	14.53 ± 0.39	15.44 ± 0.49	12.79 ± 1.43	2.21 ± 0.23

Table 4: Smoke data of the reference cigar (1RLC) stored at -20°C

Months	TPM	puffs	CO	Nicotine	NNN	NNK
	mg/cigar	n/cigar	mg/cigar	mg/cigar	ng/cigar	ng/cigar
0	150.1 ± 14.1	79.7 ± 5.7	236.98 ± 16.31	8.77 ± 1.15	5881 ± 567	1612 ± 237
1	156.0 ± 13.5	80.3 ± 4.2	235.05 ± 18.89	9.31 ± 0.99	5842 ± 252	1626 ± 104
2	153.7 ± 7.9	74.8 ± 3.9	237.60 ± 14.01	9.09 ± 1.09	5644 ± 276	1473 ± 91
3	153.7 ± 10.1	77.7 ± 3.0	246.12 ± 11.85	9.06 ± 1.16	5624 ± 79	1453 ± 89
6	152.3 ± 11.5	75.8 ± 6.6	247.31 ± 6.95	8.81 ± 0.61	5626 ± 995	1581 ± 334
9	147.7 ± 9.0	81.6 ± 2.9	248.85 ± 7.30	8.52 ± 0.68	5775 ± 893	1721 ± 203
12	150.8 ± 9.9	74.1 ± 4.5	241.47 ± 10.82	9.09 ± 1.30	5618 ± 583	1793 ± 226
18	145.9 ± 8.4	75.4 ± 3.9	233.18 ± 14.19	9.19 ± 0.75	5687 ± 443	1826 ± 133
24	145.9 ± 9.3	73.7 ± 4.2	243.63 ± 13.85	9.06 ± 0.94	6053 ± 670	1801 ± 204
36	143.8 ± 10.1	76.2 ± 5.6	247.13 ± 8.84	9.00 ± 1.08	5801 ± 571	1837 ± 301

TPM, puff counts and CO (n=10); nicotine NNN, and NNK (n=5)

Table 5: Unburned tobacco data of the reference cigar (1RLC) stored at 4°C (n=5)

Months	weight	Pre-conditioning	Nicotine	NNN	NNK
	g	Moisture (%)	mg/g	ug/g	ug/g
0	7.782 ± 0.458	13.39 ± 0.48	14.47 ± 0.98	12.84 ± 2.15	1.73 ± 0.33
1	7.562 ± 0.337	13.78 ± 0.64	15.08 ± 0.39	10.91 ± 1.21	1.58 ± 0.24
2	7.876 ± 0.222	13.99 ± 0.28	14.99 ± 0.49	12.95 ± 0.35	1.83 ± 0.63
3	7.744 ± 0.358	13.74 ± 0.50	15.09 ± 0.34	12.05 ± 1.13	1.71 ± 0.31
6	7.626 ± 0.222	13.52 ± 0.25	14.42 ± 0.38	12.73 ± 1.05	1.73 ± 0.31
9	7.754 ± 0.399	14.51 ± 0.83	14.38 ± 0.29	12.48 ± 1.47	2.16 ± 0.25
12	7.615 ± 0.342	13.87 ± 1.16	14.56 ± 0.69	10.59 ± 1.12	2.05 ± 0.72
18	7.916 ± 0.37	13.42 ± 1.46	14.88 ± 0.44	10.94 ± 0.30	1.72 ± 0.11
24	7.771 ± 0.265	13.51 ± 1.01	14.40 ± 0.88	12.61 ± 0.56	1.82 ± 0.19
36	7.822 ± 0.297	11.91 ± 1.77	14.33 ± 0.76	12.24 ± 2.08	1.93 ± 0.44

Table 6: Smoke data of the reference cigar (1RLC) stored at 4°C

Months	TPM	puffs	CO	Nicotine	NNN	NNK
	mg/cigar	n/cigar	mg/cigar	mg/cigar	ng/cigar	ng/cigar
0	150.1 ± 14.1	79.7 ± 5.7	236.98 ± 16.31	8.77 ± 1.15	5881 ± 567	1612 ± 237
1	158.0 ± 8.5	75.6 ± 3.9	235.90 ± 12.38	9.19 ± 0.72	5687 ± 443	1762 ± 118
2	154.1 ± 7.3	78.5 ± 4.0	231.57 ± 13.53	9.18 ± 0.33	5833 ± 474	1558 ± 42
3	151.9 ± 12.3	77.3 ± 4.2	239.88 ± 16.22	9.15 ± 0.90	5706 ± 636	1626 ± 232
6	143.4 ± 9.4	76.2 ± 3.7	243.91 ± 7.20	8.36 ± 1.01	5558 ± 837	1622 ± 225
9	146.4 ± 7.0	76.9 ± 4.1	245.11 ± 7.53	8.38 ± 0.56	5644 ± 513	1780 ± 178
12	160.5 ± 12.3	75.3 ± 2.8	242.59 ± 8.40	9.08 ± 1.28	5944 ± 341	1849 ± 138
18	146.5 ± 11.8	75.3 ± 6.0	234.82 ± 16.09	9.22 ± 0.88	5971 ± 677	1901 ± 218
24	144.6 ± 9.6	78.2 ± 3.5	241.89 ± 14.58	8.92 ± 1.14	5962 ± 425	1809 ± 278
36	143.9 ± 7.7	77.4 ± 4.9	246.72 ± 11.88	9.18 ± 0.55	5790 ± 592	1792 ± 189

TPM, puff counts and CO (n=10); nicotine NNN, and NNK (n=5)

Table 7: Unburned tobacco data of the reference cigar (1RLC) stored at room temperature (n=5)

Months	weight	Pre-conditioning	Nicotine	NNN	NNK
	g	Moisture (%)	mg/g	ug/g	ug/g
0	7.782 ± 0.458	13.39 ± 0.48	14.47 ± 0.98	12.84 ± 2.15	1.73 ± 0.33
1	7.626 ± 0.257	13.67 ± 0.27	16.24 ± 1.93	13.01 ± 2.93	1.48 ± 0.12
2	7.579 ± 0.34	13.87 ± 0.23	14.95 ± 0.74	11.45 ± 1.05	1.55 ± 0.41
3	7.508 ± 0.424	13.05 ± 0.17	15.30 ± 0.44	12.18 ± 0.85	1.89 ± 0.13
6	7.857 ± 0.296	13.20 ± 0.22	14.59 ± 1.16	13.17 ± 0.49	1.99 ± 0.44
9	7.645 ± 0.309	12.45 ± 0.74	14.06 ± 1.17	11.83 ± 0.67	1.98 ± 0.46
12	7.576 ± 0.231	12.37 ± 0.67	14.99 ± 0.87	10.60 ± 2.54	1.93 ± 0.82
18	7.789 ± 0.298	10.18 ± 1.08	14.83 ± 0.70	10.46 ± 0.72	1.68 ± 0.12
24	7.499 ± 0.241	9.97 ± 0.31	14.35 ± 0.81	13.19 ± 0.93	1.91 ± 0.24
36	7.623 ± 0.365	9.05 ± 0.56	14.66 ± 0.8	11.71 ± 1.87	1.88 ± 0.34

Table 8: Smoke data of the reference cigar (1RLC) stored at room temperature

Months	TPM	puffs	CO	Nicotine	NNN	NNK
	mg/cigar	n/cigar	mg/cigar	mg/cigar	ng/cigar	ng/cigar
0	150.1 ± 14.1	79.7 ± 5.7	236.98 ± 16.31	8.77 ± 1.15	5881 ± 567	1612 ± 237
1	157.5 ± 10.9	76.6 ± 4.3	238.23 ± 15.69	9.14 ± 0.69	5875 ± 669	1674 ± 258
2	156.6 ± 7.0	74.6 ± 4.6	236.51 ± 11.19	9.15 ± 1.10	5578 ± 701	1465 ± 13
3	157.4 ± 10.6	76.9 ± 4.1	242.08 ± 13.69	9.03 ± 1.38	5979 ± 211	1672 ± 180
6	145.5 ± 11.5	79.3 ± 3.4	241.13 ± 8.06	8.01 ± 0.88	5770 ± 1027	1718 ± 346
9	148.0 ± 7.8	77.5 ± 4.1	239.22 ± 8.23	8.52 ± 0.95	5338 ± 287	1674 ± 127
12	148.4 ± 9.5	73.7 ± 1.7	235.02 ± 13.62	8.70 ± 0.76	5281 ± 420	1733 ± 150
18	142.7 ± 14.4	75.8 ± 5.1	231.61 ± 15.99	8.55 ± 0.99	5094 ± 745	1770 ± 138
24	146.2 ± 9.0	73.6 ± 3.9	238.02 ± 13.84	8.93 ± 1.15	5709 ± 283	1872 ± 164
36	146.3 ± 8.9	74.1 ± 3.3	245.29 ± 12.98	8.97 ± 0.86	6155 ± 553	2012 ± 303

TPM, puff counts and CO (n=10); nicotine NNN, and NNK (n=5)

Table 9: Unburned tobacco data of the reference cigarillo (1RSC) stored at -20°C (n=5)

Months	weight	Pre-conditioning	Nicotine	NNN	NNK
	g	Moisture (%)	mg/g	ug/g	ug/g
0	2.721 ± 0.164	14.81 ± 0.26	10.72 ± 0.97	17.24 ± 1.58	3.07 ± 0.63
1	2.714 ± 0.164	14.75 ± 0.19	10.73 ± 0.63	16.07 ± 0.79	3.08 ± 0.72
2	2.656 ± 0.056	14.52 ± 0.15	10.87 ± 0.52	17.76 ± 2.10	3.21 ± 0.73
3	2.689 ± 0.100	14.66 ± 0.28	10.61 ± 0.66	16.64 ± 1.75	3.40 ± 0.78
6	2.684 ± 0.143	14.38 ± 0.52	11.08 ± 0.74	17.08 ± 0.70	2.64 ± 0.38
9	2.670 ± 0.225	14.05 ± 0.38	10.75 ± 0.41	15.84 ± 0.35	2.91 ± 0.22
12	2.647 ± 0.13	14.00 ± 0.44	11.16 ± 0.36	16.68 ± 0.61	3.46 ± 0.11
18	2.665 ± 0.167	14.14 ± 0.31	11.11 ± 0.81	15.57 ± 1.31	2.84 ± 0.36
24	2.695 ± 0.086	14.94 ± 0.19	11.13 ± 0.39	18.77 ± 2.10	2.89 ± 0.52

Table 10: Smoke data of the reference cigarillo (1RSC) stored at -20°C

Months	TPM	puffs	CO	Nicotine	NNN	NNK
	mg/cigar	n/cigar	mg/cigar	mg/cigar	ng/cigar	ng/cigar
0	79.5 ± 1.0	43.0 ± 2.7	103.15 ± 2.76	3.08 ± 0.21	3212 ± 308	1258 ± 190
1	80.0 ± 2.2	42.5 ± 2.4	100.83 ± 4.33	3.06 ± 0.10	3539 ± 157	1302 ± 99
2	78.2 ± 3.6	42.3 ± 2.1	98.44 ± 3.54	3.10 ± 0.20	3411 ± 293	1377 ± 170
3	79.7 ± 3.1	40.4 ± 1.2	99.64 ± 3.63	3.42 ± 0.20	3481 ± 331	1354 ± 116
6	78.0 ± 3.9	39.9 ± 2.2	100.57 ± 2.87	3.35 ± 0.37	3504 ± 284	1202 ± 79
9	73.8 ± 3.6	40.2 ± 2.2	101.14 ± 3.59	3.18 ± 0.25	3300 ± 435	1395 ± 168
12	78.8 ± 3.5	40.5 ± 2.4	102.33 ± 4.81	3.20 ± 0.22	3573 ± 300	1368 ± 107
18	78.2 ± 2.6	40.7 ± 2.5	101.86 ± 3.75	3.51 ± 0.36	3407 ± 287	1328 ± 166
24	79.6 ± 3.4	40.9 ± 2.0	101.27 ± 3.09	3.65 ± 0.30	3376 ± 400	1301 ± 168

TPM, puff counts and CO (n=10); nicotine NNN, and NNK (n=5)

Table 11: Unburned tobacco data of the reference cigarillo (1RSC) stored at 4°C (n=5)

Months	weight	Pre-conditioning	Nicotine	NNN	NNK
	g	Moisture (%)	mg/g	ug/g	ug/g
0	2.721 ± 0.164	14.81 ± 0.26	10.72 ± 0.97	17.24 ± 1.58	3.07 ± 0.63
1	2.715 ± 0.090	14.61 ± 0.31	11.29 ± 1.29	17.27 ± 1.18	3.12 ± 0.22
2	2.684 ± 0.116	14.06 ± 0.63	11.64 ± 1.12	16.07 ± 1.47	2.67 ± 0.36
3	2.728 ± 0.131	14.36 ± 0.56	11.25 ± 0.90	14.89 ± 1.39	2.50 ± 0.40
6	2.637 ± 0.140	13.64 ± 0.44	11.25 ± 0.54	16.21 ± 1.12	2.51 ± 0.2
9	2.594 ± 0.133	12.89 ± 1.06	11.29 ± 1.40	16.59 ± 1.03	3.36 ± 0.51
12	2.747 ± 0.175	13.17 ± 0.57	11.46 ± 0.98	17.25 ± 0.61	3.44 ± 0.31
18	2.724 ± 0.110	13.41 ± 0.23	10.83 ± 0.71	15.89 ± 1.75	2.98 ± 0.29
24	2.708 ± 0.167	13.94 ± 0.10	11.30 ± 0.70	19.16 ± 1.63	2.92 ± 0.35

Table 12: Smoke data of the reference cigarillo (1RSC) stored at 4°C

Months	TPM	puffs	CO	Nicotine	NNN	NNK
	mg/cigar	n/cigar	mg/cigar	mg/cigar	ng/cigar	ng/cigar
0	79.5 ± 1.0	43.0 ± 2.7	103.15 ± 2.76	3.08 ± 0.21	3212 ± 308	1258 ± 190
1	80.1 ± 2.0	42.3 ± 2.2	101.36 ± 4.54	3.01 ± 0.10	3233 ± 186	1230 ± 72
2	78.2 ± 2.5	42.0 ± 2.5	100.25 ± 3.09	3.18 ± 0.12	3344 ± 340	1277 ± 142
3	77.0 ± 2.8	42.2 ± 1.8	98.14 ± 4.45	3.28 ± 0.25	3303 ± 290	1226 ± 91
6	77.4 ± 3.3	39.8 ± 2.0	100.32 ± 2.44	3.45 ± 0.18	3378 ± 282	1213 ± 112
9	75.4 ± 3.7	39.2 ± 2.4	101.80 ± 4.03	3.23 ± 0.16	3313 ± 508	1388 ± 205
12	77.3 ± 4.1	41.8 ± 2.5	101.40 ± 4.86	3.23 ± 0.25	3450 ± 452	1352 ± 131
18	77.5 ± 3.2	41.1 ± 2.4	101.78 ± 3.17	3.36 ± 0.42	3446 ± 408	1305 ± 119
24	79.4 ± 3.3	41.6 ± 2.2	101.09 ± 3.51	3.63 ± 0.29	3278 ± 179	1330 ± 52

TPM, puff counts and CO (n=10); nicotine NNN, and NNK (n=5)

Table 13: Unburned tobacco data of the reference cigarillo (1RSC) stored at room temperature (n=5)

Months	weight	Pre-conditioning	Nicotine	NNN	NNK
	g	Moisture (%)	mg/g	ug/g	ug/g
0	2.721 ± 0.164	14.81 ± 0.26	10.72 ± 0.97	17.24 ± 1.58	3.07 ± 0.63
1	2.596 ± 0.182	14.28 ± 0.16	11.54 ± 0.65	18.34 ± 0.58	3.02 ± 0.54
2	2.646 ± 0.120	13.86 ± 0.54	11.04 ± 0.65	16.12 ± 2.07	2.82 ± 0.57
3	2.670 ± 0.144	14.02 ± 0.46	10.94 ± 0.53	16.39 ± 1.81	2.80 ± 0.23
6	2.705 ± 0.131	13.30 ± 0.39	10.74 ± 0.26	16.65 ± 1.23	2.53 ± 0.28
9	2.626 ± 0.096	12.79 ± 0.27	11.05 ± 0.80	15.95 ± 1.63	3.20 ± 0.27
12	2.618 ± 0.143	12.94 ± 0.45	10.46 ± 0.15	16.87 ± 0.78	3.47 ± 0.24
18	2.642 ± 0.166	12.94 ± 0.50	10.39 ± 0.45	16.30 ± 1.39	2.46 ± 0.09
24	2.719 ± 0.087	11.83 ± 1.22	10.74 ± 0.49	19.29 ± 1.35	3.18 ± 0.51

Table 14: Smoke data of the reference cigarillo (1RSC) stored at room temperature

Months	TPM	puffs	CO	Nicotine	NNN	NNK
	mg/cigar	n/cigar	mg/cigar	mg/cigar	ng/cigar	ng/cigar
0	79.5 ± 1.0	43.0 ± 2.7	103.15 ± 2.76	3.08 ± 0.21	3212 ± 308	1258 ± 190
1	81.2 ± 4.3	41.9 ± 3.0	100.91 ± 4.08	3.05 ± 0.13	3253 ± 110	1251 ± 23
2	78.8 ± 2.3	41.3 ± 2.5	100.32 ± 3.5	3.20 ± 0.22	3366 ± 295	1285 ± 63
3	75.3 ± 3.0	41.4 ± 2.2	96.72 ± 4.13	3.29 ± 0.20	3208 ± 226	1204 ± 44
6	77.3 ± 3.7	40.1 ± 1.8	100.01 ± 3.07	3.22 ± 0.48	3380 ± 303	1198 ± 97
9	76.5 ± 5.1	40.1 ± 1.7	101.34 ± 4.39	3.22 ± 0.27	3310 ± 518	1352 ± 184
12	77.3 ± 3.9	40.2 ± 2.2	102.00 ± 5.27	3.59 ± 0.38	3463 ± 109	1366 ± 76
18	77.8 ± 2.4	40.1 ± 3.2	100.89 ± 3.69	3.33 ± 0.12	3367 ± 211	1281 ± 125
24	79.1 ± 3.1	42.3 ± 1.3	99.87 ± 3.08	3.23 ± 0.34	3026 ± 148	1259 ± 81

TPM, puff counts and CO (n=10); nicotine NNN, and NNK (n=5)

Table 15: Unburned tobacco data of the reference filtered cigar (1RFC) stored at -20°C (n=5)

Months	weight	Pre-conditioning	Nicotine	NNN	NNK
	g	Moisture (%)	mg/g	ug/g	ug/g
0	1.127 ± 0.029	12.51 ± 0.60	14.52 ± 0.80	1.49 ± 0.09	0.38 ± 0.02
1	1.121 ± 0.025	12.56 ± 0.36	14.65 ± 0.46	1.72 ± 0.06	0.41 ± 0.04
2	1.129 ± 0.030	13.15 ± 0.49	14.30 ± 0.76	1.61 ± 0.19	0.41 ± 0.06
3	1.133 ± 0.028	12.33 ± 0.17	15.85 ± 0.85	1.74 ± 0.27	0.49 ± 0.13
6	1.129 ± 0.039	12.62 ± 0.23	15.25 ± 0.48	1.61 ± 0.10	0.42 ± 0.01
9	1.124 ± 0.013	12.07 ± 0.64	14.56 ± 0.76	1.50 ± 0.11	0.36 ± 0.03
12	1.126 ± 0.029	11.76 ± 0.44	13.97 ± 1.23	1.49 ± 0.08	0.41 ± 0.01
18	1.125 ± 0.024	11.85 ± 0.41	14.83 ± 1.50	1.43 ± 0.10	0.36 ± 0.03
24	1.141 ± 0.018	12.89 ± 0.70	14.52 ± 0.58	1.86 ± 0.06	0.37 ± 0.08

Table 16: Smoke data of the reference filtered cigar (1RFC) stored at -20°C

Months	TPM	puffs	CO	Nicotine	NNN	NNK
	mg/cigar	n/cigar	mg/cigar	mg/cigar	ng/cigar	ng/cigar
0	24.2 ± 1.0	18.5 ± 0.6	35.01 ± 4.42	1.30 ± 0.10	204 ± 10	108 ± 8
1	24.1 ± 0.5	18.2 ± 0.8	35.98 ± 1.17	1.35 ± 0.07	201 ± 11	112 ± 7
2	24.2 ± 0.9	18.6 ± 0.9	35.36 ± 1.13	1.41 ± 0.04	198 ± 8	111 ± 8
3	24.1 ± 0.9	18.6 ± 0.5	35.38 ± 1.23	1.39 ± 0.05	193 ± 10	102 ± 5
6	23.2 ± 0.5	18.0 ± 0.7	35.25 ± 1.00	1.43 ± 0.05	202 ± 12	105 ± 6
9	23.5 ± 0.6	17.9 ± 0.7	35.31 ± 1.29	1.42 ± 0.09	200 ± 4	102 ± 7
12	23.7 ± 1.0	18.4 ± 0.9	35.76 ± 0.99	1.32 ± 0.09	194 ± 14	103 ± 6
18	24.0 ± 0.9	18.8 ± 1.0	35.38 ± 0.83	1.31 ± 0.08	197 ± 14	107 ± 9
24	23.89 ± 0.68	19.0 ± 1.0	35.05 ± 1.76	1.28 ± 0.09	203 ± 14	107 ± 5

TPM, puff counts and CO (n=10); nicotine NNN, and NNK (n=5)

Table 17: Unburned tobacco data of the reference filtered cigar (1RFC) stored at 4°C (n=5)

Months	weight	Pre-conditioning	Nicotine	NNN	NNK
	g	Moisture (%)	mg/g	ug/g	ug/g
0	1.127 ± 0.029	12.51 ± 0.60	14.52 ± 0.80	1.49 ± 0.09	0.38 ± 0.02
1	1.122 ± 0.025	11.71 ± 0.32	15.00 ± 0.51	1.71 ± 0.18	0.46 ± 0.10
2	1.119 ± 0.020	11.90 ± 0.41	14.49 ± 0.49	1.58 ± 0.15	0.44 ± 0.04
3	1.122 ± 0.014	11.51 ± 0.44	15.38 ± 1.74	1.67 ± 0.12	0.46 ± 0.03
6	1.123 ± 0.028	10.35 ± 1.26	15.62 ± 0.75	1.53 ± 0.07	0.42 ± 0.01
9	1.117 ± 0.025	10.02 ± 0.85	14.62 ± 0.18	1.52 ± 0.04	0.34 ± 0.01
12	1.135 ± 0.022	10.23 ± 0.50	14.62 ± 0.71	1.49 ± 0.04	0.39 ± 0.03
18	1.120 ± 0.033	10.05 ± 0.35	13.66 ± 1.50	1.41 ± 0.08	0.36 ± 0.04
24	1.135 ± 0.055	10.78 ± 0.97	14.96 ± 0.45	1.82 ± 0.11	0.36 ± 0.04

Table 18: Smoke data of the reference filtered cigar (1RFC) stored at 4°C

Months	TPM	puffs	CO	Nicotine	NNN	NNK
	mg/cigar	n/cigar	mg/cigar	mg/cigar	ng/cigar	ng/cigar
0	24.2 ± 1.0	18.5 ± 0.6	35.01 ± 4.42	1.30 ± 0.10	204 ± 10	108 ± 8
1	24.5 ± 0.5	18.6 ± 0.6	35.89 ± 1.02	1.35 ± 0.06	201 ± 6	110 ± 6
2	23.9 ± 0.8	18.5 ± 0.7	35.55 ± 0.87	1.38 ± 0.04	194 ± 4	107 ± 5
3	23.9 ± 0.8	18.4 ± 0.5	35.48 ± 1.00	1.38 ± 0.02	190 ± 5	102 ± 3
6	23.2 ± 1.1	18.1 ± 0.6	35.36 ± 0.81	1.42 ± 0.08	198 ± 14	103 ± 5
9	23.2 ± 0.6	18.1 ± 0.7	35.27 ± 1.32	1.37 ± 0.11	200 ± 26	102 ± 6
12	23.6 ± 0.7	18.2 ± 0.8	35.90 ± 1.17	1.32 ± 0.06	189 ± 10	103 ± 6
18	23.5 ± 0.9	18.8 ± 0.9	35.17 ± 0.93	1.32 ± 0.07	193 ± 7	108 ± 7
24	23.7 ± 0.6	18.6 ± 0.8	35.23 ± 1.50	1.25 ± 0.11	202 ± 22	109 ± 6

TPM, puff counts and CO (n=10); nicotine NNN, and NNK (n=5)

Table 19: Unburned tobacco data of the reference filtered cigar (1RFC) stored at room temperature (n=5)

Months	weight	Pre-conditioning	Nicotine	NNN	NNK
	g	Moisture (%)	mg/g	ug/g	ug/g
0	1.127 ± 0.029	12.51 ± 0.60	14.52 ± 0.80	1.49 ± 0.09	0.38 ± 0.02
1	1.120 ± 0.025	11.70 ± 0.51	14.96 ± 0.64	1.40 ± 0.22	0.37 ± 0.06
2	1.122 ± 0.033	12.15 ± 0.42	14.61 ± 1.02	1.59 ± 0.14	0.42 ± 0.02
3	1.123 ± 0.015	10.93 ± 0.49	15.77 ± 0.52	1.80 ± 0.04	0.49 ± 0.04
6	1.118 ± 0.022	8.83 ± 0.30	14.40 ± 0.93	1.58 ± 0.05	0.43 ± 0.02
9	1.112 ± 0.029	8.23 ± 0.21	14.03 ± 0.48	1.55 ± 0.02	0.37 ± 0.01
12	1.131 ± 0.018	7.95 ± 0.17	13.97 ± 1.23	1.58 ± 0.06	0.39 ± 0.03
18	1.097 ± 0.035	7.81 ± 0.28	13.66 ± 1.54	1.46 ± 0.05	0.36 ± 0.03
24	1.123 ± 0.026	8.42 ± 0.17	14.16 ± 0.55	2.26 ± 0.16	0.38 ± 0.08

Table 20: Smoke data of the reference filtered cigar (1RFC) stored at room temperature

Months	TPM	puffs	CO	Nicotine	NNN	NNK
	mg/cigar	n/cigar	mg/cigar	mg/cigar	ng/cigar	ng/cigar
0	24.2 ± 1.0	18.5 ± 0.6	35.01 ± 4.42	1.30 ± 0.10	204 ± 10	108 ± 8
1	24.3 ± 0.5	18.7 ± 0.5	35.36 ± 0.66	1.39 ± 0.07	203 ± 2	111 ± 6
2	23.9 ± 0.9	18.1 ± 0.6	35.33 ± 0.90	1.41 ± 0.02	194 ± 15	111 ± 11
3	23.3 ± 0.7	18.6 ± 0.6	35.04 ± 0.99	1.40 ± 0.06	188 ± 6	102 ± 4
6	23.0 ± 0.7	17.8 ± 0.4	35.42 ± 0.74	1.43 ± 0.10	191 ± 11	103 ± 6
9	23.3 ± 0.6	17.9 ± 0.6	34.91 ± 0.80	1.36 ± 0.09	190 ± 18	100 ± 7
12	23.7 ± 0.8	18.5 ± 0.9	35.33 ± 1.20	1.35 ± 0.08	207 ± 11	111 ± 8
18	22.8 ± 1.0	18.8 ± 0.9	34.96 ± 0.95	1.27 ± 0.06	191 ± 9	108 ± 9
24	23.5 ± 0.9	18.7 ± 1.0	35.07 ± 1.42	1.20 ± 0.17	208 ± 27	113 ± 5

TPM, puff counts and CO (n=10); nicotine NNN, and NNK (n=5)

Table 21: THC contents in the cannabinoids -infused beverages on the market

	THC labeled on can	Volume labeled on the can	Actual volume	THC Per CAN
	mg/can	mL/can	mL/can	mg/can
NORTHHIGHER VIBES	2	355	370	4.36
CROOKED LANE	5	355	353	5.79
WYNK	5	355	355	5.28
LARK	5	355	354	0.96
BETTER THAN BOOZE	5	360	354	1.97
CRESCENT	5	355	355	1.79
Coastalo	10	355		8.14
Green Goddess	20	355	363	12.63
Torch	60	355	365	53.55
Habit	100	354	333	68.33

Figure 1: The reference large cigar (1RLC), cigarillo (1RSC), and the filtered cigar (1RFC)

Figure 2: Waters Xevo TQD Triple Quadrupole Mass Spectrometry

Figure 3: Tested Cannabinoids -infused beverages on the market

CORESTA COLLABORATIVE STUDIES (2025)

Investigator(s): Huihua Ji (KTRDC)

Report type: KTRDC in-house research project – final

Lay Summary: KTRDC analytical laboratory is a member of several CORESTA analytical working groups (Tobacco and Tobacco Products Analytes, Smoke Analytes, Cigar Smoking Methods, Heated Tobacco Products, and E-Vapor). We have been involved in CORESTA subgroups' collaborative studies on tobacco and tobacco product analyses, cigarette, cigar, heated tobacco products, and e-cigarette mainstream smoke analyses, and the development of analytical methods for many years. In 2025, we participated in two CORESTA collaborative studies: the Stability study of 2016 CORESTA Reference Products - 2025 Analysis and the 18th Collaborative Study (2025) on cigars' repeatability and reproducibility.

Introduction

Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) is an international organization of tobacco scientists. CORESTA supports international cooperation in scientific research related to tobacco and tobacco products. KTRDC analytical laboratory is a member of several CORESTA analytical working groups (Tobacco and Tobacco Products Analytes, Smoke Analytes, Cigar Smoking Methods, Heated Tobacco Products, and E-Vapor). We have been involved in CORESTA subgroups' collaborative studies on tobacco and tobacco product analyses; cigarettes, cigars, heated tobacco products, and e-cigarette mainstream smoke analyses; and the development of analytical methods for many years.

Summary of Progress

<u>CORESTA Collaborative Study --- Stability study of 2016 CORESTA Reference Products - 2025 Analysis</u>

In 2016, CORESTA Tobacco and Tobacco Products Analytes subgroup (TTPA) produced four smokeless reference products - CORESTA Reference Products (CRPs), including CRP1.1 (Swedish snus pouch), CRP2.1 (American-style loose moist snuff), CRP3.1 (American-style loose dry snuff powder), and CRP4.1 (American-style chopped loose-leaf chewing tobacco). TTPA conducted a preliminary collaborative study to characterize the CRPs in 2016. Since then, TTPA has had a collaborative study to characterize the CRPs to evaluate the stability of CRPs every two years. In 2025, TTPA initiated the fifth stability study for the 2016 CRPs by determining nicotine, pH, moisture (oven volatiles), and tobacco-specific nitrosamines (TSNAs) using the CORESTA Recommended Methods (CRMs) 62, 69, 76, and 75, respectively. The KTRDC laboratory participated in this study. Our data is shown in Table 1. The results of this collaborative study indicated that most analytes and measures for CRPS did not show statistically significant differences, except moisture for CRP3.1 and pH for CRP1.1. A small increase in the moisture of CRP3.1 and a slight drop in pH in CRP1.1 are consistent with the previous results.

CORESTA Collaborative Study --- 18th Collaborative Study (2025) on cigars' repeatability and reproducibility

KTRDC analytical laboratory personnel collaborated with the CORESTA Cigar Smoking Methods Subgroup (CSM) to develop methods for the analysis of HPHCs (harmful and potentially harmful constituents) in cigar mainstream smoke. Before 2023, CSM conducted a collaborative study on cigars' repeatability and reproducibility every year. Starting in 2023, CSM began conducting this study every two years. In 2025, CSM conducted the 18th collaborative study on the repeatability and reproducibility of cigar TNCO analysis under the cigar smoke methods of CORESTA Recommended Method (CRMs) 46, 64, 65, 66, 67, and 68. This collaborative study included the certified reference large cigar (1RLC) from the UKY, CORESTA Monitor 9 (CM9) and CM10, and three UKY cigar reference products, including the filtered cigar (1C2), the cigarillo (1C3), and the large cigar with natural wrapper (1C4). We participated in this study. We used the Borgwaldt linear cigar smoke machine LM4E to smoke the filtered cigars, cigarillos, and large cigars under the cigar smoke regime. Our results are shown in Table 2. Currently, the collaborative study is still in the data collection stage.

Plans for Future Work

KTRDC analytical laboratory will continue to participate in the CORESTA and ISO collaborative studies on tobacco product analysis and the development of analytical methods in the future.

References

CORESTA Recommended Method N° 46: Atmosphere for Conditioning and Testing Cigars of all Sizes and Shapes

CORESTA Recommended Method N° 62: Determination of Nicotine in Tobacco and Tobacco Products by Gas Chromatographic Analysis

CORESTA Recommended Method N° 64: Routine Analytical Cigar-Smoking Machine - Specifications, Definitions and Standard Conditions

CORESTA Recommended Method N° 65: Determination of Total and Nicotine-Free Dry Particulate Matter using a Routine Analytical Cigar-Smoking Machine – Determination of Total Particulate Matter and Preparation for Water and Nicotine Measurements

CORESTA Recommended Method N° 66: Determination of Nicotine in the Mainstream Smoke of Cigars by Gas Chromatographic Analysis

CORESTA Recommended Method N° 67: Determination of Water in the Mainstream Smoke of Cigars by Gas Chromatographic Analysis

CORESTA Recommended Method N° 68: Determination of Carbon Monoxide in the Mainstream Smoke of Cigars by Non-Dispersive Infrared Analysis

CORESTA Recommended Method N° 69: Determination of pH in Tobacco and Tobacco Products

CORESTA Recommended Method N° 75: Determination of Tobacco-Specific Nitrosamines in Mainstream Smoke by LC-MS/MS

CORESTA Recommended Method N° 76: Determination of Moisture Content (Oven Volatiles) of Tobacco and Tobacco Products

Figures and Tables

Table 1: Characterization of CRPs

	Replicate	Nicotine	NNK	NNN	NAT	NAB	Oven volatiles	pH
Product		**mg/g**	**µg/g**	**µg/g**	**µg/g**	**µg/g**	**%**	**-**
CRP1.1	1	7.117	0.04217	0.1899	0.1328	0.005559	52.36	8.29
CRP1.1	2	6.923	0.04523	0.1749	0.1297	0.006447	52.32	8.24
CRP1.1	3	6.925	0.03880	0.1762	0.1117	0.005886	50.71	8.34
CRP2.1	1	10.25	1.852	3.410	3.628	0.2515	52.36	7.72
CRP2.1	2	10.70	1.924	3.408	4.046	0.2624	51.31	7.70
CRP2.1	3	10.42	1.994	3.148	4.244	0.2524	51.34	7.72
CRP3.1	1	16.33	2.780	6.456	3.982	0.2912	8.978	6.88
CRP3.1	2	16.40	2.622	5.840	4.144	0.2904	9.372	6.88
CRP3.1	3	16.41	2.438	6.022	3.830	0.3107	9.016	6.90
CRP4.1	1	9.363	0.8479	3.586	1.642	0.1102	20.96	6.12
CRP4.1	2	8.920	0.7769	3.272	1.746	0.1052	22.60	6.12
CRP4.1	3	9.084	0.7837	3.754	1.548	0.1082	23.06	6.13

Table 2: TNCO in cigar mainstream smoke

Sample ID	TPM	Nicotine	Water	NFDPM	CO
	mg/cigar	mg/cigar	mg/cigar	mg/cigar	mg/cigar
CM9	17.30	1.79	1.38	14.13	12.24
	16.60	1.61	1.15	13.85	12.82
	16.90	1.67	1.30	13.94	12.58
	17.30	1.79	1.50	14.01	12.36
	17.00	1.69	1.43	13.88	13.12
1C2	26.45	1.16	1.70	23.59	38.62
	27.05	1.32	2.03	23.70	39.14
	26.15	1.20	1.76	23.19	37.05
	26.60	1.26	1.94	23.39	36.97
	25.70	1.17	1.60	22.93	38.66
1C3	74.45	2.28	6.71	65.46	107.36
	71.25	2.10	6.48	62.67	111.52
	69.50	1.97	6.06	61.47	106.31
	72.80	2.15	6.66	63.99	105.72
	70.65	2.04	6.60	62.01	102.88
1C4	67.80	3.35	6.63	57.83	114.35
	72.70	3.96	7.16	61.58	122.72
	65.20	3.17	5.91	56.12	116.34
	69.80	3.69	7.03	59.07	108.24
	71.90	3.86	6.61	61.43	120.08
1RLC	153.50	9.57	30.59	113.34	242.38
	147.20	8.35	27.95	110.90	230.52
	145.30	8.18	25.19	111.92	219.94
	160.70	10.30	33.20	117.20	237.67
	141.30	7.84	21.09	112.36	220.16

TOBACCO PROFICIENCY TESTING AT CTRP (2025)

I Investigator(s): Ruth McNees (KTRDC), Matthew Craft (KTRDC), Stacey Slone (KTRDC), Ling Yuan (KTRDC), Orlando Chambers (CAFE)

Report type: KTRDC in-house research project – final report
Externally funded research project

Lay Summary: In 2016, using funds from the Food and Drug Administration (FDA) grant UC2FD005049, the Center for Tobacco Research Products (CTRP) was established to improve the quality and traceability of measurements in analytical labs specializing in tobacco smoke chemistry. In 2017, the CTRP proficiency testing (PT) program was accredited by American Association of Laboratory Accreditation (A2LA) in accordance with the recognized ISO 17043:2010, and recertified in September 2025 to the revised ISO 17043:2023, to be an Accredited Proficiency Test Provider. Based on the success of the cigarette PT program, CTRP has expanded the tobacco reference products that can be offered for PT schemes to include four smokeless tobacco products which were produced using funds from FDA grant UC2FD005671. CTRP is currently looking to expand the tobacco reference products that can be offered for PT schemes to include the newly produced certified reference cigars which have been produced using funds from FDA grant UC2FD006890. CTRP has provided and received data for 32 rounds of PT which covered a broad range of Harmful of Potentially Harmful Compounds (HPHCs) in cigarettes and smokeless tobacco products.

Introduction

Since 1968, the University of Kentucky has provided reference tobacco products as standards for non-clinical investigational purposes by tobacco manufacturers, contract and government laboratories, and academic institutions. In 2014, the University of Kentucky Center for Tobacco Reference Products (CTRP) was awarded a Cooperative Agreement with the US Food and Drug Administration's Center for Tobacco Products (CTP), within the FDA, to develop a cigarette tobacco reference products program (CTRP, 2014). The 1R6F certified reference cigarette was produced in March of 2015 as a blended reference cigarette typical of an American cigarette. Approximately 50 million test pieces were produced to meet the need for quality control testing in the tobacco industry and to support research in both academic and industrial settings. The 1R6F certified reference cigarette represents a suitable matrix for the quantitative analysis of salient parameters in blended cigarette products and is the first certified reference tobacco products (Certificate of Analysis available at https://ctrp.uky.edu/home) (CTRP, 2019). It should be noted that the 1R6F was the first certified tobacco reference available to the tobacco research community with proven homogeneity and stability for use as PT material.

The four certified reference smokeless tobacco products were produced in 2017. The compositions of the reference products resemble smokeless tobacco products commonly sold in the United States. The four reference products produced include 20,000 cans of Swedish snus (1S4), 23,000 cans of snus (1S5), 16,500 pouches of loose leaf chewing tobacco (3S1), and 23,000 cans of moist snuff (3S3). The various quantities

were produced to meet the need for quality control testing in the tobacco industry and to support research in both academic and industrial settings. It is known that the levels of salient monitoring parameters in smokeless tobacco products vary due to inherent variability in tobacco as an agricultural crop. The four certified reference smokeless tobacco products represent a suitable matrix for the quantitative analysis of salient parameters in products commonly sold in the United States and are the first certified reference smokeless tobacco products (certificate of analysis for each product available at ctrp.uky.edu).

In 2019, the University of Kentucky was awarded a third FDA grant for the production of certified reference cigars which will be included in the proficiency testing program. To determine preliminary design parameters for the reference cigars, thorough analysis of commercially available cigar products representing the product categories large cigar, cigarillo, and filtered cigar was performed. The results were discussed with FDA and various stakeholders including cigar manufacturers, commercial analytical laboratories, and tobacco researchers. Incorporating feedback from the various groups facilitated the development of final design parameters. The first certified reference cigar, the large cigar (1RLC) was produced and shipped to the long-term storage facility in Erlanger, KY. Selected cases of cigars were transported to KTRDC for labelling and shipping to the three contract laboratories that were chosen to perform the analyses that was used to generate the Certificate of Analysis (CoA). The data from the contract laboratories was analyzed through rigorous statistical methods and results were used to populate the CoA which was reviewed and approved by the FDA. The 1RLC was listed on the CTRP website with the CoA and the CTRP customers were notified via email through the Listserv. The newly produced certified reference cigarillo (1RSC) and filtered cigar (1RFC) were produced and shipped to the long-term storage facility in Erlanger, KY. Selected cases for both products were transported to KTRDC for labelling and shipping to the contract laboratories that were chosen to perform the analyses that was used to generate the Certificate of Analysis (CoA). Due to changes in contract laboratory availability, the approach to characterize the 1RSC and 1RFC was done using 4 datasets with less replicates than the original 3 dataset approach originally designed, this resulted in additional data points and captured the expected real-world variability in the analysis of cigar products. Following statistical analysis of the data provided by the contract laboratories, a CoA was generated for the 1RSC and 1RFC and the products were listed on the CTRP website and CTRP customers were notified via email from the Listserv

Summary of Progress

Development of Proficiency Testing

Proficiency testing (PT), which indicates the analytical performance of a laboratory for a particular method and analyte in comparison with other participating laboratories, has become a necessary part of third-party performance evaluation for analytical laboratories (Wojciechowski, 2016). Clinical laboratories in the United States have been required to participate in PT for laboratory evaluation since 1994. Since 2005, the standard governing the general requirements for the competence of testing and calibration laboratories (ISO 17025) has required participation in PT (Abdel Massih, 2016). The PT program coordinated by CTRP includes analysis of the 1R6F reference cigarette and the four certified reference smokeless products. The calendar for the PT program and certificates of analysis for each of the certified products can be found at the CTRP website (https://ctrp.uky.edu/home). A protocol is published online

for each PT round that identifies the particular parameters to be analyzed and references on the recommended analytical methods for the parameters. Once a PT kit is selected and purchased, the test material is sent to the participant for analysis. Participants are given access to download an Excel template for data submission and assigned a unique identifier. Once the data are submitted to CTRP, statistical analysis is performed, and an interim report is assembled and issued for participating laboratories to review and comment on the results. After a period of time, comments and revised data are compiled and analyzed for inclusion in the final report, which is used by individual laboratories to maintain accreditation if necessary.

Statistical Analysis of the Data Submitted

Once the data deadline is passed for each round, the data are partitioned into 4 subsets: Linear Non-intense, Linear Intense, Rotary Non-intense and Rotary Intense. Each subset of data is reviewed for outliers using multiple outlier detection tests including Cochran's, Grubb's Single, and Mandel's h & k. Instead of removing outlying data points, robust means and standard deviations are calculated for each analyte using Algorithm A (ISO, 1998). These parameter-specific standard deviations are then used to calculate the standard deviation for proficiency assessment

$$\sigma_{PT} = \sqrt{s_R^2 - s_r^2(1 - 1/m)},$$

where m is the number of replicates in the proficiency study, per ISO 13528. Where n is the number of participants, $\sigma_{PT}/\sqrt{n}$ estimates uncertainty of the consensus mean.

The parameter-specific robust mean and the standard deviation for proficiency assessment, which will be the uncertainty for proficiency testing, are then used to calculate a z-score,

$$z = \frac{x_i - x_{pt}}{\sigma_{pt}},$$

as per ISO 13528 for each participant and tabled values are provided of the parameter, participant, and z-score value and an indication of whether the z-score value falls in the alert (z value above 3 in absolute value) or warning (z value between 2 and 3 in absolute value) regions. The set regions will serve as performance evaluation criteria for each participant's calculated z-score.

Summary of PT program for July 1, 2024 to June 30, 2025

1. CIG-2024C Proficiency Testing Round (Element 12, 13, and 15)

This round of testing includes the chemical constituents (Formaldehyde, Acetaldehyde, Acetone, Acrolein, Propionaldehyde, Crotonaldehyde, 2-butanone, n-butyraldehyde, and Puff Count) in both the Non-intense and Intense smoking regimes and physical parameters (cigarette resistance to draw (pressure drop open), cigarette resistance to draw (pressure drop closed), filter pressure drop (fully encapsulated), total ventilation, filter ventilation, tobacco weight, cigarette weight, air permeability, firmness, circumference, cigarette length, filter plug length, and tipping paper length) using the 1R6F cigarette.

Study Timeline

- June 6, 2024 PT round Opened
- July 4, 2024 Data submission portal Opened
- August 29, 2024 Data submission portal Closed

- November 27, 2024 Issuance of Interim Report
- December 14, 2024 Issuance of Final Report

Equipment Type	Purchased	Uploaded
Linear	10	8
Rotary	10	9
Total	20	17

Preventative / Corrective Action: None

2. SMK-2024D Proficiency Testing Round (Element 18)

This round of testing will include parameters (Total Nicotine, Free Nicotine, NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, NNN (N-nitrosonornicotine), NAT (N-nitrosoanatabine), NAB (N-nitrosoanabasine), pH, Moisture, Acetaldehyde, Crotonaldehyde, Formaldehyde, Benzo[α]pyrene (BaP), Cadmium, and Arsenic) using two certified reference smokeless tobacco products: 1S4 (Swedish Style Snus) and 3S3 (Moist Snuff).

Study Timeline

- August 22, 2024 PT round Opens
- October 24, 2024 Data submission portal Opened
- January 02, 2025 Data submission portal Closed
- April 3, 2025 Issuance of Interim Report
- May 6, 2025 Issuance of Final Report

	Purchased	Uploaded
Total	16	14

Preventative / Corrective Action: None

3. CIG-2025A Proficiency Testing Round (Elements 1, 2, and 16)

This round of testing includes the chemical constituents (Chemical Nicotine-Free Dry Particulate Matter (NFDPM/Tar), Nicotine, Carbon Monoxide, Water, Hydrogen Cyanide (HCN), Oxides of Nitrogen (NOx), Total Particulate Matter (TPM), and Puff Count using both the 1R6F and 2R5F as proficiency test material smoked in both the Non-intense and Intense smoking regimes.

Study Timeline

- January 9, 2025 PT round Opened
- February 20, 2025 Data submission portal Opened
- May 16, 2025 Data submission portal Closed
- June 26, 2025 Issuance of Interim Report
- July 17, 2025 Issuance of Final Report

Equipment Type	Purchased	Uploaded
Linear	17	14
Rotary	18	17
Total	35	31

Preventative / Corrective Action: None

4. CIG-2025B Proficiency Testing Round (Elements 3, 4, and 15)

This round of testing includes the chemical constituents (NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone), NNN (N-nitrosonornicotine), NAT (N-nitrosoanatabine), NAB (N-nitrosoanabasine), Total Particulate Matter (TPM), and Puff Count and physical parameters cigarette resistance to draw (pressure drop open), cigarette resistance to draw (pressure drop closed), filter pressure drop (fully encapsulated), total ventilation, filter ventilation, tobacco weight, cigarette weight, air permeability, firmness, circumference, cigarette length, filter plug length, and tipping paper length) using the 1R6F as proficiency test material smoked in both the Non-intense and Intense smoking regimes.

Study Timeline

- March 27, 2025 PT round Opened
- May 1, 2025 Data submission portal Opened
- July 17, 2025 Data submission portal Closed
- August 15, 2025 Issuance of Interim Report
- October 13, 2025 Issuance of Final Report

Equipment Type	Purchased	Uploaded
Linear	12	9
Rotary	12	10
Total	24	19

Preventative / Corrective Action: None

4. CIG-2025C Proficiency Testing Round (Element 14)

This round of testing includes analysis of cigarette filler for chemical constituents (Formaldehyde, Acetaldehyde, Acetone, Acrolein, Propionaldehyde, Crotonaldehyde, 2-butanone, n-butyraldehyde, and Puff Count) 1R6F as proficiency test material smoked in both the Non-intense and Intense smoking regimes and physical parameters (Cigarette Resistance to Draw (pressure drop open), Cigarette Resistance to Draw (pressure drop closed), Filter Pressure Drop (fully encapsulated), Total Ventilation, Filter Ventilation, Tobacco Weight, Cigarette Weight, Air Permeability, Firmness, Circumference, Cigarette Length, Filter Plug Length, and Tipping Paper Length) using the 1R6F as proficiency test material.

Study Timeline

- May 29, 2025 PT round Opened
- July 10, 2025 Data submission portal Opened
- September 19, 2025 Data submission portal Closed
- October 13, 2025 Issuance of Interim Report

- November 10, 2025 Target Date for Issuance of Final Report

	Purchased	Uploaded
Total	26	21

Preventative / Corrective Action: None

Plans for Future Work

The calendar for the CTRP PT program through the end of 2026 is available at the website (https://ctrp.uky.edu/home) and covers parameters to be tested using the 1R6F certified reference cigarette and the four certified reference smokeless tobacco products. Based on feedback from various stakeholders, CTRP worked with the FDA to produce four certified reference smokeless tobacco products (3S1, Loose Leaf Chewing tobacco; 3S3, Moist Snuff; 1S4, Swedish Style Snus; and 1S5, Snus). These products are representative of the most commonly used smokeless tobacco products on the market currently available to consumers. The products are currently available for purchase by qualifying institutions and as part of the CTRP proficiency testing program. Additionally, these products have been characterized by CORESTA Tobacco and Tobacco Products Analytes Sub-Group (Ballentine, 2021). CTRP worked with multiple cigar manufacturers to produce four different cigars (1C1, Large Machine-Made Cigar; 1C2, Machine Made Filtered Little Cigar; 1C3, Small Machine Made Cigarillo; and 1C4, Large Machine Made Cigar with a natural wrapper) for use in collaborative studies. These products have been used to help establish a baseline of analytes of interest, including HPHCs and design parameters used for the production of certified reference cigars produced in a cooperative agreement with the FDA. A number of HPHCs, including the tobacco-specific nitrosamines (TSNAs), pH, ammonia, water activity, arsenic, and cadmium in unburned cigars were measured by CORESTA Tobacco and Tobacco Products Analytes Sub-Group (Prepelitskaya, 2021), while conditioned cigar weight and tar, nicotine, carbon monoxide (TNCO) in mainstream smoke were measured in an additional study (Morton, 2021). These studies were used to determine the minimum number of samples needed for the characterization of the newly produced certified reference cigars. Characterization is complete for the large certified reference cigar (1RLC), the certified reference filtered cigar (1RFC), and certified reference cigarillo (1RSC). These products are listed on the website and made available to the tobacco research community following final approval of the characterization data by the FDA. Additionally, all three cigar products will be added to the proficiency testing program with the first round of testing to occur in 2026.

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SECTION 1: RESEARCH
PART A: PROJECT REPORTS 2024-2025

ii. FACULTY RESEARCH SUPPORT

HORMONE AND STRESS REGULATION OF TOBACCO GROWTH (2024)

Investigator(s): Jan Smalle (P&SS)

Report type: KTRDC faculty research support – interim report

Lay Summary: The aim of this project is to identify hormonal and stress factors that influence the growth and physiology of plants. We use a range of plant species to be able to identify core plant physiological and developmental processes that are applicable to tobacco. During the past year, we have explored the role of metabolites of the phenylpropanoid biosynthesis pathway in the hormone-like promotion of leaf growth and the suppression of fungal infection. Our data have revealed that these compounds exert similar effects in a range of plant species. In parallel, we have investigated the evolutionary origin of plant hormones, an analysis that was started several years earlier, and that potentially sheds light on hormone function, with the ultimate aim to be able to use these important growth regulators more precisely and effectively in agriculture. We have concluded that plant hormones are derived from metabolic byproducts that are stably correlated with core complex abilities, and that over evolutionary time they became comprehensive promoters of these same abilities.

Summary of Progress

<u>The potential use of phenylpropanoid pathway intermediates in promoting leaf growth and in the suppression of microbial growth</u>

Leaf crops are crucial to global agriculture, providing essential nutrition for humans and livestock, and serving as a resource of interest for both the tobacco and biofuels industry. The domestication of these crops has historically focused on increasing leaf size and other traits that enhance leaf biomass and crop quality. While breeding efforts continue, immediate gains in leaf crop agriculture could be achieved through treatment regimes that accelerate leaf growth and increase final leaf size. We have previously shown that the plant natural product *trans*-cinnamic acid (*t*-CA) has a leaf growth promotion effect in the model plant Arabidopsis thaliana [1], and that this effect is also observed in other plant species, where it can cause an up to two-fold increase in leaf growth. This significant growth enhancement could shorten the length of crop cycles and simultaneously increase yield by preventing crop losses due to issues that arise late in development. During the past year we have tested the effects of *t*-CA on tobacco plants and also found a leaf growth promotion effect. Importantly, this growth promotion was achieved by simply spraying young tobacco seedlings with one *t*-CA dose, suggesting that this could be an easy (i.e. economical) way for boosting tobacco yields, as leaves are (obviously) the main plant part of interest for this crop species.

In addition to this growth promotion effect, we have also further analyzed the potential use of *t*-CA in suppressing microbial growth on agricultural produce [2]. We previously found that it has a potent suppression effect on the growth of fungi, which are a major cause for post-harvest spoilage. Our work

during the past year revealed that this anti-microbial (i.e. anti-spoilage effect) can be enhanced by the inclusion of certain edible surfactants and organic nanoparticles that serve to improve the delivery and effectiveness of *t*-CA on produce.

The evolutionary origin of plant hormones.

As sessile organisms, plants adapt to environmental challenges through flexible developmental and physiological programs. Hormones play a central role in this adaptability, integrating environmental signals into coordinated responses that regulate growth and stress tolerance. Comparative studies across photosynthetic lineages reveal that several core hormone functions are remarkably conserved, despite major evolutionary changes in hormone perception, biosynthesis, metabolism, and transport. This conservation suggests that plant hormones have played a pivotal evolutionary role—not only preserving essential biological functions but also enabling increased complexity in plant form and function. A similar dual role is observed in evolutionary endocrinology in animals, where hormones contribute to the emergence and regulation of complex traits. During the past year, we have performed a comprehensive review of hormone regulation in plants, and we proposed that hormones such as cytokinins, auxins, brassinosteroids, strigolactones, and abscisic acid originated as metabolic derivatives closely tied to core physiological functions essential for survival and reproduction, including reproductive success, nutrient sensing, and dehydration tolerance [3]. Over time, these compounds were progressively integrated into increasingly sophisticated regulatory networks, where they now serve as central coordinators and key targets of evolutionary selection. This model advances our understanding of hormone evolution by providing a structured framework to interpret the persistence, specialization, and integration of plant hormones across evolutionary timescales.

Our interpretation of hormone emergence and evolution involves a three-phase model represented in Figure 1. These three phases include: (1) The Association Phase where pre-hormone metabolites correlate with key adaptive traits such as reproduction or stress tolerance; (2) The Causation Phase, in which these compounds acquire regulatory roles and begin actively promoting these traits; and (3) The Integration Phase, marked by the evolution of hormone-specific biosynthesis, transport, metabolism, and high-sensitivity receptor- mediated signaling. The red arc in Figure 1 indicates the strengthening regulatory role of the hormone across evolutionary time.

In Figure 2, an example of this model is provided in the case of salicylic acid emergence and evolution. This figure maps the emergence of SA via the phenylpropanoid pathway, from *trans*-cinnamic acid (*t*-CA) through benzoic acid (BA), highlighting its ancestral link to stress defense and its subsequent regulatory specialization. The evolution of multiple SA receptors and biosynthetic routes reflects its dual role in biotic and abiotic stress adaptation.

Plans for Future Work:

We aim to further investigate the potential use of *t*-CA for the promotion of leaf growth in tobacco and the suppression of fungal infections. In addition, we will further explore the applicability of our model for hormone evolution by analyzing to what extent it can be applied to the hormones gibberellic acid, ethylene and jasmonic acid..

Figures

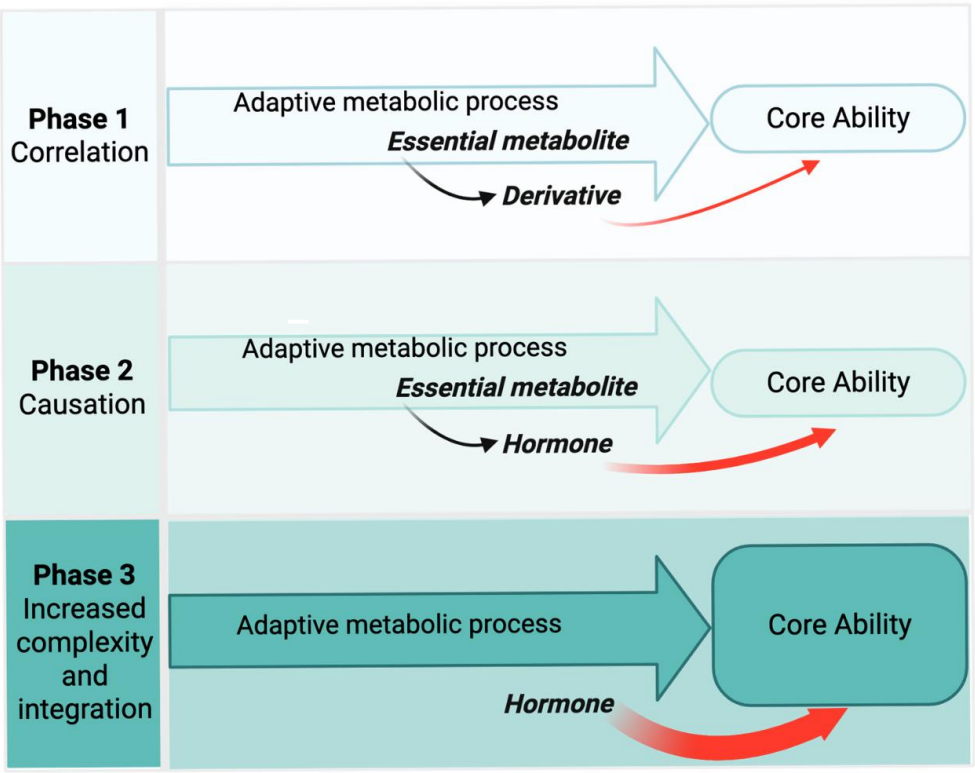

Figure 1: Model of plant hormone emergence and evolution.

From Kurepa & Smalle, (2025) *Int. J. Mol. Sci.,* 26, 7190

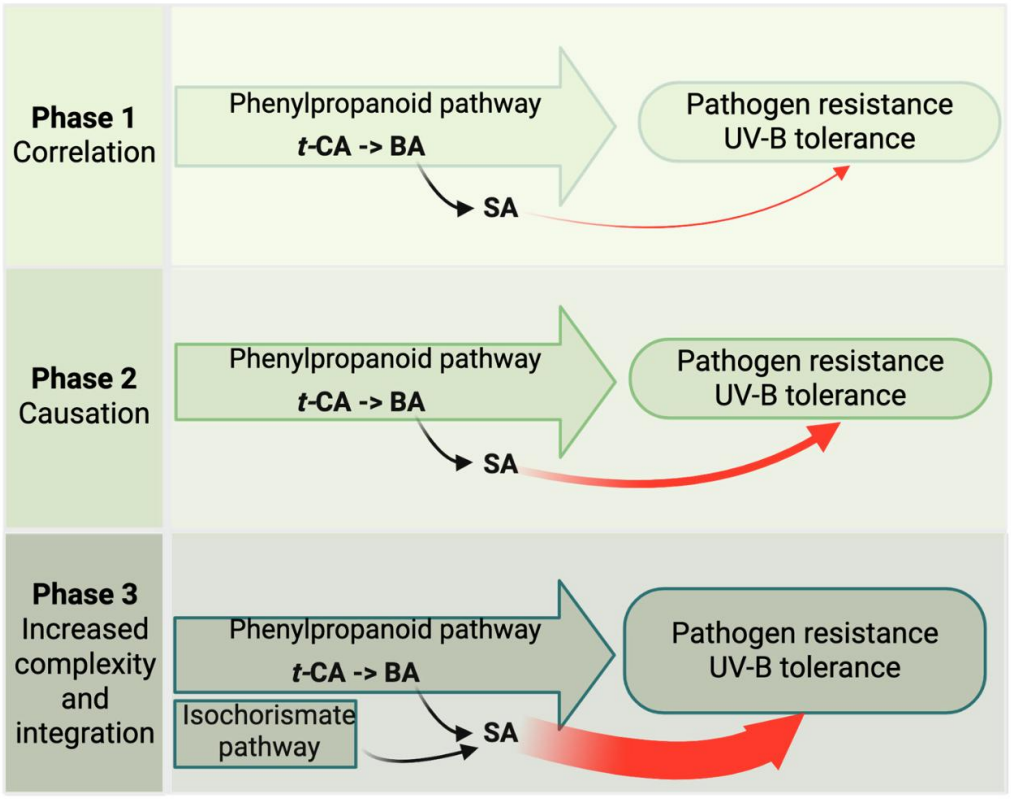

Figure 2: Emergence and diversification of salicylic acid (SA) as a hormone module.

From Kurepa & Smalle, (2025) *Int. J. Mol. Sci.,* 26, 7190

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SECTION 1: RESEARCH

PART A: PROJECT REPORTS 2024-2025

iii. SUMMIT PILOT-PROJECT GRANT REPORTS

STUDYING THE RELEASE OF NATURAL TOBACCO FLAVORANTS IN COMMON TOBACCO VARIETIES AND *ARTEMISIA ANNUA* AT HNB AND E-LIQUIDS TEMPERATURES (100-325°C) (2021-2024)

Investigator(s): Antoaneta Mihaylova-Kroumova (KTRDC), George Wagner (P&SS)

Report type: KTRDC tobacco summit pilot-project grant – final

Lay Summary: The tobacco industry is driven by regulatory pressures to mitigate the risks associated with tobacco products. Numerous studies indicate a significant reduction in harmful compounds released from heat-not-burn (or heated) and other non-combusted tobacco products. These heated tobacco products employ specialized devices designed to heat tobacco sticks to lower temperatures. The latest products from various companies heat tobacco within the range of 50°C to 350°C. In a previous report, we investigated the release of volatile flavor compounds (including nicotine) from Burley, Virginia, and Samsun tobacco varieties at heat-not-burn temperatures (100°C, 200°C, 300°C, and 400°C). Our findings suggested that to maximize the release of volatiles from the smoking device, the heating temperature should gradually increase up to 400°C, and a mix of tobacco varieties, as in traditional cigarettes, should be utilized. The objective of the current study is to explore the potential of *Artemisia annua L.* to enter the tobacco commercial space, particularly in emerging markets such as vapor and heat-not-burn products. We examined Artemisia volatiles at heat-not-burn temperatures and assessed the opportunities to deliver valuable medicinal and flavor compounds through heated devices. Employing a methodology similar to the one used for tobacco, we investigated volatile emissions from both fresh and dry Artemisia leaves, using the Agilent Thermal Separation Probe (TSP), coupled with GC/MS. The TSP was instrumental in comparing volatiles generated after preliminary heating of the material for less than 30 seconds at a specific inlet temperature, approximating heat-not-burn and vaping processes where consumers begin puffing immediately upon heating the material in the smoking device. In the volatiles of fresh Artemisia, seven compounds detected at 100°C were also present at 200°C, including caryophyllene, germacrene D, 2-bornanone (camphor), eucalyptol, camphene, p-cymene, and beta-pinene. Camphor abundance was the highest, followed by germacrene D and camphene. All compounds found at 100°C and 200°C possess flavor/fragrance properties or medicinal benefits. Similar results were observed from dry Artemisia at 200°C, 275°C, and 325°C. At 200°C, camphor predominated, followed by endo-Borneol, caryophyllene, and deoxyartemisinin. At 275°C and 325°C, the major compounds were camphor, neophytadiene, and 2-methoxy-4-vinylphenol. Many volatile compounds released from both fresh and dry Artemisia exhibited medicinal properties and others showing flavor and fragrance properties. The consideration of using Artemisia in heated tobacco devices for recreational or medicinal purposes is plausible, with suggested heating temperatures ranging from 100°C to 325°C.

Introduction

Since the early 2000s, tobacco companies have been developing newer nicotine and tobacco products, including heat-not-burn tobacco products (HnB), also known as heated tobacco products. This trend is primarily motivated by regulations aimed at reducing the risks associated with combustible tobacco products. As of November 2023, the latest heated tobacco products (HTP) on the market include: Imperial Brands: iSenzia (315-345°C) (November 2023) https://imperialbrandsscience.com/our-ngp-portfolio/products/, IQOS ILUMA (PMI) (November 2023), heating up to 50°C

(https://heetstore.com › products › iqos-iluma-prime/); Ploom X and Ploom X Advanced (JTI) (up to 320°C); ILUMA, Levia (IQOS) with nicotine, but without tobacco that could eliminate/reduce the regulatory (e.g., flavor bans) and fiscal (e.g., taxation) disadvantages associated to a tobacco containing product: Terea Crafted HTP (IQOS) with botanicals enhanced tobacco taste (up to 350oC). Natural ingredients of the botanical component (i.e. no artificial flavors) include rosemary & menthol, star anise, clove, and will offer a naturally flavored heated tobacco product despite a flavor ban. (https://tobaccoinsider.com/heated-tobacco-products/). These new IQOS products aims to address both regulatory constraints (e.g., flavor bans) and fiscal challenges (e.g., taxation) associated with traditional tobacco-containing products.

Several studies have demonstrated the harm reduction potential of HnB tobacco products compared to conventional combustible products. Consensus in the scientific community suggests that these products deliver significantly fewer toxicants (Simonavicius et al., 2019; Peitsch et al., 2018; Mallock et al., 2018). For instance, Mallock et al. (2018) reported an 80.5 to 99.4% reduction in acetaldehyde, acrolein, formaldehyde, 1,3-butadiene, benzene, isoprene, styrene, and toluene compared to combustible cigarettes.

A key question regarding the novel, less harmful tobacco products is how the lower temperature of non-combusted products affects their natural flavor delivery. Additionally, there is a need to explore how new products can deliver sufficient flavor without traditional additives, some of which have been banned (https://www.fda.gov/tobacco-products/products-ingredients-components/menthol-and-other-flavors-tobacco-products). Given that tobacco flavor significantly influences product sales, the incorporation of natural flavor compounds may offer a regulatory advantage. Notably, the FDA has recently permitted the marketing of e-cigarette products containing naturally flavored e-liquids, such as RJR/BAT vuse e-liquid roasted tobacco (https://www.fda.gov/news-events/press-announcements/fda-permits-marketing-e-cigarette-products-marking-first-authorization-its-kind-agency)

The flavor components of non-combusted products have been investigated to identify flavor additives. Krusemann et al. (2018) conducted a comprehensive study comparing cigarettes with flavor additives, cigarettes with non-characterizing flavor products, and reference products (cigarettes and natural tobacco leaves). Thirty-six natural flavor compounds were identified in at least one of the reference products, serving as a major reference library for this study.

In a previous interim report, we evaluated the release of volatile compounds and nicotine from various tobacco varieties at heat-not-burn temperatures (100 to 400°C), including cured Virginia K326, Cured

Burley tobacco TN 90LC, and Cured Samsun. Our findings indicated the release of fewer known natural volatiles compared to headspace analysis (Krusemann et al., 2018), where lower incubation temperatures (140°C) and significantly longer incubation times (30 minutes) were employed. New volatile compounds, including limonene (fruity flavor), neophytadiene (flavor enhancer), and solanone, were observed at 100 to 400°C, which were not detected in tobacco smoke at combustion temperatures (Rodgmann and Perfetti, 2009). Nicotine was released abundantly at 300-400°C, limonene at 400°C, and solanone at 200°C. We concluded that to experience all volatile compounds during smoking, the heating rod temperature of heated tobacco devices needs to gradually increase up to 400°C during the smoking time and blending different tobacco varieties is critical for achieving a fuller aroma experience, as seen in combustible cigarettes.

The current study aims to explore the potential of *Artemisia annua* (*A. annua*)to enter the tobacco commercial space, particularly in emerging markets such as vapor and heat-not-burn products. We examined Artemisia volatiles at HnB temperatures and assessed opportunities to deliver valuable medicinal and flavor compounds via HnB devices.

Artemisia annua L., sweet wormwood, is a highly aromatic herb originating from Asia and Eastern Europe. It serves as the sole source of traditional Chinese herbal medicine known as Qing Hao, utilized for over 2000 years to alleviate fevers, inflammation, headaches, bleeding, and to counteract malarial attacks (Vidic et al., 2018). Previous analyses of *A. annua* volatiles have focused on essential oils. A review by Bilia et al. (2014) summarized extract compositions, highlighting mainly monoterpenoids and sesquiterpenes. These profiles exhibited significant variations in the three main components: artemisia ketone, 1,8-cineole (eucalyptol), and camphor, depending on the global phytogeographic origin. To date, there isn't literature regarding the study of Artemisia volatiles at HnB and e-liquid vaping temperatures.

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Summary of Progress

Materials and Methods

Plant material

Seeds of the Swiss *Artemisia annua L.* variety, known as Apollon, were germinated and cultivated at Coldstream Farm, University of Kentucky's experimental station in Lexington, KY. Subsequently, the material was harvested and was dried at ambient temperature for a few days.

Sample collection

The Artemisia material was supplied by Colin Fisher and Jeff Kinney from KTRDC. Harvested on September 26, 2022, the Artemisia plants underwent a process where whole leaves were removed from the stem and subsequently dried in a barn. Following the drying process, the leaves were milled into 1 mm particles.

Studying the volatile compounds generated at HnB temperatures

We applied the same novel approach that was described in the previous report to study known, and other flavour-related volatile emissions in tobacco. We utilized a Thermal Separation Probe (TSP) inlet attached to Agilent ultra-inert GC column DB-624UI. The inlet temperature is manually regulated to correspond to the temperatures of tobacco heated devices and e-liquid devices (100°C-325°C). According to some information sources there are several herbs that release volatiles the best at lower temperatures (sub-

150°C) include catnip, lavender, and lemon balm, some herbs require higher temperature (150–190°C) peppermint, green tea and yerba mate and some – above 190°C: valerian, damiana herb, and chamomile. https://www.zamnesia.com/blog-the-ultimate-vaping-temperature-guide . In contrast, tobacco heated sticks create vapor at higher temperatures, up to 360oC (…). In the TSP, volatile compounds are separated from the matrix before entering the GC column. The analysis was done with Agilent 8890/ 5977B GC/MS system. The operation conditions were the same as described in the previous annual report (2022). For each inlet temperature, samples were run in duplicates and the average values are presented in the tables. Compounds were identified with NIST 17 library. The compound hits showing percent match higher than 80 were considered reliable. The potential flavor value of those compounds was determined using the flavor library published by Krusemann et al. (2018). Where the volatiles were not found in this library, the Good Scent Company (http://www.thegoodscentscompany.com) and other internet sources were searched.

We employed the same innovative approach detailed in the previous report to investigate both known and other flavor-related volatile emissions in tobacco. This involved utilizing a Thermal Separation Probe (TSP) inlet connected to an Agilent ultra-inert GC column (DB-624UI). The manually regulated inlet temperature corresponds to the temperatures of tobacco-heated devices and e-liquid devices (100°C-325°C). According to various information sources, different herbs release volatiles optimally at varying temperatures: catnip, lavender, and lemon balm perform best at lower temperatures (sub-150°C), while peppermint, green tea, and yerba mate require higher temperatures (150–190°C), and valerian, damiana herb, and chamomile show optimal release above 190°C (https://www.zamnesia.com/blog-the-ultimate-vaping-temperature-guide). Notably, tobacco-heated sticks generate vapor at higher temperatures, reaching up to 360°C.

Within the TSP, volatile compounds are separated from the matrix before entering the GC column. The analysis was conducted using the Agilent 8890/5977B GC/MS system, with operation conditions consistent with those described in the previous annual report (2022). Compound identification utilized the NIST 17 library, with hits showing a percent match higher than 80 considered reliable.

The potential flavor value of these compounds was determined using the flavor library published by Krusemann et al. (2018). For volatiles not found in this library, additional searches were conducted using resources such as the Good Scent Company (http://www.thegoodscentscompany.com) and other internet sources.

Results and discussion

Fresh Artemisia leaves

The total ion profile of volatiles, emitted at 100oC is presented in Figure 1. The individual volatiles are shown in Tables 1 (100°C). The data at 100°C are presented here from a single chromatographic run, while the data at 200°C were an average from triplicates. There were 3-fold more compounds at 100°C. Seven compounds—caryophyllene, germacrene D, 2-bornanone (camphor), eucalyptol, camphene, p-cymene, and beta-pinene—were consistently detected at both temperatures. As it can be seen from the tables, all the compounds at both temperatures have flavor/fragrance properties, or medicinal benefits. The dominant compounds at 100°C were camphor and camphene, known for their medicinal uses. Following closely were verbenol, germacrene D, and beta-guaiene, contributing to the flavor and fragrance profile. Some volatiles identified were also found in tobacco, including caryophyllene, aromadendrene, caparratriene, avocadinofuran, and hydroxyquinone. Additionally, the precursor of artemisinin, amorphadiene, was observed at 100°C.

Figure 1 provides a visual representation of the total ion profiles at 100°C, offering insights into the composition and abundance of volatile compounds released during the analysis.

Dry Artemisia leaf powder

The examination of volatiles from dry Artemisia material was conducted at temperatures of 200°C, 275°C, and 325°C, with samples analyzed in triplicate. The total ion profile of the sample run at 275°C is depicted in Figure 2. Detailed chemical profiles of the volatiles are recorded in Tables 3 (200°C), 4 (275°C), and 5 (325°C), where the peak abundance (in mln.) is averaged. At 200°C, 20 compounds were identified, at 275°C, 28 compounds, and at 325°C, 25 compounds. Many of these compounds were consistent with those found in fresh leaves and showed some similarities across all three temperatures, including caryophyllene, germacrene, copaene, camphor, camphene, deoxyartemisinin, and eucalyptol.

Most of the compounds exhibit medicinal properties, as well as flavor, fragrance, antifungal, and antibacterial properties (e.g., camphene, germacrene D). Notably, deoxyartemisinin, while lacking significant antimalarial activity, demonstrates anti-inflammatory and antiulcer properties (de Faveri Favero et al., 2014). Bilia et al. (2014) provided a comprehensive summary of the volatile composition of Artemisia essential oils collected globally. Their findings highlighted significant variations in the profiles, particularly in the three main components: artemisia ketone, 1,8-cineole (eucalyptol), and camphor. These differences were attributed to the diverse phytogeographic origins of the Artemisia plants.

In our study, we did not observe the presence of artemisia ketone, a component deemed "typical of artemisinin-rich *A. annua* cultivars" (Reale et al., 2011). It's noteworthy that artemisinin, a non-volatile compound, was naturally not detected in our volatile compound analysis.

Summary

The investigation into *Artemisia annua L.* volatiles, generated by heating the leaves at temperatures ranging from 100 to 325°C for less than 30 seconds, revealed the presence of numerous compounds, reaching up to 28 at certain temperatures. Notably, twelve of these compounds were reported to exhibit strong antibacterial and antifungal activity (Bilia et al., 2014).

Comparisons between the volatile compounds released from fresh and dry Artemisia indicated similarities, with some compounds displaying medicinal properties, while others exhibited flavor and fragrance characteristics. These findings open up the possibility of considering Artemisia for use in heated tobacco devices, catering to both recreational and medicinal purposes. The heating temperature, originally reaching up to 325°C, can be potentially optimized by reducing it to 300°C or even 275°C. It's worth noting that the minimal temperature required for the release of volatile compounds is 100°C. The identification of similar volatile profiles across different temperatures in our study suggests the possibility of reducing the maximum temperature of vaping devices, such as to 275°C or 300°C. This finding holds significance for device design, as it implies that desirable compound release from Artemisia can be achieved at lower temperatures, potentially enhancing the safety and efficiency of vaping devices.

Plans for Future Work

We will complete studying Artemisia volatiles from fresh and dry material at 100^{o}C, 200^{o}C, 275^{o}C and 325^{o}C temperatures to confirm the reliability of the approach.

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Figures and Tables

Table 1: Volatile compounds from fresh Artemisia at 100^0C

Library/ID	Area	Properties
	Peak area$x10^6$	
Caryophyllene	**7,965**	**anti-inflammatory, antimicrobial, antibacterial, and antioxidant properties.**
Germacrene D	**16,67**	**Insect pheromones, antibacterial and antifungal activities**
cis-Sabinene hydrate =Bicyclo[3.1.0]hexan-2-ol,	**1,658**	**effective against cancer, may alleviate some of the side effects of chemotherapy**
(+)-2-Bornanone aka Camphor	**224,382**	**anti-inflammato, antibacterial, antifungal properties, cough, pain, and itching ,**
Eucalyptol	**9,269**	**controls airway mucus hypersecretion and asthma via anti-inflammatory cytokine inhibition**
Camphene	**39,356**	**reduces pain and inflammation, and has antifungal properties**
Copaene	**1,414**	**anti-proliferative, antioxidant, anti-genotoxic and cytotoxic activities**
Aromandendrene	**1,876**	**anti-cancer terpenoid, especially when combined with β-caryophyllene.**
.alpha.-Pinene	**2,743**	**α-Pinene is an anti-inflammatory and is likely antimicrobial, improves memory; in canabis**
Caparratriene	**4,458**	**significant growth inhibitory activity against CEM leukemia cells**
.beta.-Myrcene	**1,445**	**flavouring and aroma agent , antioxidant, anti-ageing, anti-inflammatory, analgesic properties**
.gamma.-Terpinene	**2,052**	**antioxidant**
Amorphadiene - =(1R,4R,4aS,8aR)-4,7-Dimethyl	**2,192**	**precursor of artemisinin**
L-Borneol =Bicyclo[2.2.1]heptan-2-ol, 1,7,7-tr	**1,431**	**relief of minor aches and pains of muscles and joints,**
Carveol	**1,864**	**possesses potent antiparkinsonian activity in animal models**
Germacrene B, =1,5-Cyclodecadiene, 1,5-dimet	**3,555**	**an essential oil component in a variety of plants; antioxidant, antiradical and antimicrobial**
cis-.beta.-Farnesene	**2,911**	**in purfum industry, natural insect repellent**
Avocadynofuran	**1,142**	**natural product in avocado**
.alpha.-Campholenal	**1,528**	**natural product, flavoring agent**
***epi C*edrol** (3R,3aS,6S,7R)-3,6,8,8-Tetramethyl	**5,747**	**natural product, found in *Asphodelus albus***
1-Octen-3-ol	**1,132**	**natural product; atracts biting insects**
3-Hexen-1-ol, (E)-	**1,337**	**Used for a green topnote in herbaceous fragrances.**
.beta.-Guaiene	**10,355**	**fragrance and flavor**
Benzyl valerate = Pentanoic acid, phenylmethy	**1,608**	**fragrance and flavor**
Verbenol	**13,363**	**flavoring agent, food additive, pheromone**
Hydroquinone	**2,527**	**skin bleaching cream antioxidant,**
p-Cymene	**16,299**	**flavoring, fragrance**
(-)-beta Pinene =Bicyclo[3.1.1]heptane, 6,6-dim	**2,877**	**air freshener, fragrance**

The data presents average of three repeats.

Table 2: Volatile compounds from fresh Artemisia at 200^0C

Library/ID	Average	StDev	Health benefits
	Peak area x10^5		
Caryophyllene	4.747	0.415	anti-inflammatory, antimicrobial, antibacterial, and antioxidant properties
Germacrene D	23.226	3.337	insect pheromones, antibacterial and antifungal activities
(+)-2-Bornanone	51.957	3.496	anti-cavity drugs for cardiotonic agents, stimulants, coolants, antipruritic agents
Camphene	4.579	1.351	reduces pain and inflammation, and has antifungal properties
(-)-beta-Pinene; OR Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene	1.529	0.287	air freshener, fragrance
beta Pinene; OR Bicyclo[3.1.1]hept-2-en-6-one, 2,7,7-trimethyl-	2.083	0.64	food improvement
Eucalyptol	4.636	0.994	controls airway mucus hypersecretion and asthma via anti-inflammatory cytokine inhibition
cis-Sabinene hydrate; OR Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-m	2.213	0.666	treatment of bladder, kidney, and urinary stones
p-Cymene	0.889	0.296	flavoring, fragrance
Aromandendrene	1.624	0.07	anti-cancer terpenoid, especially when combined with β-caryophyllene.

Each data presents average of three repeats.

Table 3: Volatile compounds from dry Artemisia at 200^{0}C

Library/ID	Aver	StdDev	Health benefit
	Peak area x10^6/mg		
Carveol	2.399	0.691	possesses potent antiparkinsonian activity in animal models
Copaene	1.921	0.903	Anti-proliferative, antioxidant, anti-genotoxic and cytotoxic activities
Caryophyllene	15.061	2.421	anti-inflammatory, antimicrobial, antibacterial, and antioxidant properties.
cis-Sabinene hydrate; OR Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-	1.499	0.137	effective against cancer, may alleviate some of the side effects of chemotherapy
(+)-2-Bornanone	103.146	9.646	anti-inflammato, antibacterial, antifungal properties, cough, pain, and itching ,
Spathulenol; OR 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-m	8.001	0.548	flavor and fragrance, in oregano
Camphene	1.518	0.748	reduce pain and inflammation, and has antifungal properties
o-Cymene	3.650	0.953	prevents coughs and eliminate phlegm
Eucalyptol	4.639	1.297	controls airway mucus hypersecretion and asthma via anti-inflammatory cytokine inhibition
2-Methoxy-4-vinylphenol	3.163	0.429	It has a role as a pheromone, a flavouring agent and a plant metabolite.
cis-.beta.-Farnesene	6.123	1.562	in purfum industry, natural insect repellent
Coumarin	5.940	2.503	multiple medical use: inhibit blood clots, deep vein thrombosis, and pulmonary embolism
Sabinene OR Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	1.061	0.271	flavor and fragrance
beta Pinene, OR Bicyclo[3.1.1]hept-2-en-6-one, 2,7,7-trimethyl-	4.486	0.744	antibacterial, antidepressant, cytotoxic, and antimicrobial.
Pinocarvone	0.920	0.085	antiproliferative, antioxidant and antibacterial activities
Germacrene D	22.962	2.226	Insect pheromones, antibacterial and antifungal activities
endo-Borneol	8.853	0.390	increases the depth of sleep and the perception of well-being
iso-Cadinene OR Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-meth	1.048	0.153	fragrance and flavor
Deoxyartemisinin	14.832	3.221	anti-inflammatory and antiulcer activities
Pyrrolidine, 1-acetyl-	3.804	1.686	aroma and flavor

Each data presents an average of three repeats.

Table 4: Volatile compounds from dry Artemisia at 275^{0}C

Library/ID	Aver	Stdev	Health benefits or other properties
	Peak area x 10^6		
Caryophyllene	**17.237**	*2.104*	**anti-inflammatory, antimicrobial, antibacterial, and antioxidant properties.**
Germacrene D	**30.186**	*2.550*	Insect pheromones, antibacterial and antifungal activities
Neophytadiene	**57.088**	*2.265*	**an anti-inflammatory agent, an antimicrobial agent, a plant and an algal metabolite.**
Eucalyptol	**4.528**	*0.416*	**anti-inflammatory cytokine inhibition**
(+)-2-Bornanone aka Camphor	**112.886**	*7.703*	**topical medication for cough, pain, and itching,** insect bites,
cis-.beta.-Farnesene	**7.218**	*1.785*	in purfum industry, natural insect repellent
2-Furanmethanol	**21.541**	*4.773*	**flavoring**
Copaene	**5.099**	*0.619*	**anti-proliferative, antioxidant, anti-genotoxic and cytotoxic activities**
Indole	**17.846**	*3.436*	flavor and fragrance in oregano
Coumarin	**23.995**	*4.796*	**anticoagulant: inhibits blood clots, deep vein thrombosis, and pulmonary embolism**
Spathulenol = 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimeth	**16.815**	*1.999*	flavor and fragrance in oregano
Camphene	**3.201**	*0.619*	**reduces pain and inflammation, and has antifungal properties**
Phenol, 2-methoxy-	**21.027**	*4.969*	**disinfectant properties and an expectorant**
2-Methoxy-4-vinylphenol	**47.594**	*8.544*	pheromone, a flavouring agent and a plant metabolite.
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)-	**2.455**	*0.739*	fragrance and flavor
Phytol	**3.261**	*1.563*	**antinociceptive, anti-inflammatory, immune-modulating, and antimicrobial effects**
Phytol	**3.542**	*1.166*	antinociceptive, anti-inflammatory, immune-modulating, and antimicrobial effects
Deoxyartemisinin	**30.411**	*5.562*	anti-inflammatory and antiulcer activities
.gamma.-Terpinene	**1.059**	*0.245*	antioxidant
Phenol	**40.478**	*0.350*	**relieve pain and irritation caused by sore throat, sore mouth, or canker sores.**
endo-Borneol	**10.522**	*1.635*	**increases the depth of sleep, the perception of well-being**.
Jasmone = 2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)-	**1.594**	*0.235*	antioxidant, natural Artemisia compound,jasmin flavor
Hydroquinone	**6.674**	*2.494*	skin bleaching cream antioxidant
beta-Copaene = (1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenet	**0.272**	*0.083*	in citrus extracts
(Z)-tagetenone =(Z)-2,6-Dimethylocta-2,5,7-trien-4-one	**0.532**	*0.131*	natural substance
1-(1'-Pyrrolidinyl)-2-propanone	**15.177**	*3.756*	natural substance
6,9-Guaiadene=1R,3aS,8aS)-7-Isopropyl-1,4-dimethyl-1,2,3,3a,6,8a-	**9.932**	*0.591*	flavor/fragrance in Cannabis vape oil
Benzeneacetaldehyde	**7.107**	*4.326*	honey sweet, floral, chocolate, cocoa, spicy
N,N-Dimethylaminoethanol	**19.856**	*8.022*	**a nootropic- improves cognitive functions**

Each data presents an average of three repeats.

Table 5: Volatile compounds from dry Artemisia at 325^0C

Library/ID	Average/mg	Stdev	Health benefit
	Peak area, x 10^6		
Caryophyllene	20.833	2.741	anti-inflammatory, antimicrobial, antibacterial, and antioxidant properties.
Neophytadiene	51.573	6.059	an anti-inflammatory agent, an antimicrobial agent
(+)-2-Bornanone (Camphor)	105.909	15.413	topical medication,oinment
Germacrene D	27.359	4.930	Insect pheromones, antibacterial and antifungal activities
2-Furanmethanol	25.321	8.135	flavor in food industry
Eucalyptol	5.852	1.885	controls airway mucus hypersecretion and asthma via anti-inflammatory cytokine inhibition
Copaene	6.448	0.950	anti-proliferative, antioxidant, anti-genotoxic and cytotoxic activities
cis-β-Farnesene	9.384	4.883	in purfum industry, natural insect repellent
γ-Selinene = Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-n	14.103	6.428	in many natural oils, mosquito repellent
Camphene	5.044	2.082	reduces pain and inflammation, and has antifungal properties
Jasmone = 2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)-	1.520	0.495	antioxidant properties, jasmine flavor, natural product in Artemisia
Spathulenol = 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-	17.924	1.280	flavor and fragrance in oregano
p-Cymene	7.349	2.021	prevent coughs and eliminate phlegm
Phenol, 2-methoxy-	28.048	1.291	disinfectant properties and used as an expectorant.
2-Methoxy-4-vinylphenol	56.863	2.560	pheromone, a flavouring agent and a plant metabolite.
Indole	33.483	0.759	flavor and fragrance
Coumarin	26.252	2.017	anticoagulant
Phytol	4.598	2.172	antinociceptive, anti-inflammatory, immune-modulating, and antimicrobial effects
Deoxyartemisinin	21.275	15.387	anti-inflammatory and antiulcer activities
Phenol	10.662	0.853	antiseptic, treat sore throat
p-Cresol	2.543	2.268	Local antiseptic, parasiticide, disinfectant; an intestinal antiseptic
endo-Borneol	13.253	1.712	increases the depth of sleep, the perception of well-being.
N,N-Dimethylaminoethanol	18.163	6.604	nootropic agent, benefits spatial and working memory, treat ADHD
γ-Terpinene	1.118	0.260	antioxidant
Cyperolactone	1.925	0.675	aroma component of Cypriol Oil
δ-amorphene = 1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaph	2.367	1.579	natural product
(+)-Coniine = Cyclopenta[c]quinolin-4-one, 1,2,3,5-tetrahydro-	1.098	0.141	the poison of Socraties

Each data presents an average of three repeats.

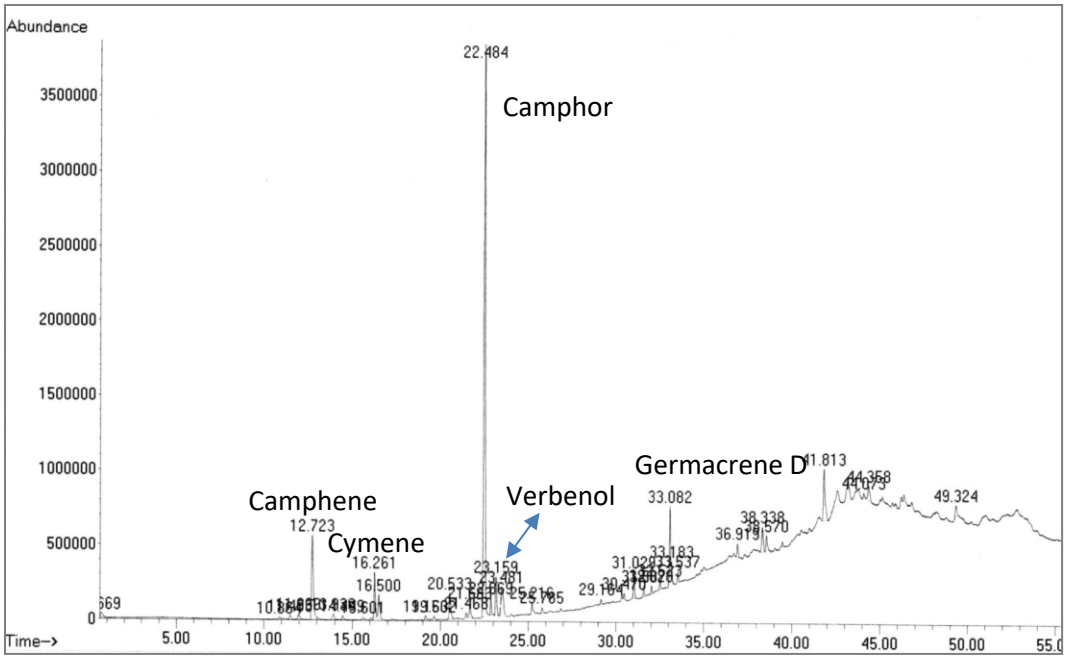


Figure 1: Total ion profile of fresh Artemisia at 100^{0}C**.**

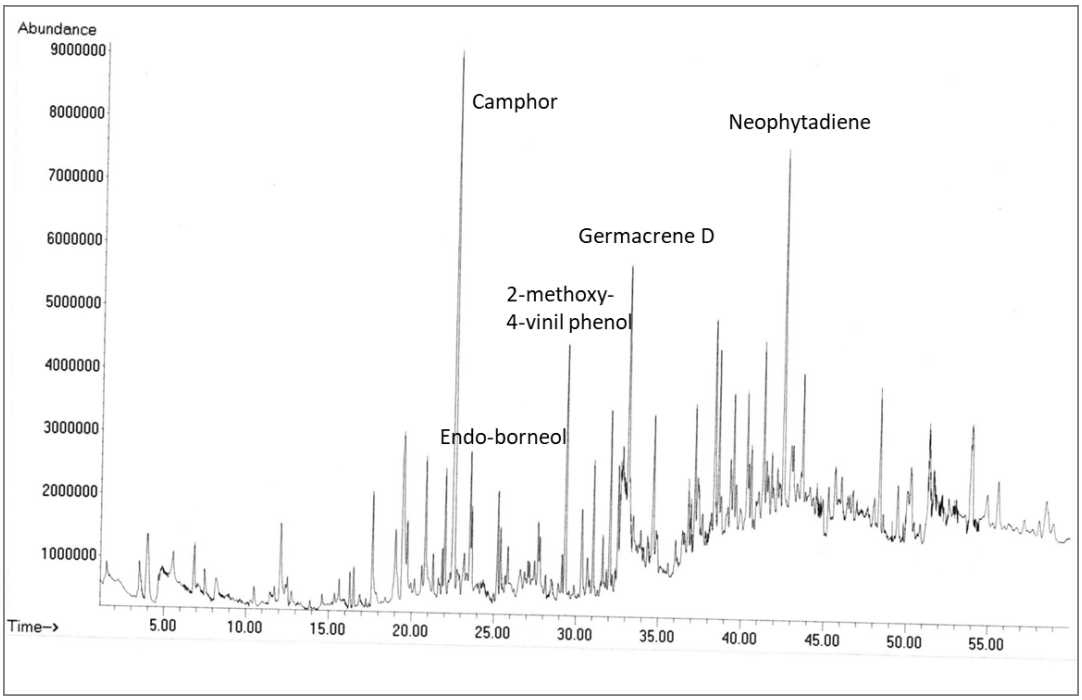


Figure 2: Total ion profile of dry Artemisia at 275^{0}C**.** For simplicity, only few major compounds are labeled.

SECTION 1: RESEARCH

PART A: PROJECT REPORTS 2024-2025

iv. EXTERNALLY FUNDED RESEARCH PROJECT REPORTS

NOT REPORTED FOR 2024-2025

Due to technical issues and international travel, reports for field research funded by external sources were not available at the time of publication of this report.

Due to confidentiality and in progress work for publication, reports for laboratory research funded by external sources were not available at the time of publication of this report.

SECTION 1: RESEARCH
PART B: LISTINGS OF RESEARCHERS AND PUBLICATIONS

LISTING OF KTRDC-SUPPORTED FACULTY, RESEARCHERS AND SENIOR RESEARCH STAFF IN 2024-25

A. Research faculty who have appointments in other departments and colleges at the University of Kentucky, and who have collaborated with KTRDC researchers in 2024-25.

Faculty	Title	Department
A. Bailey	Extension Professor	Plant & Soil Sciences
R. Pearce	Extension Professor	Plant & Soil Sciences
J. Smalle	Associate Professor	Plant & Soil Sciences
G. Wagner	Professor Emeritus	Plant & Soil Sciences
L. Yuan*	Executive Director	Plant & Soil Sciences

* Received KTRDC salary support

B. Senior Research Scientists supported by funding from KTRDC.

Scientist	Title
A. Fisher	Research Director
C. Fisher	Scientist III
H. Ji	Scientist III
R. McNees	Scientist II
A. Mihaylova-Kroumova	Scientist III
S. Pattanaik	Scientist III
B. Patra	Scientist III
S. Singh	Scientist II
D. Zaitlin	Scientist III

C. University faculty and research personnel who received 2024-25 research funding from KTRDC.

Researcher	Title	Department
J. Smalle	Faculty	Plant & Soil Sciences
G. Wagner	Professor	Plant & Soil Sciences
D. Orren	Professor	Toxicology and Cancer Biology

PUBLISHED ARTICLES AND ABSTRACTS

July 1, 2024 – December 31, 2025

Note: Every effort is made to obtain a complete and accurate listing of publications deriving from KTRDC-sponsored research (i.e. that deriving from KTRDC grants or KTRDC-funded principal investigator positions). However, submissions for this list are the responsibility of the individual investigators, and KTRDC may not always be able to verify the completeness and appropriateness of the citations provided. Also, it should be noted that the Center places considerable emphasis on mission-critical, applications-related work, a type of research which typically does not generate large numbers of scientific publications. Finally, at any time there can be many manuscripts and patent applications in preparation, submitted, or under review – these are not listed here until finally published/issued, and until a full citation is available.

Published Articles and Book Chapters:

Fisher, A. and Bailey, W.A. 2025. TSNAs in burley and dark tobacco. Chapter in "2025-2026 Burley and dark tobacco production guide; A cooperative effort of the University of Kentucky; the University of Tennessee; Virginia Tech; and NC State University". p 68-74 https://publications.mgcafe.uky.edu/sites/publications.ca.uky.edu/files/ID160_1.pdf

Davis, E.B.; Jacobs, A.A.; Flythe, D.M.; Hamilton, A.T.; Ji, H; Schrick, F.N; Goodman, J. P. 2025 Effects of isoflavone supplementation, via red clover hay, on the growth and postgraze physiological recovery of beef steers grazing endophyte-infected tall fescue pastures. Journal of Animal Science. Volume 103, 2025, skaf304, https://doi.org/10.1093/jas/skaf304

Klotz, J.; Checura, C.; Greene, M.; Ji, H.; 2025 Duckett, S. Serotonin can stimulate vasorelaxation in ovine lateral saphenous veins precontracted with ergovaline. Journal of Animal Science. Volume 103, 2025, skaf108, https://doi.org/10.1093/jas/skaf108

Kurepa, J.; Smalle, J. (2025). The Evolution of Plant Hormones: From Metabolic Byproducts to Regulatory Hubs. *Int. J. Mol. Sci*., 26 (15), 7190. https://www.mdpi.com/1422-0067/26/15/7190

Ling, C.; Yang, J.; Xu, J.; Tang, W.; Liu, Y.; Wang, Y.; Li, P.; He, Y.; Ouyang, Z.; Chen, S.; Xing, F.; Wang, X.; Liu, P.; Liu, Y.; Wang, R.; Liu, X.; Yin, X.; Huo, H.; Li, D.; Smalle, J.; Liu, Y.; Wang, Y. (2025). Natural variation of AcEGY3 mediates chloroplastic ROS homeostasis to confer kiwifruit thermotolerance. *Nat. Commun.* 16 (1), 6184. https://www.nature.com/articles/s41467-025-61593-5

Liu, Y.; Shi, J.; Patra, B.; Singh, S.K.; Wu, X.; Lyu, R.; Liu, X.; Li, Y.; Wang, Y.; Zhou, X.; Pattanaik, S.; Yuan, L.; 2025. Transcriptional reprogramming deploys a compartmentalized 'Timebomb' in *Catharanthus roseus* to fend Off chewing herbivores. Plant, Cell & Environment. 48(5):3236-3256. doi: 10.1111/pce.15324

Mihaylova-Kroumova, A., Korenkov, V., Wagner, G., & Kinney, J. (2025). Exploring the Potential of Artemisia Annua for Therapeutic Uses in Heated Tobacco Devices, *Journal of Herbs, Spices & Medicinal Plants*, 31(3), 340–361. https://doi.org/10.1080/10496475.2025.2488336

Wu, X.; Singh, S.K.; Patra, B.; Wang, J.; Pattanaik, S and Yuan, L. 2025. An Overview of the Regulation of specialized metabolism in tobacco. Current Plant Biology 41:100431. doi: 10.1016/j.cpb.2024.100431

Zhou, Y.; Liu, Y.; Lyu, R.; Singh, S.K.; Sui, X.; Hou, X.; Pattanaik, S.; Yuan, L. 2025. Post-translational control of biotic stress-related nicotine biosynthesis by a MAP kinase signaling cascade. The Crop Journal (in press). doi: 10.1016/j.cj.2025.08.012

Zhou, Y.; Singh, S.K.; Patra, B.; Liu, Y.; Pattanaik, S.; Yuan, L. 2025. Mitogen-activated protein kinase-mediated regulation of plant specialized metabolism. Journal of Experimental Botany. 76(2):262-276. doi: 10.1093/jxb/erae400

Presentations:

Fisher, A. 2024. Integrated pest management (IPM) 2024 report. Presented: CORESTA Congress, Edinburgh, United Kingdom, October 2024.

Fisher, A. 2025. Integrated pest management (IPM) 2025 report. Presented: CORESTA Agronomy and Phytopathology Conference, Surabaya, Indonesia, September 2025.

Fisher, A.; Fisher, C.; Ji, H.; Kinney, J.; Slone, S.; Yang, S. 2025. Does later topping improve the quality of low alkaloid burley tobacco? . Abstract 146. Presented: 78th Tobacco Science Research Conference, Knoxville, Tennessee, September 2025.

Fisher, A.; Fisher, C.; Ji, H.; Kinney, J.; Slone, S.; Yang, S. 2025. Does later topping improve the quality of low alkaloid burley tobacco? Abstract AP03. Presented: CORESTA Agronomy and Phytopathology Conference, Surabaya, Indonesia, September 2025.

Fisher, A.; Patra, B.; Fisher, C.; Ji, H.; Kinney, J.; Slone, S.; Yang, S. 2024. Stacking a novel low nicotine gene with the LA *nic1nic2* mutants lowers nicotine to ultra-low levels. Abstract 83. Presented: 77th Tobacco Science Research Conference, Atlanta, Georgia, September 2024.

Fisher, A.; Patra, B.; Fisher, C.; Ji, H.; Kinney, J.; Slone, S.; Yang S. 2024. Stacking a novel low nicotine gene with the LA *nic1nic2* mutants lowers nicotine to ultra-low levels. Abstract AP50. Presented: CORESTA Congress, Edinburgh, United Kingdom, October 2024.

Fisher, A. M.; Patra, B.; Fisher, C. R.; Yang, S.; Slone, S.; Ji, H.; Kinney, J. 2024 Stacking a novel low nicotine gene with the LA nic1nic2 mutants lowers nicotine to ultra-low levels. Abstract AP50. Presented: CORESTA Congress, Edinburgh, Scotland, October 2024

Fisher, A.M.; Slone, S; Patra, B.; Fisher, C.R.; Ji, H.; Kinney, J.; Yang, S. 2024. Stacking a novel low nicotine gene with the LA nic1nic2 mutants lowers nicotine to ultra-low levels. Abstract 83. Presented: 77th Tobacco Science Research Conference, Atlanta, Georgia, September 2024

Fisher A.; Fisher C.; Ji, H.; Kinney, J.; Slone, S.; Yang, S. 2025. Does later topping improve the quality of low alkaloid burley tobacco? Abstract AP 03. Presented: CORESTA Conference of the Agronomy/Phytopathology, Surabaya, Indonesia, October 2025

Fisher, A.M.; Fisher. C.; Ji, H.; Kinney, J.; Slone, S.; Yang, S. 2025. Does Later Topping Improve the Quality of Low-Alkaloid Burley Tobacco? Abstract 146. Presented: 78th Tobacco Science Research Conference, Knoxville, Tennessee, September 2025

Ji, H.; Jin, Z.; Fenton, L. 2024. Stability of Certified Reference Cigars during Long-Term Storage. Abstract STPOST 71. Presented: CORESTA Congress, Edinburgh, Scotland, October 2024

Ji. H. 2024. Analysis of Commercial Cigars for Development of Design Parameters for Reference Cigars. Abstract W4. Presented: 77th Tobacco Science Research Conference, Atlanta, Georgia, September 2024

Ji, H.; Jin, Z.; Fenton, L. 2024. Stability of Certified Reference Cigars during Long-Term Storage. Abstract 13. Presented: 77th Tobacco Science Research Conference, Atlanta, Georgia, September 2024

Ji, H.; Wu, Y. 2025. Determination of NNN and NAT enantiomers in tobacco and tobacco products. Abstract MP 657. Presented: 73rd American Society for Mass Spectrometry Conference, Baltimore, Maryland, June 2025

Ji, H.; Wu, Y. 2025. Determination of NNN and NAT enantiomers in tobacco and tobacco products. Abstract 30. Presented: 78th Tobacco Science Research Conference, Knoxville, Tennessee, September 2025

McNees, R.; Ji, H.; Craft, M.; Slone, S.; Hall, J.T.; Yuan, L. Chambers, O. 2025. Center for Tobacco Reference Products. Abstract STPOST 43. Presented: CORESTA Conference of the Smoke Science and Product Technology Sub-Groups, Annecy, France, October 2025

McNees, R.; Ji, H.; Craft, M.; Slone, S.; Hall, J.T.; Yuan, L. Chambers, O. 2025. Center for Tobacco Reference Products. Abstract 9. Presented: 78th Tobacco Science Research Conference, Knoxville, Tennessee, September 2025

Pattanaik, S. Proficiency Testing for Detection of Transgenic Tobacco: GMO Sub-Group Report 1. CORESTA Agronomy/Phytopathology Conference, Surabaya, Indonesia, September 2025.

Pattanaik, S. Proficiency Testing for Detection of Transgenic Tobacco: GMO Sub-Group Report 2. CORESTA Agronomy/Phytopathology Conference, Surabaya, Indonesia, October 2025.

Pattanaik, S. Proficiency Testing for Detection of Transgenic Tobacco: GMO Sub-Group Report 1. CORESTA Agronomy/Phytopathology Conference, Edinburgh, Scotland, October 13, 2024.

Pattanaik, S. Proficiency Testing for Detection of Transgenic Tobacco: GMO Sub-Group Report 2. CORESTA Agronomy/Phytopathology Conference, Edinburgh, Scotland, October 17, 2024.

Patra, B.; Fisher, C.; Singh, S.K.; Kinney, J. 2024. The roots of tobacco plants dictate the leaf quality. AP49, CORESTA Agronomy/Phytopathology Conference, Edinburgh, Scotland, October 2024.

Patra, B.; Fisher, A.; Singh, S.K.; Kinney, J. 2024. Understanding the molecular mechanism underlying low alkaloid accumulation in mutant burley breeding line with novel spontaneous mutation(s). AP51, CORESTA Agronomy/Phytopathology Conference, Edinburgh, Scotland, October 2024.

Patra, B.; Fisher, C.; Singh, S.K.; Kinney, J. 2024. The roots of low alkaloid tobacco probably determine its poor leaf quality. AP82, Tobacco Science Research Conference, Atlanta, September 2024.

Patra, B.; Fisher, A.; Singh, S.K.; Kinney, J. 2024. Understanding the molecular mechanism underlying low alkaloid accumulation in mutant burley breeding line with novel spontaneous mutation(s). AP84, Tobacco Science Research Conference, Atlanta, September 2024.

Pochucha, A.; Westberg, H.; Pattanaik, S.; Anderson, D. 2025 Proficiency Testing. CORESTA Agrochemical Analysis-GMO Subgroup Webinar Presentation, Lexington, February, 2025.

Wu, Y.; Ji, H. 2024. Distribution of Tobacco Constituents PON and NNK in Cured Burley Tobacco Leaf Tissue. Abstract 109. Presented: 77th Tobacco Science Research Conference, Atlanta, Georgia, September 2024

Zhou, Y.; Liu, Y.; Pattanaik, S.; Patra, B.; Lyu, R.; Ji, H.; Singh, S.; Yuan, L. 2024. Post translational regulation of nicotine biosynthesis by map kinase cascade NTMEKK1B-NTMKK2ANTMPK4. Abstract 85. Presented: 77th Tobacco Science Research Conference, Atlanta, Georgia, September 2024

Patents / Patents Pending:

Yuan, L.; Singh, S.K.; Pattanaik, S.; Lawson, D. 2025 bZIP Transcription Factors Regulate Conversion of Nicotine to Nornicotine and reduce levels of tobacco specific (TSNA) precursors. US Patent No. 12,344,853

SECTION 2: ADMINISTRATION

SECTION 2: ADMINISTRATION

PART A: KTRDC STRUCTURE

MEMBERS OF THE KENTUCKY TOBACCO RESEARCH BOARD

2023-2024	2024-2025
OFFICERS Todd Clark, Chair Al Pedigo, Vice Chair Angela Martin, Treasurer	**OFFICERS** Todd Clark, Chair Al Pedigo, Vice Chair Angela Martin, Treasurer
BOARD MEMBERS Larry Clark (Shane Wiseman) Todd Clark Sierra Enlow Richard Heath (David Hale) Jason Howell Bill McCloskey F. T. Samuel (Maria Labreveux) Jonathan Shell (Warren Beeler) Laura Stephenson (James Matthews) Monique Quaterman (Kristine McNeil) Al Pedigo (Jason Wade) Ilhem Messaoudi (Linda Dwoskin)*	**BOARD MEMBERS** Larry Clark (Shane Wiseman) Todd Clark Sierra Enlow Myron Dossett (David Hale) Jason Howell Bill McCloskey F. T. Samuel (Maria Labreveux) Jonathan Shell (Warren Beeler) Laura Stephenson (James Matthews) Monique Quaterman (Kristine McNeil) Al Pedigo (Jason Wade) Ilhem Messaoudi (William Stoops)*

- Non-voting member

ORGANIZATION AND REPORTING

The provisions contained within KRS 248.510 to 248.580 created the Kentucky Tobacco Research Board (KTRB), which oversees the administrative and research activities of the KTRDC. The KTRB is responsible to the Governor of the Commonwealth through the Legislature and the Legislative Research Commission. The KTRDC also operates under the administrative and financial guidelines of the University of Kentucky.

Reporting Procedures and Accountability

The Managing Director of the KTRDC under the KTRB has the following reporting and accountability responsibilities:

1. Quarterly Progress Reports to the Legislative Research Commission.
2. Quarterly Progress Reports to the KTRB.
3. Annual Progress Reports to the Governor of the Commonwealth, the Legislative Research Commission, and the KTRB.
4. Responsive to an annual financial audit by an external auditing firm as directed by the University of Kentucky financial administration.
5. Operates under all administrative and policy guidelines of the University of Kentucky.
6. Continued interaction with the office of the Dean of the Martin Gatton College of Agriculture, Food and Environment (MG-CAFÉ), University of Kentucky.

KTRDC RESEARCH COMMITTEE 2024-25

Ms. Anne Fisher
Dr. Colin Fisher
Ms. Huihua Ji
Dr. Ruth McNees
Dr. Antoaneta Mihaylova-Kroumova
Dr. Barunava Patra
Dr. Sitakanta Pattanaik
Dr. Jan Smalle
Dr. Ling Yuan

SECTION 2: ADMINISTRATION

PART B: FINANCIAL INFORMATION

TOBACCO RESEARCH INCOME

INCOME COMPARISON

Fiscal Years	2018-2019	2019-2020	2020-2021	2021-2022	2022-2023	2023-2024	2024-2025	2025-2026
July	$120,890.40	$141,864.01	$136,565.92	$102,816.87	$113,853.04	$-	$97,579.97	$84,421.42
August	$126,982.37	$145,789.42	$11,873.82	$148,863.59	$121,485.75	$235,814.07	$113,878.38	$94,787.78
September	$178,553.92	$132,169.60	$261,157.23	$138,395.19	$143,503.64	$116,834.55	$112,212.24	$109,920.98
1st QUARTER	**$426,426.69**	**$419,823.03**	**$409,596.97**	**$390,075.65**	**$378,842.43**	**$352,648.62**	**$323,670.59**	**$289,130.18**
October	$97,793.84	$150,849.00	$141,682.93	$138,913.78	$131,512.77	$84,290.07	$86,565.34	$96,740.93
November	$128,963.50	$117,280.34	$135,157.14	$101,844.54	$101,050.68	$132,736.05	$88,478.89	$104,074.91
December	$175,277.00	$151,323.23	$159,616.92	$138,232.14	$113,515.64	$81,648.61	$90,136.26	$97,484.98
2nd QUARTER	**$402,034.34**	**$419,452.57**	**$436,456.99**	**$378,990.46**	**$346,079.09**	**$298,674.73**	**$265,180.49**	**$298,300.82**
January	$564,217.88	$120,247.87	$93,056.96	$116,044.01	$111,657.62	$101,501.91	$78,472.89	$-
February	$141,118.46	$114,095.14	$125,797.09	$89,271.71	$78,955.86	$77,922.09	$125,701.11	$-
March	$122,472.86	$403,962.17	$143,903.75	$140,521.53	$119,175.49	$105,636.69	$45,037.80	$-
3rd QUARTER	**$827,809.20**	**$638,305.18**	**$362,757.80**	**$345,837.25**	**$309,788.97**	**$285,060.69**	**$249,211.80**	**$-**
April	$146,789.57	$117,862.64	$144,970.47	$127,449.97	$79,639.90	$119,161.48	$94,449.22	$-
May	$63,797.02	$141,525.18	$100,238.76	$148,769.94	$120,890.24	$88,889.28	$80,887.20	$-
June	$250,352.13	$138,849.18	$211,130.06	$121,204.33	$149,991.92	$154,407.96	$103,211.51	$-
4th QUARTER	**$460,938.72**	**$398,237.00**	**$456,339.29**	**$397,424.24**	**$350,522.06**	**$362,458.72**	**$278,547.93**	**$-**
TOTAL INCOME	**$2,117,208.95**	**$1,875,817.78**	**$1,665,151.05**	**$1,512,327.60**	**$1,385,232.55**	**$1,298,842.76**	**$1,116,610.81**	**$587,431.00**

INCOME AND FINANCIAL REPORT

FISCAL YEAR 2024-25 REPORTING SECOND QUARTER KTRDC ANNUAL FINANCIAL REPORT

Funds Center	Funds Center Name	Category	Annual Budget	Prior Month Balance	Current Month Actual	YTD Actual	* YTD Encumbrance	Available Budget
1235410080	KTRDC HOLDING ACCOUNT	Revenue	($1,723,000.00)	($489,946.02)	($97,484.98)	($587,431.00)		($1,135,569.00)
1235410080	Result	Total	($1,723,000.00)	($489,946.02)	($97,484.98)	($587,431.00)		($1,135,569.00)
1235410090	KENTUCKY TOBACCO RESEARCH BOARD	Operating Expenses	$1,000.00					$1,000.00
1235410090	Result	Total	$1,000.00					$1,000.00
1235410100	KTRDC ADMINISTRATION	Salaries	$260,000.00	$75,960.40	$14,564.23	$90,524.63	$91,571.33	$77,904.04
1235410100	KTRDC ADMINISTRATION	Benefits		$31,191.00	$6,142.50	$37,333.50	$38,687.56	($76,021.06)
1235410100	KTRDC ADMINISTRATION	Operating Expenses		$1,885.95	$231.95	$2,117.90	$0.00	($2,117.90)
1235410100	KTRDC ADMINISTRATION	Recharges		$18.41	$2.37	$20.78		($20.78)
1235410100	Result	Total	$260,000.00	$109,055.76	$20,941.05	$129,996.81	$130,258.89	($255.70)
1235410110	KTRDC PERSONNEL	Salaries		$170,545.16	$30,891.25	$201,436.41	$159,081.95	($360,518.36)
1235410110	KTRDC PERSONNEL	Benefits		$48,643.34	$9,316.19	$57,959.53	$51,163.68	($109,123.21)
1235410110	KTRDC PERSONNEL	Expense Transfers			$25.78	$25.78		($25.78)
1235410110	KTRDC PERSONNEL	Operating Expenses	$1,000,000.00	$3,452.82	$1,691.10	$5,143.92		$994,856.08
1235410110	KTRDC PERSONNEL	Recharges		$22,534.76	$3,230.25	$25,765.01		($25,765.01)
1235410110	Result	Total	$1,000,000.00	$245,176.08	$45,154.57	$290,330.65	$210,245.63	$499,423.72
1235410120	KTRDC PUBLICATIONS AND TRAVEL	Operating Expenses	$25,000.00	$3,874.41	$2,268.46	$6,142.87	$0.00	$18,857.13
1235410120	KTRDC PUBLICATIONS AND TRAVEL	Recharges		$730.76	$22.93	$753.69		($753.69)
1235410120	Result	Total	$25,000.00	$4,605.17	$2,291.39	$6,896.56	$0.00	$18,103.44
1235410130	KTRDC BUILDING MAINTENANCE	Operating Expenses	$50,000.00	$12,266.17	$1,327.18	$13,593.35	$0.00	$36,406.65
1235410130	KTRDC BUILDING MAINTENANCE	Recharges		$1,604.07		$1,604.07		($1,604.07)
1235410130	Result	Total	$50,000.00	$13,870.24	$1,327.18	$15,197.42	$0.00	$34,802.58
1235410180	KTRDC SHOP	Operating Expenses	$2,000.00	$725.56		$725.56	$0.00	$1,274.44
1235410180	KTRDC SHOP	Recharges		$3.90		$3.90		($3.90)
1235410180	Result	Total	$2,000.00	$729.46		$729.46	$0.00	$1,270.54
1235410240	KTRDC LABORATORY EQUIPMENT	Operating Expenses	$40,000.00	$9,244.24	$1,848.85	$11,093.09		$28,906.91
1235410240	Result	Total	$40,000.00	$9,244.24	$1,848.85	$11,093.09		$28,906.91

INCOME AND FINANCIAL REPORT

FISCAL YEAR 2024-25 **REPORTING SECOND QUARTER** **KTRDC ANNUAL FINANCIAL REPORT**

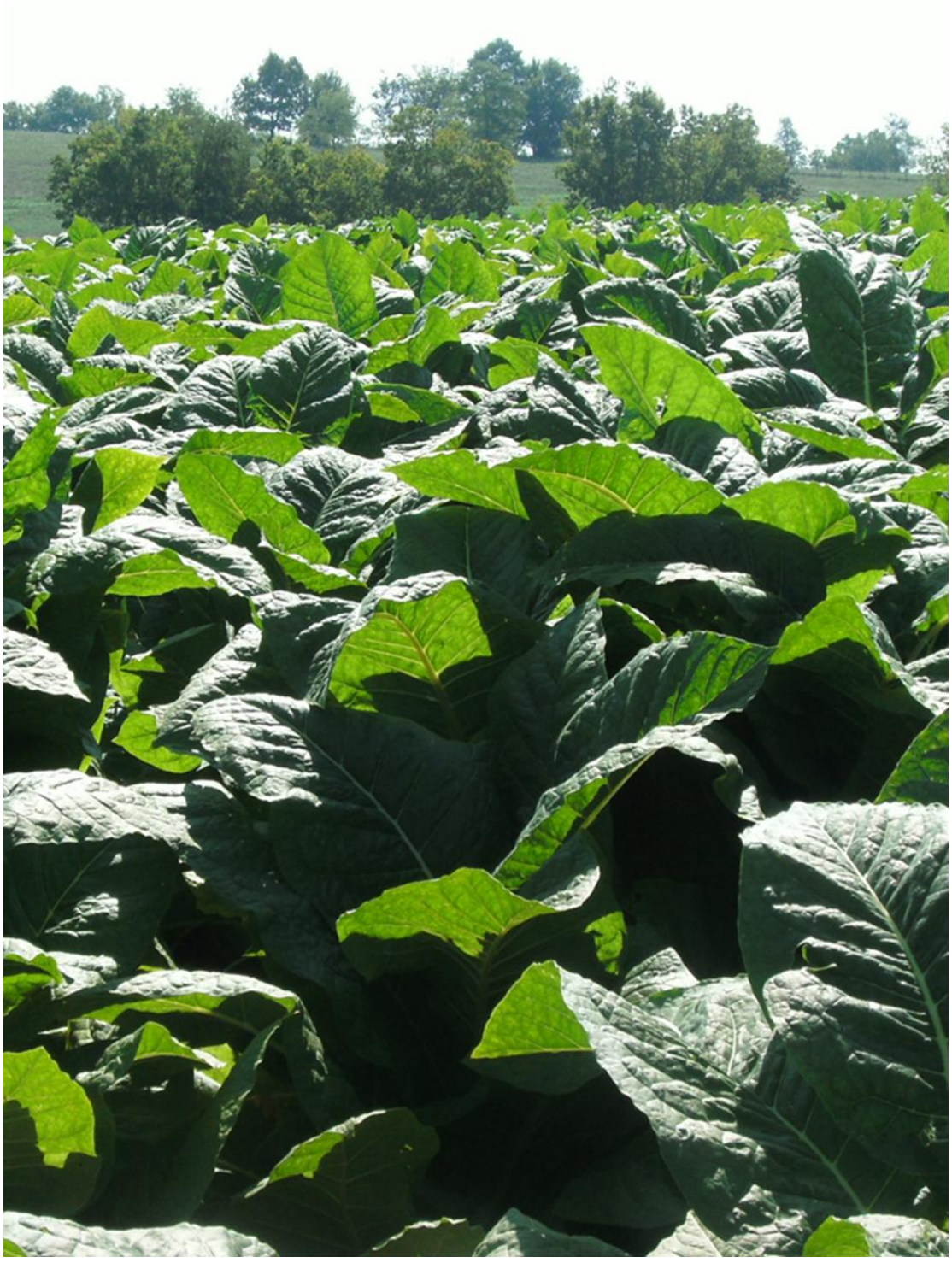
Funds Center	Funds Center Name	Category	Annual Budget	Prior Month Balance	Current Month Actual	YTD Actual	* YTD Encumbrance	Available Budget
1235410250	KTRDC UNALLOCATED RESERVE FOR RESEARCH	Operating Expenses	$90,000.00					$90,000.00
1235410250	Result	Total	$90,000.00					$90,000.00
1235410280	KTRDC GENERAL LABORATORY	Operating Expenses	$50,000.00	$7,631.84	$1,379.01	$9,010.85	$0.00	$40,989.15
1235410280	KTRDC GENERAL LABORATORY	Recharges		$530.01	$1.11	$531.12		($531.12)
1235410280	Result	Total	$50,000.00	$8,161.85	$1,380.12	$9,541.97	$0.00	$40,458.03
1235411040	KTRDC DISCRETIONARY	Operating Expenses	$20,000.00	$2,135.47		$2,135.47		$17,864.53
1235411040	KTRDC DISCRETIONARY	Recharges		$24.16		$24.16		($24.16)
1235411040	Result	Total	$20,000.00	$2,159.63		$2,159.63		$17,840.37
1235411310	KTRDC OUTREACH & COMMUNICATIONS	Operating Expenses	$20,000.00					$20,000.00
1235411310	Result	Total	$20,000.00					$20,000.00
1235411340	GENETIC MANIPULATION OF TOBACCO TO	Salaries		$10,415.00	$1,019.50	$11,434.50		($11,434.50)
1235411340	GENETIC MANIPULATION OF TOBACCO TO	Benefits		$628.10	$40.33	$668.43		($668.43)
1235411340	GENETIC MANIPULATION OF TOBACCO TO	Operating Expenses	$30,000.00					$30,000.00
1235411340	GENETIC MANIPULATION OF TOBACCO TO	Recharges		$1,220.52		$1,220.52		($1,220.52)
1235411340	Result	Total	$30,000.00	$12,263.62	$1,059.83	$13,323.45		$16,676.55
1235411360	PLANT BIOTECH METABOLIC	Operating Expenses	$30,000.00	$11,182.05	$1,208.71	$12,390.76	$0.00	$17,609.24
1235411360	PLANT BIOTECH METABOLIC	Recharges		$84.56	$14.09	$98.65		($98.65)
1235411360	Result	Total	$30,000.00	$11,266.61	$1,222.80	$12,489.41	$0.00	$17,510.59
1235411370	KTRDC PLANT BIOTECH - MOLECULAR	Operating Expenses	$30,000.00	$15,395.39	$1,568.71	$16,964.10	$278.00	$12,757.90
1235411370	KTRDC PLANT BIOTECH - MOLECULAR	Recharges		$2,896.75	$2,180.87	$5,077.62		($5,077.62)
1235411370	Result	Total	$30,000.00	$18,292.14	$3,749.58	$22,041.72	$278.00	$7,680.28

INCOME AND FINANCIAL REPORT

FISCAL YEAR 2024-25 **REPORTING SECOND QUARTER** **KTRDC ANNUAL FINANCIAL REPORT**

Funds Center	Funds Center Name	Category	Annual Budget	Prior Month Balance	Current Month Actual	YTD Actual	* YTD Encumbrance	Available Budget
1235411380	MOLECULAR GENETICS	Operating Expenses	$30,000.00	$3,874.43		$3,874.43	$0.00	$26,125.57
1235411380	MOLECULAR GENETICS	Recharges		$27.66		$27.66		($27.66)
1235411380	Result	Total	$30,000.00	$3,902.09		$3,902.09	$0.00	$26,097.91
1235411410	KTRDC GREENHOUSE	Operating Expenses	$15,000.00	$1,375.56	$3.80	$1,379.36	$1,768.60	$11,852.04
1235411410	KTRDC GREENHOUSE	Recharges		$1,990.47	$879.38	$2,869.85		($2,869.85)
1235411410	Result	Total	$15,000.00	$3,366.03	$883.18	$4,249.21	$1,768.60	$8,982.19
1235411570	TOBACCO MOLECULAR FARMING AGRONOMICS	Recharges		$0.00		$0.00		$0.00
1235411570	Result	Total		$0.00		$0.00		$0.00
1235412360	FLAVONOID - SMALLE	Salaries		$6,148.87	$2,580.98	$8,729.85	$10,526.49	($19,256.34)
1235412360	FLAVONOID - SMALLE	Benefits		$3,029.84	$1,253.29	$4,283.13	$5,119.14	($9,402.27)
1235412360	FLAVONOID - SMALLE	Operating Expenses	$30,000.00					$30,000.00
1235412360	Result	Total	$30,000.00	$9,178.71	$3,834.27	$13,012.98	$15,645.63	$1,341.39

* Projected salary encumbrances, not included in available budget



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