1. No-till and rotation improve soil aggregation and associated carbon and nitrogen stocks in burley tobacco production. B. Pearce; Zou, C.; Grove, J.; Coyne, M. University of Kentucky, Lexington KY USA

Burley tobacco (Nicotiana tobacum L.) production in Kentucky is typically tillage-intensive and sometimes mono-cropped. Intensive tillage has been reported to degrade soil structure and deplete soil organic carbon (SOC) and total nitrogen (TSN) stocks. This study assessed the effect of tillage, crop rotation, and N fertilizer application on soil structure, water stable aggregation, and aggregate-associated SOC and TSN concentrations and stocks for rotations including burley tobacco. Four burley tobacco production systems (main plots) were established in 2007 on a Bluegrass-Maury silt loam soil including: i) no-tillage continuous tobacco (NT-Tobacco); ii) conventional tillage continuous tobacco (CT-Tobacco); iii) 2-yr sod (*Festuca arundinacea* Schreb.) 1-yr no-tillage tobacco (NT-Rotation); iv) 2-yr sod and 1-yr conventional tillage tobacco (CT-Rotation). In 2012, two N rates (0 and 280 kg N ha-1) were applied to split plots. Soil samples were taken in spring 2013 to determine water stable aggregate size distributions and associated SOC and TSN concentrations and stocks. Compared to conventional tillage and tobacco monoculture, no-tillage and rotation with sod significantly increased macroaggregate fractions (> 250 µm) and associated SOC and TSN stocks at the expense of the silt-clay size class (< 53 µm) and associated SOC and TSN stocks. Although previous fertilizer N application had no significant effect on whole-soil SOC and TSN stocks, a significant interaction with crop rotation on macroaggregate distribution and macroaggregate-associated SOC and TSN stocks was observed. Using NT and/or rotation practices in burley tobacco production maintains desirable soil physical and chemical properties via macroaggregate stabilization, which leads to the conservation of SOC and TSN stocks. (Reprinted with permission)

2. Residual nitrogen investigations for strip-tilled flue-cured tobacco (*Nicotiana tabacum L.*) production in Virginia. E. B. Brown, Reed, T. D.; Teutsch, C. D., Tracy, B. F., Virginia Tech, Blackstone VA USA

Approximately 75% of tobacco production in Virginia is grown in fields classified as highly erodible. Strip-tillage, which has been demonstrated to be suitable for tobacco in southern Virginia, utilizes specialized equipment to cultivate narrow strips into which tobacco is transplanted. Tobacco is typically strip-tilled into the residue of a previous crop or a small grain cover crop. This study was conducted to evaluate soil nitrogen levels and nitrogen uptake by tobacco with different strip-tillage cover management programs. The experiment was conducted in 2014 and 2015 at the Southern Piedmont Agricultural Research and Extension Center near Blackstone, VA on a complex of Durham and Worsham sandy loams. Treatments evaluated the topdressing of a wheat cover crop with 22 or 45 kg N/ha. Subsequent fertilization of the tobacco compared the standard N rate (81 kg/ha) to treatments reduced by the corresponding amount of topdressing applied to the wheat. Additional treatments included strip-till following a wheat cover crop with no topdressing and a conventional tillage control. Soil nitrate levels decreased steadily after topping in 2014, but significant differences were only found among the treatments at two of the sample dates and were not consistent. Treatments having reduced tobacco nitrogen rates had significantly lower petiole nitrate levels throughout the four harvests in 2014 and the first two harvests in 2015 compared to all other treatments. Conventional tillage, 45 N topdress, and no N topdress had higher normalized difference vegetation indices (NDVI) than all other treatments through the first three harvests in 2014. Treatments having reduced tobacco nitrogen rates had significantly lower NDVI than all other treatments through the first two harvests of 2015. Results of this study indicated that the wheat cover crop residue did not adversely affect the uptake of nitrogen by the tobacco crop. (Reprinted with permission)

Key terms: nitrogen, strip-tillage.

3. Evaluation of NCDA boron sufficiency ranges for flue-cured tobacco. C. Jernigan; Hardy, D. H.; Hicks, K. A.; McGinnis, M. S., North Carolina Department of Agriculture and Consumer Services, Raleigh NC USA; Fisher, L. R.; Vann, M. C., North Carolina State University, Raleigh NC USA

Boron (B) is an essential micronutrient for optimum plant growth of flue-cured tobacco and is required in trace amounts (<1.0 lb B/acre). Since use of premium tobacco fertilizer grades that contain trace amounts of B are not typical in today's production and flue-cured tobacco is not known to be sensitive to B deficiency, B is often not applied. In recent years however, B deficiency in North Carolina has been visually and analytically identified by sampling the most recently mature leaf (MRML). In 2015, research was initiated at five research stations to reevaluate B plant tissue sufficiency ranges using two plant parts (bud and MRML) and investigate associations with plant calcium levels. To assure adequate B sufficiency in transplants, a 1 ppm B concentration in the float bed water was used at all locations. Soil applied treatments of 0, 0.5, 1.0, 2.0, 5.0, and 10.0 lbs B /acre were initiated soon after base fertilizer (8-2-20) application, approximately 2 to 3 weeks after tranplanting. Three foliar treatments of 0.25 lbs B/acre were scheduled at second cultivation, at layby and "as needed" based on visual symptoms, with the latter not initiated. During the growing season, bi-weekly visual ratings were conducted to identify the occurrence of B deficiency and toxicity symptoms. Tissue samples were collected just prior to the two scheduled- foliar applications and at topping. Plots were harvested by stalk position and cured on-station. Yield was quantified and cured leaf was assigned a USDA grade. Crop value was determined through the combination of cured leaf yield and quality. Boron deficiency was not observed in 2015; however, B toxicity was documented at rates above 2.0 lbs B/acre. (Reprinted with permission)

Key Terms: boron, crop nutrition

4. Dark fire-cured tobacco response to boron. J. C. Rodgers and W. A. Bailey, University of Kentucky, Princeton KY USA

Boron deficiency has been documented on several farms in the dark tobacco production area of Kentucky and Tennessee, most commonly in the Purchase Area of Kentucky (Murray/Mayfield KY area). Typical symptoms of boron deficiency include leaf breakage approximately 2 inches from the stalk and possibly stunting/yellowing/malformation of the terminal bud. In some cases, high soil pH has been a contributing factor as boron becomes less available as soil pH approaches 7 or higher. Field experiments were conducted in 2014 and 2015 at a private farm near Mayfield KY where boron deficiency had been documented on the tobacco crop in 2012 and the soybean crop that followed in 2013. Treatments included transplant water-simulated (TPW) applications of boron made immediately after transplanting, or foliar applications made at 3 to 4 weeks after transplanting. Boron rates used were 0.25, 0.5, or 1.0 lb B per acre. Boron source was boric acid (Borosol® liquid, 10% B). In 2014, extensive transplant injury was seen from 0.5 and 1.0 lb B/A applied as TPW applications. Injury resulted in 34 to 66% injury from boron at 0.5 to 1.0 lb B/A, respectively, and as much as 29% stand loss at the 1 lb B/A rate. However, foliar boron applications were beneficial, with tobacco showing increasing positive visual growth responses to increasing rates of boron. 2014 yields were reflective of negative responses seen to TPW applications and positive responses seen to foliar applications. In 2015, injury from TPW applications was not obvious as it was in 2014, presumably due to much wetter conditions during and following transplanting. (Reprinted with permission)

Key terms: dark fire-cured tobacco, boron

5. Dark fire-cured tobacco response to potassium application rate and timing. R. A. Hill; Richmond, M. D.; Bailey, W. A.; Rodgers, J. C., University of Kentucky, Princeton KY USA

Field experiments were conducted in 2012, 2013, and 2014 to evaluate dark tobacco response to potassium at a site near Murray KY that showed low to medium potassium levels in soil samples taken in the spring prior to tobacco transplanting. Soil test K at the site was 102 in 2012 (low, 290 lbs K/A recommended), 136 in 2013 (low, 260 lbs

K/A recommended), and 212 in 2014 (medium-low, 190 lbs K/A recommended). Potassium was applied at the soil test recommendation, 0.5 x the soil test recommendation, or 1.5 x the soil test recommendation in either broadcast applications made the day before transplanting or banded applications (2 bands per row) made approximately 7 days after transplanting. Potassium sulfate (0-0-50) was the potassium source used in all applications. An untreated check was included that received no potassium. There was no response to potassium application timing, as vigor, yield, and quality were similar whether potassium was applied broadcast before transplanting or banded at one week after transplanting. Dark tobacco vigor, yield, and quality response to potassium rate was minimal in any year, but temporary potassium deficiency was seen in check plots that received no potassium. Treatments receiving potassium had higher yields than the untreated check. Slight increases in yield were seen in 2012 and 2014 as potassium rate increased from the 0.5 x to the soil test recommendation, but generally did not increase further as rate increased to 1.5 x the soil test recommendation. Quality was generally similar in all treatments that received potassium, and higher than in the untreated check. (Reprinted with permission)

Key terms: dark fire-cured tobacco, potassium

6. The effects of carbon nanosol on potassium adsorption characteristics and potassium uptake in the roots of tobacco. Liang, T. B.; LI, Y. L.; Yang J.; Zhang,Y. L.; and Yin, Q. S.; Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou China

Owing to particular properties such as surface effect, volume effect and quantum size effect, nanomaterials can enhance chemical fertilizer efficiency and promote plant growth. Carbon nanosol, which is produced by graphite electrolysis, also showed similar effects. In our previous studies, we found that carbon nanosol could greatly increase potassium (K) content in tobacco leaf and enhance tobacco leaf quality. However, the mechanism and approach of potassium promotion by carbon nanosol is still not clear. In this study, with adsorption and hydroponic experiments, the effects of carbon nanosol on K adsorption characteristics, K ion velocity and gene expression in tobacco roots were investigated. The results showed that carbon nanosol increased K adsorption ability, and more than 90% of the K equilibrium adsorption amount was adsorbed within three minutes. The adsorption isotherms could be modeled by both of the Langmuir and Freundlich equations, with the former much better. Carbon nanosol concentrations of 5-40 mg/L could promote potassium uptake and accumulation in tobacco. Carbon nanosol particles were observed on the surface of tobacco roots. Carbon nanosol could change the potassium ion flow direction in tobacco roots. 10mg/l carbon nanosol changed K ion flow from efflux to influx. The application of carbon nanosol also promoted the gene expression of inward potassium ion channels(*NKT*1 and *NtKC*1) and inhibited that of outward potassium ion channels (*NTORK*1). Higher K adhesive ability on the surface of tobacco roots, stronger potassium adsorption and change of K ion flow direction in tobacco roots all contributed to the increase of potassium content of tobacco leaf. (Reprinted with permission)

Key Terms: Carbon nanosol; Tobacco; Adsorption characteristics; Potassium uptake

7. Post-layby nitrogen application to fine textured soil of the North Carolina Piedmont. J. H. Mason; Vann, M. C.; Fisher, L. R., North Carolina State University, Raleigh NC USA

Current recommendations for nitrogen application to flue-cured tobacco are two separate application timings; one-half rate applied after transplanting and one-half rate applied at layby. A result of this practice is the likelihood of too much nitrogen being applied in a dry growing season or too little nitrogen applied in a wet growing season. Both scenarios can negatively impact leaf yield and quality. In previous evaluations, tobacco produced on coarse soil types had no differences irrespective of application timing. However, due to reduced leaching potential of nitrogen, tobacco produced on fine textured soils may result in reduced leaf yield and quality when following these recommendations. Research was initiated at two separate locations in 2015 to determine the most appropriate timings of nitrogen applications based on experienced growing conditions. Three targeted nitrogen rates (50, 70, and 90 lbs N/acre) were

used, with each rate being applied at 75, 100, and 125% of the targeted rate with various combinations of application timings. One-half rate of the targeted nitrogen rate was applied 7-10 days after transplanting and remaining applications occurred at layby and two weeks after layby. Leaf tissue samples were collected at layby, after topping, and after curing to quantify total nitrogen content and uptake. SPAD measurements were collected at layby and after topping to determine the correlation between nitrogen uptake and leaf color. Plots were harvested by stalk position and green leaf weight was recorded to quantify yield. Sub-samples of harvested leaf were cured in mesh peanut bags and used for leaf quality assessment. Crop value was determined through the combination of leaf yield and quality. (Reprinted with permission)

Key Terms: nitrogen, application rate, application timing

8. Evaluation of three transgenic varieties compared to three conventional varieties for low alkaloid production with three nitrogen variables. J. Cheek; Vann, M. C.; Fisher, L. R., North Carolina State University, Raleigh NC USA

Nicotine is one of the most studied and scrutinized plant secondary metabolites. Its concentration in tobacco products now falls under the jurisdiction of the Food and Drug Administration and it has been speculated that increased regulation might occur. In 2014, research was initiated at the Lower Coastal Plain Research Station, Upper Coastal Plain Research Station, and the Oxford Tobacco Research Station to evaluate three transgenic tobacco lines altered to decrease alkaloid (nicotine) production. Transgenic varieties were derived from K 326, with each having a different location at which the transgenic event took place. These three transgenic varieties, along with three conventional varieties (K 326, NC 95, LAFC53), were randomized over three different nitrogen rates, for a total of 18 treatments. Nitrogen application rates were 70%, 85%, and 100% of the recommended rate. Nitrogen was applied at a base rate of 30lbs N/acre after transplanting, with the difference being applied through 28% Urea-Ammonium-Nitrate at layby. After topping, leaf length, leaf width, SPAD readings, leaf discs, stalk height, and leaf counts were collected. Leaf yield, grade, and value were quantified after curing and composite samples from all four stalk positions were analyzed for alkaloid content. In 2015, excised leaf was collected to conduct a bioassay with tobacco budworms to determine preferential feeding differences among varieties. Yield and quality for transgenic lines were similar to traditional K-326 and higher than NC-95 and LAFC-53 at all three locations. Nicotine content was 75-80% lower in transgenic varieties when compared to traditional K-326. (Reprinted with permission)

9. Organic production of tobacco transplants: The nitrogen dilemma. B. Pearce, University of Kentucky, Lexington KY USA

Organic tobacco production presents a small but apparently growing niche market for tobacco growers. Among the challenges for organic tobacco growers is the production of healthy viable transplants. Most organic tobacco growers would prefer to use float technology to produce transplants and avoid the drudgery of pulling plants from plant beds in soil culture. Previous research has shown that the soilless media used in float beds typically lack the microbial populations necessary for rapid nitrification of ammonium to nitrate. In float beds supplied only with urea nitrogen (N), significant accumulations of nitrite-N in the weeks after fertilization resulted in severely stunted plants. Recent observations in grower float beds fertilized with organic fertilizer sources have revealed similar stunting of plants. In an effort to boost plant growth, the plant growers added sodium nitrate to the water, but growth remained stunted. Further, the presence of larvae that thrive in low oxygen environments and a large increase in alkalinity of the float water were noted. From these observations it appeared that the decomposition of the organic fertilizer depleted much of the oxygen in the water. This resulted in denitrification of the added nitrate which increased alkalinity levels. A series of experiments will be reported which examine nitrogen transformations in float environments. (Reprinted with permission)

10. Evaluation of a hard plastic greenhouse float tray for tobacco transplant production. T. D. Reed, Virginia Tech, Blackstone VA USA

Greenhouse float tobacco transplant production has long been the standard of the tobacco industry both in the U.S. and in other areas of the world. The expanded polystyrene (EPS) tray is the foundation of the float production system. However, effective sanitation and proper disposal of old trays are problematic. Additionally, trays fragment and pieces from broken trays represent a highly undesirable foreign material in cured tobacco. A prototype hard plastic float tray was evaluated at the Virginia Tech Southern Piedmont Center in 2014 and the first commercial trays (Trilogy from Beltwide Inc.) became available for the 2015 season. Though plastic is heavier than water, the tray is constructed with multiple compartments to trap air on the underside of the tray to provide floatation. Research trials were conducted to evaluate tobacco transplant production parameters in the plastic float tray and compare to the standard EPS float tray. The Trilogy is substantially heavier than EPS trays resulting in a lower float depth and increased saturation of the soilless medium. No significant differences were observed in seedling emergence but there were observations of minimally increased mortality of young seedlings in the plastic tray. The growth of transplants roots into the porous cell walls of EPS trays contributes to the difficulty of proper sanitation of the trays. Such is not the case with plastic trays, but this fact contributes to minimal anchoring of the plants in the tray cells. The result is the potential for dislodging of plants when trays are removed from the greenhouse bay and when riding the transplanter. The replacement of EPS trays with a plastic alternative represents a significant change to both tobacco transplant production and transplanting. Changes in seedling production practices and the handling of trays may be necessary to optimize their use and thus realize the potential advantages of eliminating the use of EPS trays. (Reprinted with permission)

Key terms: tobacco transplant production, greenhouse seedling trays

11. Multiplex use on tobacco to determine leaf major components, and maturation. E. Bargiacchi; Milli, G.; Romani, A.; Campo, M.; Miele, S., Italian Interuniversity Consortium for Science and Technology of Materials, Firenze Italy

The fluorescence-based sensor, Multiplex, has been calibrated over the last four years on Virginia Bright and Kentucky tobacco crops in Central Italy at Fattoria Autonoma Tabacchi and OPTA-Città di Castello comparing Multiplex field and lab measurements and HPLC/DAD/MS analyses of the hydroalcoholic extracts of the same tobacco leaves, both in different varieties, fertilization experiments, and growers' fields. Results indicated that several parameters can be determined with a $r^2 \approx 0.9$: total nitrogen, nicotine, nicotine derivatives, total polyphenols, with particular reference to monocaffeolquinic acids, quercitin- and kaempferol-ramnosyl glucosides. This sensor offers the opportunity to non-destructively investigate leaf composition and maturation for different targets: variety biochemistry, varietal adaptation to a given location, fertilization and phytopathology studies, effect of treatments with exogenous polyphenols, and the use of tobacco as a metabolic factory.
Since the 2015 season, a new aspect has been investigated: the possibility to detect early infection of some plant diseases, e.g. tobacco blue mold (*Peronospora hyoscyami* s.sp. *tabacina* Skalický), by non-invasive detection of selected compounds acting as phytoalexin, a mechanism already described in Vitis vinifera for stilbenes and Plasmopara viticola.

This reasearch has been funded by Consortium INSTM. Some data of the 2013-2014 destructive analyses were taken from the Regione Umbria's QualiTaba Project, project leader Fattoria Autonoma Tabacchi. (Reprinted with permission)

Key terms: Portable sensors, Fluorescence-based sensor, Multiplex, tobacco, metabolic factory

12. Effects of light intensity on soluble sugars and organic acids in tobacco leaf (*Nicotiana tabacum* L.). B. Cai; Zhao, H.; Cai K.; Lei, B.; Guizhou Academy of Tobacco Science, Guizhou China

The ecological environment influences the accumulation of chemical components and the formation of regional characteristics of tobacco leaf. Soluble sugars and organic acid play an important role in smoke quality of tobacco products. However, few reports about the effects of light intensity on soluble sugars and organic acid have been published. To study the effects of sunlight on the accumulation of soluble sugars and organic acid, tobacco grown in the field was shaded with sunshade net, and sampled at the harvest time and post-cured period. The samples were then measured for soluble sugars and organic acid. The results showed that except for sucrose in leaf at harvest, reduced light led to lower glucose, fructose and sucrose level in leaves at harvest or post-cured. As for organic acid, reduced light intensity would decrease all the organic acid measured in this study in leaf at harvest. Reduced light intensity led to higher oxalic acid, malic acid and citric acid in flue-cured leaf; while lower light intensity resulted in lower Palmitic acid, linoleic acid, stearic acid, mixture of linolenic acid, and oleic acid in flue-cured leaf. The results from this study shed light on the formation and development of tobacco leaf with regional characteristics and could be used as the references for the chemical compositions of shade-grown tobacco leaf, such as cigar wrapper. (Reprinted with permission)

13. Flue-cured tobacco layby herbicide evaluation. N. E. Harrell and B. M. Spivey, North Carolina Cooperative Extension Service, Wilson NC USA

Palmer amaranth (*Amaranthus palmeri*) is a competitive, aggressive, fast growing weed capable of producing over 600,000 seed per plant. At present, high populations exist in the southeastern United States making control a significant challenge for growers in all field crops, including tobacco. In addition, Palmer amaranth has developed herbicide resistance to several herbicide chemistries, including glyphosate, acetolactate synthase (ALS) inhibitors, and most recently protoporhyrinogen oxidase (PPO) inhibitors. Grasses, particularly large crabgrass (*Digitaria sanguinalis*), are also issues late-season for tobacco growers. As tobacco is harvested, more sunlight reaches the row middle, which can allow for late season germination of Palmer amaranth and crabgrass. Leaf manufacturers have expressed concern regarding weed seed and vegetation in tobacco exports and consider it non-tobacco related material (NTRM). It is particularly important for growers to control these weeds in their tobacco. To address this issue, North Carolina Cooperative Extension in Johnston and Wilson counties conducted three on-farm tests in 2014 and 2015 to evaluate different herbicides and rates applied at layby for Palmer amaranth and grass control. Herbicides evaluated were pendimethalin, napropamide, S-metolachlor and trifloxysulfuron. Materials were band applied to a single row middle just after layby with a CO2-pressurized backpack sprayer calibrated to deliver 12.6 or 17.2 gallons of solution per acre, depending upon location. Due to low Palmer amaranth density in 2014, large crabgrass was the only weed evaluated for control, with pendimethalin (58-63%, depending upon rate) and S-metolachlor (70%) providing the greatest control at higher application rates. Alternatively, in 2015, due to low large crabgrass density, Palmer amaranth was the only weed evaluated. S-metolachlor provided exceptional control of Palmer amaranth (79%) followed by pendimethalin (35-42%, depending upon application rate). Results from this study will be used to support labeling efforts of new herbicide chemistries for United States tobacco production. (Reprinted with permission)

Key terms: weed control, layby herbicide, *Amaranthus palmeri, Digitaria sanguinalis*

14. Evaluation of non-tobacco labeled herbicides for late season application. W. T. Whaley; Fisher, L. R.; Vann, M.C., North Carolina State University, Raleigh NC USA

Recently, viable seed from various weed species has been found in tobacco exports, initiating great concern in foreign markets and a zero tolerance policy. University Specialists believe the majority of weed seed contamination is a result of mechanized harvest. At present, farmers have a variety of management options, such as cultivation, herbicide application, and hand weeding, to reduce weed pressure and lower seed bank populations for future years. However, the spectrum of herbicide options for tobacco is extremely limited, specifically for post-transplanting

application. An evaluation of non-tobacco labeled herbicides for late season application is greatly needed to give farmers alternative strategies in weed management. Research was initiated at the Lower Coastal Plain Research Station (LCPRS) and the Upper Coastal Plain Research Station (UCPRS) in 2014. Treatments were arranged in a randomized complete block design and replicated four times. Eight different herbicides were evaluated, each at two different application timings: before topping and after first harvest. The eight herbicides were as follows: Dual Magnum, Spartan, Envoke, Reflex, Liberty, Callisto, Lorox, and Aim. A ninth herbicide, Poast, was applied after first harvest only and a control plot of Spartan + Command (Pre-T only) was included as well. Applications were made with a backpack sprayer containing a twenty inch boom and two Teejet VisiFlo flat spray tip nozzles. Application occurred at a spray volume of 20 gallons/acre. Spray applications covered the row middles as well as a portion of the tobacco bed. Product rates were based upon NCSU recommendations. Following application, weed control efficacy, crop injury, leaf yield, quality, value, and chemistry were quantified. In 2014, herbicide injury was observed in before topping applications of Liberty (3.75%), Callisto (12.50%), and Aim (2.50%) at UCPRS; however, leaf yield was not affected. Palmer amaranth suppression ranged from 95-100% following application for all treatments. (Reprinted with permission)

Key Terms: herbicide, weed control, Palmer amaranth

15. The influence of primary tillage and flue-cured tobacco management on Palmer amaranth populations in a three year crop rotation. M. C. Vann; Fisher, L. R., Jordan, D. L.; Stewart, A. M.; Wells, R., North Carolina State University, Raleigh NC USA

Glyphosate-resistant Palmer amaranth (PA) (*Amaranthus palmeri*) is a significant issue in agronomic crop production. Research was conducted from 2012 to 2014 and from 2013 to 2015 to quantify the impact of primary tillage, herbicide program, and prevention of PA seed production over a three year cropping sequence of flue-cured tobacco followed by cotton. Treatments imposed to tobacco were deep tillage (15 inch depth) or shallow tillage (5 inch depth), two herbicide programs (clomazone alone or clomazone plus sulfentrazone), and hand removal or no hand removal of PA. In year one, soybean was planted into plots bordering tobacco using deep and shallow tillage treatments to assess early season PA density between cropping systems. Tillage treatments were not imposed in years two or three of the study. Glyphosate and glufosinate were utilized for POST weed suppression in cotton. Weed counts were conducted two and six weeks after crop establishment to assess PA density within each treatment. Palmer amaranth visible above the late season crop canopy was removed in a single event in each year of the study. Hand weeding time was recorded for economic assessment. Crop yield and value were also recorded after harvest. Deep tillage reduced PA density in soybean and tobacco plots compared to shallow tillage; however, PA density was consistently higher in tobacco, most likely due to row ridging. In the absence of sulftentrazone, deep tillage reduced PA density in tobacco two weeks after transplanting but not at six weeks or in subsequent cotton crops. Sulfentrazone reduced PA density and increased tobacco yield and economic return. In the absence of sulfentrazone, hand weeding increased tobacco yield and economic value. Palmer amaranth suppression in cotton was improved in treatments where sulfentrazone and/or hand weeding were utilized in previous season(s). Hand weeding did not affect net economic return. (Reprinted with permission)

Key Terms: herbicide, Palmer amaranth, tillage, weed control

16. Effect of artificial ordering on TSNAs in burley tobacco during short term bulking between takedown and stripping. C. R. Fisher; Jack, A.; Bush, L.; Pearce, R., University of Kentucky, Lexington KY USA

Production guidelines caution burley tobacco growers against using water sprays to bring tobacco into case and storing high-moisture tobacco because of concerns that these practices can lead to the accumulation of TSNAs. A series of experiments over three years was done to test whether this is indeed the case. Low and a high converter burley tobacco lines were brought into order using several methods: naturally, in a conditioning chamber, with steam

and with a mist or spray of water from a hosepipe. Samples for moisture content, alkaloids, TSNAs and nitrate and nitrite nitrogen were taken at takedown, before bulking and after 14 days in the bulk. The method of ordering did not cause an increase of TSNAs in any of the tests. After 14 days in a bulk, there was no increase in TSNA accumualtion as the moisture content of the tobacco increased. The method of application however, did make a difference to moisture content of the tobacco, more because of the difficulty of distributing the water evenly if a coarse spray or even a mist were used. Takedown and stripping in Kentucky is typically done between November and February when the average low temperatures are just below freezing and the average high temperatures are 40 to 45ºF (4 to 7ºC). In warmer production areas, especially those with higher humidity, there could still be some effect of ordering on TSNA accumulation, especially at higher moisture contents. (Reprinted with permission)

17. Lower leaf removal to eliminate lower stalk grades in flue-cured tobacco. L. R. Fisher; Vann, M. C.; Brown, A. B.; Inman, M. D., North Carolina State University, Raleigh NC USA

Research was conducted to evaluate removal of four or eight leaves at topping and the effect on yield, quality, leaf chemistry, crop throw, and value per acre. Removal of four leaves resulted in minimal loss of yield and value, but did not consistently eliminate P and X grades. Removal of eight leaves consistently reduced yield and value and generally reduced P and X grades. Leaf removal had no effect on leaf chemistry. Research in 2016 will evaluate agronomic practices to improve yield when the bottom eight leaves are removed. (Reprinted with permission)

Key Terms: leaf removal, grade distribution, yield, value.

18. Evaluation of a flue-cured tobacco bulk barn exhaust air heat recovery system. J. A. Macialek; Ellington, G. H., North Carolina State University, Raleigh NC USA

Recovering the waste heat during the curing process for flue-cured tobacco has been researched in the past utilizing the energy in the exhaust air to preheat the colder intake air. The exhaust air vented from a curing barn can range in temperature from 100 to 165ºF and range in relative humidity from 15 to 90%. The exhaust air heat recovery system utilized during the 2014 and 2015 season reassess the economic feasibility of this strategy primarily due to increased airflow and leaf capacities of modern barns and current fuel prices. The system consisted of a heat exchanger (recovery coil) in the exhaust air stream at the front of the barn connected to an additional heat exchanger (preheat coil) in the fresh air stream located in the rear of the barn. A fractional horsepower pump circulates water between the two heat exchangers in a closed loop system. Fuel and kilowatt-hour meters were installed to monitor the total energy usage from the barn implemented with the system and an identical make barn without the system for direct comparisons. Spatial air temperature data was collected along with water temperatures to assess the system performance. Averaged over ten cures during the 2014 season, the barn implemented with the heat recovery system consumed approximately 228 gallons of LP gas per cure compared to 278 gallons for the check barn. An additional benefit of the heat recovery system is that the burner cycles on and off fewer times extending the life of the heat exchanger and burner. (Reprinted with permission)

Key terms: energy, heat recovery, heat exchangers

19. On-farm performance assessment of new curing barns. G. Ellington; Macialek, J.A., North Carolina State University, Raleigh NC USA

Total energy consumption and other performance related information from four different make of new curing barns was collected at the same on-farm location. Direct comparisons were made with two existing make barns. The primary objective was to provide growers with independent on-farm performance-based information to assist them with curing infrastructure decisions. The season average curing efficiency for the new barns was 14.5 lb cured leaf / gal LP gas

compared to 11.6 lb cured leaf / gal LP gas for the existing barns. This is approximately a 25% increase in energy efficiency (fuel only) and as a result a cost reduction of approximately 2¢ per pound cured based on 2014 fuel prices. Averaged over the season, the curing time for the new barns ranged 6.5 days to 7.8 days. The two existing barns averaged 8.6 days and 7.1 days, respectively. The largest new barn leaf capacity was approximately 50% more than the two existing barns. A reduction in curing time combined with an increase in leaf capacity will result in fewer barns needed for a given acreage. Grower feedback indicated limited differences, if any, observed in the cured leaf quality and management requirements between the new barns. Although energy performance alone is not the justification for selecting a given make barn, it is an important factor because of the production costs associated with curing. Other factors include the new barn cost, leaf capacity, serviceability and maintenance requirements. This independent information can assist all growers with curing infrastructure decisions. Additionally, performance-based comparisons and manufacturer competition can result in engineering innovations and barn improvements to better serve growers and the industry. (Reprinted with permission)

Key terms: curing, energy, efficiency, barn

20. **A proposed efficiency testing protocol for tobacco bulk curing barn heat exchangers.** T. D. Reed, Virginia Tech, Blackstone VA USA

The flue-cured tobacco industry in the U.S. conducted a retrofit program between the 1999 and 2000 growing seasons to convert bulk curing barns from direct-fired to indirect-fired heating. This was necessary to remove the products of combustion from the curing air space and thus avoid the formation of tobacco specific nitrosamines (TSNAs) resulting from the reaction of nitrous oxides and secondary alkaloids present in the tobacco leaf. In the vast majority of cases this process was accomplished through the replacement of traditional LPG fueled ring burners with heat exchangers and a new gas burner. The result was a substantial reduction in the overall level of TSNAs present in flue-cured tobacco. Heat exchangers must be periodically tested to ensure structural integrity and continued elimination of combustion gases. Many heat exchangers have required repair and some have been repaired multiple times as units age and thus more prone to failure. The condition of some heat exchangers currently in service warrants consideration of replacement.

Growers should consider the efficiency of a heat exchanger when replacement of a unit is necessary. Thought was not given to this factor during the earlier retrofit program. The thermal efficiency of heat exchanger has only recently been discussed with the availability of energy conservation cost share funding for replacement with high efficiency heat exchangers. However, a procedure for evaluating heat exchanger in place in a tobacco curing barn is needed to provide quantitative ratings of heat exchangers and allow for comparisons. A protocol for heat exchanger testing will be described and comparative data of different heat exchangers presented. (Reprinted with permission)

21. Using exogenous auxin application to control axillary shoot development in burley tobacco. W. J. Carmack; Miller, R. D.; Smalle, J. A., University of Kentucky, Lexington KY USA

The use of hydroponically produced tobacco (*Nicotiana tabacum* L.) transplants has been widely adopted by tobacco producers. There are many advantages to using hydroponic tobacco transplants; however, one disadvantage is their increased propensity to form basal axillary buds or shoots, often referred to as "ground suckers". There is no clear understanding as to why ground suckers are so much more prevalent in hydroponically produced transplants. One possibility is that there is a difference in levels of specific plant hormones between traditional soil-grown transplants versus hydroponically produced transplants. Of particular interest is the ratio of auxins, which are produced primarily in apical meristems and inhibit axillary shoot formation, to cytokinins, which are primarily produced in the roots of plants and promote lateral branching. The root to shoot ratio in hydroponically grown transplants is much greater compared to traditional transplants, which may result in a significantly different auxin to cytokinin ratio. A study was conducted to investigate whether the addition of synthetic auxins or cytokinins to hydroponic solutions used for

transplant production could significantly affect ground sucker formation. Greenhouse grown plants of burley cultivars TN 86 and Hybrid 403, which have high and low propensities to produce ground suckers, respectively, were treated with 2, 10, or 50 nM concentrations of the synthetic auxin1-Naphthalene acetic acid (NAA) or the synthetic cytokinin 6-Benzylaminopurine (BA). Preliminary results indicated that the 2nM concentration of NAA resulted in a significant reduction in axillary shoot formation in TN 86 compared to the control solution that contained no added NAA or BA. Conversely, for Hybrid 403, all concentrations of NAA or BA resulted in increased axillary shoot formation in comparison to the control. Additional greenhouse and field studies are currently underway to confirm or refute these preliminary findings. (Reprinted with permission)

22. Chemical topping of burley tobacco. M. D. Richmond; Bailey, W. A.; Pearce, R. C., University of Kentucky, Lexington KY USA

The act of topping tobacco (*Nicotiana tabacum* L.) involves the removal of the terminal bud or inflorescence of the tobacco plant. This practice ordinarily is accomplished by manually removing the top of each tobacco plant in an entire field which is labor intensive and costly. A study was initiated in 2015 at the UK Agriculture Experiment Station near Lexington, KY and the Murray State University West Farm near Murray, KY to evaluate chemical topping in burley tobacco. This study builds on preliminary work performed in the last two years. The objectives of this study are to determine if burley tobacco can be chemically topped, the appropriate rate of chemicals, optimum timing of application, and appropriate burley tobacco varieties for this practice. To pursue our objectives, a study was designed with burley tobacco varieties TN90 (medium maturity), KT210 and KT215 (late maturity) to determine what maturity and timing of application is most effective. A second study was initiated to investigate the efficacy of suckericide applications using combinations of maleic hydrazide (MH), butralin, and fatty alcohols. These studies focus on the impact of treatments on the agronomic and quality traits of burley tobacco. All treatments that included maleic hydrazide had significantly less total weight and average weight of suckers per plant regardless of manual topping or chemical topping, compared to the untreated check. Early observations suggested that pre-bud (10% button) and early-bud (50% button) timings are best suited for chemical topping practices. Treatments that targeted 10% bloom stages did control sucker growth but did not completely halt inflorescence growth. Impacts on leaf yield and preliminary observations for the agronomic portion of these experiments will be discussed. (Reprinted with permission)

Key terms: chemical topping, burley tobacco

23. Comparison of sucker control methods for dark fire-cured tobacco. W. D. Pitt; Bailey, W. A.; Sims, B. D.; Walker, E. R., University of Tennessee, Springfield TN USA

Dark tobacco is known to be have more vigorous sucker growth than burley tobacco, and is also more prone to crooked stalks and lodging from wind damage, which makes chemical sucker control more difficult. Standard dark tobacco sucker control programs are still based on manual stalk rundown applications of contact fatty alcohols and local systemics made with drop line applicators or backpack sprayers. These manual application methods require between 15 and 20 man-hours of labor per acre depending on the condition of the tobacco. A major focus of dark tobacco sucker control research has been to find improved methods to mechanically apply suckercides to reduce these labor requirements, and also find best use practices for maleic hydrazide (MH) in dark tobacco. Unlike other types of tobacco grown in the U.S., dark tobacco sucker control programs do not rely on maleic hydrazide (MH). Although MH use has increased in dark tobacco in recent years, its use is still limited and it is a secondary component of dark tobacco sucker control programs. When MH is used close to topping as is standard in burley tobacco, excessive yellowing of upper leaves and potentially lighter colored cured leaf can result. Research has shown that if MH application is delayed until no sooner than 5 days after final topping, and a reduced rate of MH is applied at that time (1.88 lbs MH per acre for dark tobacco instead of the standard 2.25 lbs MH per acre used for burley tobacco), marketable leaves will show minimal or no effects of MH application. However, this delay in MH application

requires that at least two applications of other suckercides still be made before MH is applied. This presentation will summarize 12 years of dark tobacco sucker control research that has been conducted in Kentucky and Tennessee. (Reprinted with permission)

Key terms: dark tobacco, sucker control

24. Maleic hydrazide residues resulting from applications at three times of day and three rates of MH. J. M. Moore and S. LaHue, University of Georgia, Tifton GA USA

The presence of significant residues of maleic hydrazide (MH) in cured tobacco produced in the US continues to be of concern to tobacco leaf dealers and manufacturers who purchase tobacco or manufacture tobacco products for export. Globally, the tobacco industry evaluates MH residues based on the established CORESTA guideline residue levels (GRL) for MH of 80 ppm. Over the last 25 years, changes in the application techniques and amounts of MH applied have resulted in lower residue levels. There still appear to be factors in addition to the application rate of the chemical that affect the resulting MH residues.

Trials were conducted on the University of Georgia Tifton Campus Bowen Farm to evaluate MH application with variables including application at each of three times of day (7 am, Noon, 4 pm) and application at each time of day of three MH rates (1.12 lb ai, 2.25 lb ai, 3.375 lb ai). MH residues were determined from repeated sampling of green leaves from the top of the tobacco plants starting the day after application and following each successive rainfall event. Samples were also analyzed from the cured leaf from each of four harvests. Sucker control was evaluated for each treatment based on sucker counts and weights prior to the final harvest.

Results of the MH residue samples analyzed showed a strong correlation between early day application and lower residue levels at each of the MH application rates. Residues were reduced by more than half with the first rainfall which removed some of the unabsorbed MH from the leaf surface regardless of the application time and amount applied. Successive rainfall events reduced the MH residue level further. (Reprinted with permission)

Key terms: sucker control, maleic hydrazide, MH residues

25. Evaluation of androgenic and gynogenic doubled haploid lines for use as parental lines for hybrid burley tobacco varieties. R. D. Miller and E. DeOliveira, University of Kentucky, Lexington KY USA

Ten androgenic haploid (ADH) lines and ten gynogenic haploid (GDH) lines, developed via anther culture and an interspecific cross with *Nicotiana africana*, respectively, were derived from the inbred burley tobacco (*Nicotiana tabacum* L.) cultivar TN 90LC. Eight ADH and four GDH lines were also derived from burley parental line GR 149LC. Mid-vein culture was then utilized to double the chromosome number of each haploid line. For TN 90LC, each of the ten ADDH and ten GDDH doubled haploid lines were randomly paired with TN 90LC to form a triplet. The ten TN 90 triplets were evaluated for agronomic traits at three locations utilizing a randomized complete block with three replications. The GR 149LC ADDH and GDDH lines were evaluated in a similar fashion. For TN 90LC, on average the ADDH lines yielded 3.9% less and the GDDH lines yielded 1.9% more than the inbred source. For GR 149LC, the average yields of the ADDH lines, GDDH lines, and inbred source were 3357, 3343, and 3351 Kg/ha, respectively. Individual ADDH and GDDH lines that produced yields equal to or greater than their inbred source were identified for both TN 90 and GR 149. Each of the ADH and GDH TN 90 lines was crossed with TKS 2002LC, the female parental line of the hybrid variety KT 204LC. Similarly, each of the GR149 ADH and GDH lines was crossed with ms TN 90LC, the female parental line of the hybrid variety TN 97LC. The hybrid varieties were then evaluated in the same manner described for the parental lines. For both the KT 204LC and TN 97LC families, no yield differences were observed in hybrids having either ADDH or GDDH lines as the male parent in comparison to hybrids having the original inbred line as the male parent. (Reprinted with permission)

Key terms: Haploids, doubled haploids, androgenic, gynogenic

26. Optimal generation to develop tobacco doubled haploids when selecting for quantitatively inherited disease resistance. R. D. Miller and E. DeOliveira, University of Kentucky, Lexington KY USA

Doubled haploidy (DH) is a plant breeding strategy used to decrease the time required to reach genetic homozygosity. In order to obtain homozygosity as quickly as possible, DH tobacco (*Nicotiana tabacum* L.) lines are usually generated from greenhouse grown plants in the F1 generation. Although this is the quickest way to reach homozygosity, it does not allow for selection for quantitatively inherited disease resistance in a segregating breeding population. When quantitatively inherited disease resistance is the primary objective of the breeding project, the DH process may be more effective by utilizing only F2 plants displaying good resistance in nurseries having high levels of the disease of interest. In the current study, DH lines were obtained from two crosses where the target was high race 1 black shank resistance. For cross one, ten anther derived (ADH) and ten *Nicotiana Africana* derived (GDH) lines were developed from F1 plants grown in the greenhouse, and ten ADH and five GDH lines were developed from F2 plants displaying high disease resistance in a field nursery having high levels of race 1 black shank. For cross two, ten F1 ADH, eight F1 MDH, ten F2 ADH, and seven F2 MDH lines were developed. When the resultant DH lines were evaluated in three race 1 black shank nurseries, significant differences for disease incidence twelve weeks after transplanting were detected between F1 versus F2 generated ADH and GDH lines for both crosses. For cross 1, the average disease incidence for F1 generated DH lines was 29.1%, compared to 10.3% for F2 generated lines. For cross two, disease incidence was 44.3% and 12.4% for F1 versus F2 generated lines, respectively. Although a low frequency of highly resistant lines were observed among the F1 generated DH lines, their incidence was much higher among F2 generated lines. (Reprinted with permission)

Key terms: Doubled haploids, race 1 black shank resistance, Fusarium wilt resistance

27. Field evaluations of burley tobacco lines containing alleles minimizing nicotine to nornicotine conversion. C. G. Shelton and R. D. Miller, University of Kentucky, Lexington KY USA

CYP82e4, CYP82e5, and CYP82e10 alleles that minimize the conversion of nicotine to nornicotine have been introgressed into numerous burley tobacco (*Nicotiana tabacum* L) varieties and parental lines developed by the Kentucky-Tennessee Tobacco Improvement Initiative (KTTII). A backcross breeding protocol was utilized, with the objective being the creation of "e3" varieties that differ only for nornicotine and nitroso-nornicotine content in comparison to the original low converter (LC) varieties. Field studies were conducted in Kentucky and Tennessee during the 2013 growing season where multiple iterations of seven KTTII parental lines, and four iterations of five hybrid commercial KTTII varieties, were grown and compared to their original LC counterparts. Considerable variation for agronomic and/or race 1 black shank resistance was detected among the iterations within specific breeding line and hybrid families. After selection and/or additional backcrosses within parental line families during 2013, e3 parental lines and varieties were again compared with their LC counterparts in 2014 field trials. Much less variation was detected between the e3 and LC versions of breeding lines and varieties in the 2014 field trials. The e3 alleles proved to be very effective in reducing the conversion of nicotine to nornicotine, resulting in a reduction in the formation of nornicotine, and the concomitant formation of nitroso-nornicotine, by approximately 85-90%. As a result of this project, e3 versions of TN 86, KT 209, KT 210, and KT 212 were released as commercial varieties in March, 2015, and it is anticipated that e3 versions of TN 90, KT 204, and KT 206 will be released in March, 2016. (Reprinted with permission)

Key terms: Conversion, nornicotine, nitroso-nornicotine

28. Breeding for increased nicotine content in tobacco. R. S. Lewis, North Carolina State University, Raleigh NC USA

A considerable amount of research has been conducted to develop genetic methods for reducing nicotine content in tobacco. There may also be roles for tobacco genotypes possessing genetic variability for increased nicotine content, however. For example, germplasm with increased nicotine content may be useful for (1) development of higher-yielding tobacco cultivars with alkaloid levels desired by the industry, (2) manufacture of 'next-generation' tobacco products, or (3) maximizing nicotine levels for the purpose of extraction. Working towards the goal of transferring novel allelic variability affecting increased alkaloid accumulation to flue-cured tobacco, we initially hybridized a NCSU flue-cured tobacco breeding line with tobacco germplasm lines TI464 and TI959 previously identified as producing high alkaloid levels relative to their yielding abilities. Selection was practiced among and within families during four generations of backcrossing for desirable combinations of high alkaloid accumulation and high yield levels. Phenotypically stable lines were produced by subsequent self-pollination and tested for yield and alkaloid content. A high number of lines with unique combinations of high nicotine content and yielding ability were identified. These conventionally-derived materials were also compared in field tests with transgenic tobacco lines genetically engineered to overexpress NtMYC2a, a transcription factor affecting the expression of genes involved in alkaloid biosynthesis. The percent increase in nicotine content realized through conventional breeding was comparable to that achieved using genetic engineering. (Reprinted with permission)

Key Terms: nicotine, tobacco, breeding

29. Working towards an increased understanding of the genetics of soil-borne disease resistance in tobacco. K. Drake; and Lewis, R. S., North Carolina State University, Raleigh NC USA; Bakaher, N.; Goepfert, S.; Philippon, B.; and Mark, R.; Philip Morris International, Neuchatel, Switzerland; Peterson, P. D; Clemson University, Florence SC USA

Black shank and bacterial wilt, caused by *Phytophthora nicotianae* and *Ralstonia solanacearum*, respectively, are the most important diseases affecting tobacco production in the United States. An increased understanding of the genetic systems controlling resistance to these diseases, as well as identification of DNA markers associated with genomic regions controlling this resistance, could aid in the development of improved varieties. Flue-cured tobacco cultivar 'K346' exhibits high levels of resistance to both diseases. In effort to understand the genetics of soil-borne resistance in this cultivar, we developed a recombinant inbred line (RIL) mapping population consisting of 186 lines derived from a cross between K346 and disease-susceptible tobacco accession 'TI1068.' The population was genotyped with more than 300 microsatellite markers and evaluated for two years for field resistance to both black shank and bacterial wilt. Several genomic regions of K346 origin were found to affect resistance to both pathogens, a finding that may at least partially explain previously observed correlations between resistance to black shank and bacterial wilt among current cultivars and within breeding populations. QTLs discovered to affect disease resistance in K346 were compared to those previously identified for 'Florida 301' and 'Beinhart 1000.' (Reprinted with permission)

Key Terms: tobacco, breeding, black shank, bacterial wilt

30. Generation, evaluation, and use of tobacco lines nearly isogenic for QTLS affecting black shank resistance. J. Ma, Lewis, R. S., North Carolina State University, Raleigh NC USA; Edwards, K.; Humphry, M.; British American Tobacco, United Kingdom

The black shank disease, caused by *Phytophthora nicotianae*, is typically the most economically-important disease affecting tobacco production in the United States. Host plant resistance is the most effective and cost-efficient means of control if the resistance can be incorporated into new cultivars without sacrificing yield and/or quality

characteristics. Marker-assisted identification of novel quantitative trait loci (QTL) affecting non- race-specific resistance to this disease might assist breeders in efforts to increase the range and level of black shank resistance in future tobacco cultivars. Previous mapping work identified two major resistance QTL on linkage groups 7 and 15 that contribute to the very high level of resistance in cigar tobacco cultivar 'Beinhart 1000.' Marker assisted backcrossing into the BC4 and BC6 generations has been used to generate versions of 'Hicks' and 'K326' that are nearly isogenic for one or both of these two major QTL. These NILs have been evaluated in field experiments for multiple years, and data indicate that these QTLs provide moderate levels of resistance, with a synergistic effect between the two loci. Efforts to fine map the genomic positions of these two QTL will be discussed. (Reprinted with permission)

Key Terms: tobacco, breeding, black shank, marker assisted selection, *Phytophthora nicotianae*

31. The demethylase mutants - panacea or new problems? A. M. Jack; Ji, H.; Fannin, N.; Fisher, C.; Schoergendorfer, A., University of Kentucky, Lexington KY USA

The demethylase mutants contain knockout versions of three genes controlling nicotine to nornicotine conversion (CYP82E4, CYP82E5v2 and CYP82E10). These triple mutants have many advantages; lower conversion, lower NNN, cheaper and easier identification, elimination of expensive seed screening. However, there are several potential disadvantages. As a result of the lower conversion, nicotine levels in the mutants can be too high; three triple mutant lines failed the RQT (Regional Quality Test) on nicotine in 2013. The enantiomeric ratio is different in ultra-low converter lines, with a higher ratio of the more toxic S isomer of both nornicotine and NNN; this could result in higher absolute levels of S-NNN. We have developed a method to measure absolute amounts of R and S-NNN; previously, it was only possible to measure the ratio. It is also possible that the mutations could have an adverse effect on plant growth. In this study, we compared single (e4), double (e4e5) and triple (e4e5e10) mutants with the RNAi transgenic and equivalent wild types. Nicotine was not significantly higher in the triple mutants and transgenic than in the LC equivalent, nor was the absolute amount of S-NNN, despite the higher ratio of S-NNN. There were some consistent differences in growth parameters. The triple mutants generally had longer, narrower leaves and were taller with fewer leaves. The triple mutant and transgenic were later flowering with longer internodes in one variety but not in the other. In general, we conclude that the advantages of these triple mutants outweigh the potential disadvantages; they have a marked potential for harm reduction because the S-NNN, as well as the total amount of NNN, is greatly reduced in the triple mutants. (Reprinted with permission)

32. Oxathiapiprolin, a new fungicide active ingredient (a.i.) for control of diseases caused by Oomycetes. V. Mascarenhas; Kuhn, P.; Mclean, H.; Tally, A.; Teeter, K.; Lowder, S., Syngenta Crop Protection, Greensboro NC USA

Oxathiapiprolin is a new fungicide active ingredient (a.i.) that provides excellent control of important Oomycete diseases including black shank (*Phytophthora nicotianae*) on tobacco. The mode of action is novel and mediated via interaction with an oxysterol binding protein with an essential, but as yet uncharacterized, function. Oxathiapiprolin has been assigned FRAC Code U15. With high potency and a single site mode of action, there is the potential for development of resistance to the fungicide. In order to mitigate this possibility, label directions will incorporate strict stewardship guidelines that include a limitation on the number of applications containing oxathiapiprolin to one third of the total sprays in a program. Field trials over the last three years have focused on evaluation of different rates of oxathiapiprolin (35-140 gai/ha) applied solo or in mixture with Ridomil Gold® SL (RGSL), in transplant water or at first cultivation or at layby. In programs with two or three applications overall, the remaining sprays were RGSL solo. Programs that included oxathiapiprolin provided black shank control as good or better than competitor treatments (RGSL; Presidio®) and with good crop selectivity. Federal registration of oxathiapiprolin is anticipated during the third quarter of 2015, with full launch early in 2016. A family of products based on oxathiapiprolin and under the tradename ORONDIS™ will be available to growers. Tobacco is included in the first wave of crops. In

addition to black shank, oxathiapiprolin will also be labeled on tobacco blue mold (*Peronospora tabacina*). (Reprinted with permission)

Key terms: Oomycete, disease control, fungicide, oxathiapiprolin, Orondis

33. Black shank control in Virginia using Ridomil Gold and/or Presidio and/or Orondis in 2014 and 2015. C. S. Johnson, Virginia Tech, Blackstone VA USA

Field experiments were conducted at the Southern Piedmont Agricultural Research and Extension Center in 2014 and 2015 to compare black shank control on burley tobacco cultivar NC BH129 following one to three applications of Ridomil Gold (mefenoxam) and/or Presidio (fluopicolide) and/or Orondis (oxathiapiprolin). The field used was naturally-infested by the black shank pathogen (*Phytophthora nicotianae*), has been in continuous tobacco culture since 1974, and contains populations of pathogen races 0 and 1. Both experiments were arranged in a randomized complete block design with 6 replications. Other than an untreated control (UTC), each experimental treatment included one to three fungicide applications in an "application program" that often involved alternating use of Ridomil Gold, Presidio, or Orondis. Fungicides were applied as transplant water (TPW) treatments or field sprays at the first cultivation (1C - ~2 weeks after transplanting) and/or at layby (L) in 2014. Broadcast preplant –incorporated (PPI) sprays were also included in the 2015 study. All fungicide programs increased plant survival in 2014 compared to the UTC, but survival to harvest was lower than 90% only where Ridomil Gold was used TPW and again 1C or TPW. Disease control was over 95% for all programs that included three fungicide applications, and for programs that included two applications of Orondis and/or Presidio. In 2015, black shank control was not significantly better than the untreated control when Ridomil Gold was applied TPW and at 1C or L, when Presidio was only applied TPW, or when Presidio was applied 1C after Ridomil Gold TPW. Disease control was highest in 2015 when Presidio was applied TPW followed by Orondis or alternated with Orondis or Ridomil Gold at 1C followed by a final Presidio application at L. Both Presidio and Orondis appear to be promising new additions to Ridomil Gold as black shank fungicides. (Reprinted with permission)

34. Evaluation of Presidio and Ridomil Gold for black shank control in dark fire-cured tobacco. A. B. Keeney; Bailey, W. A.; Rodgers, J. C.; Hill, R. A., University of Kentucky, Princeton KY USA

The dark tobacco production region of western Kentucky and Tennessee sustained major losses to black shank (*Phytophthora nicotianae*) in 2014. Since black shank resistance is relatively low in dark tobacco varieties compared to other tobacco types, growers rely heavily on rotation and fungicide use for black shank management. Presidio (fluopicolide) was registered for use in 2015. Presidio may offer an alternative to Ridomil Gold and may also compliment Ridomil Gold in black shank management programs. A field trial was conducted at an established black shank site near Hopkinsville KY in 2015. The trial was a split plot design with variety as main plots and fungicide treatment as sub-plots. Variety main plots were 'Narrowleaf Madole' (no black shank resistance) and 'KT D6' (moderate black shank resistance, 3/10 to race 0 and 3/10 to race1). Fungicide treatments included Presidio at 4 oz/A in simulated transplant water (TPW) applications, Presidio at 4 oz/A TPW followed by (fb) Ridomil Gold at 16 oz/A as a banded application at first cultivation (CULT) fb Presidio at 4 oz/A at last cultivation (LAYBY), Ridomil Gold at 8 oz/A TPW fb Presidio at 4 oz/A CULT fb Ridomil Gold at 16 oz/A LAYBY, or Ridomil Gold at 8 oz/A TPW fb 16 oz/A CULT fb 16 oz/A LAYBY. An untreated check for each variety that received no fungicide application was also included. Although there was some variability in black shank pressure at this location in 2015, Presidio appeared to provide black shank control in both Narrowleaf Madole and KT D6 that was at least equivalent to Ridomil Gold when used alone or in combination with Ridomil Gold. (Reprinted with permission)

Key terms: dark fire-cured tobacco, black shank, fungicides

35. Adaptation by *Phytophthora nicotianae* to partial resistance in tobacco. K. L. McCorkle; Lewis, R. S.; Shew, H. D., North Carolina State University, Raleigh NC USA

Black shank, caused by *Phytophthora nicotianae*, is an important disease of tobacco worldwide. The most cost effective tool for control of black shank is host resistance, but resistance may be overcome by pathogen adaptations such as the rapid development of race 1 that quickly followed extensive deployment of the Ph gene. Loss of Ph gene efficacy for the management of black shank prompted a new look at other sources and types of resistance. Partial resistance provides various levels of protection to all races of the pathogen, but severe losses may still occur. Currently, all partial resistance in commercial tobacco varieties is from variety 'Florida 301'. Variety 'Beinhart 1000' is under investigation as an additional source of partial resistance in new varieties of tobacco. Since resistance genes place selection pressure on the pathogen, the objectives of our study were to determine how isolates adapt to varying sources and levels of partial resistance and if adaptations are specific to the source. Ten tobacco lines with resistance from Florida 301, Beinhart 1000, or single genes Wz or Php were grown in the greenhouse. Plants were inoculated with race 0 and race 1 isolates for six continuous generations. Adapted isolates were compared across generations for each variety and pathogen race combination. An increase in pathogen aggressiveness was observed over generations for both races on multiple varieties. More aggressive isolates had decreased incubation periods (IP) and increased percent root rot (%RR). Observed changes in IP and %RR were gradual for all varieties except when the pathogen was exposed to tobacco with the Wz gene; when exposed to the Wz gene, there was a rapid change in IP and %RR between generations two and three. Understanding how *P. nicotianae* adapts to resistance will improve recommendations for variety rotation to manage the pathogen and the disease. (Reprinted with permission)

Key terms: Aggressiveness, host resistance, pathogen adaptation, Wz

36. Nicotiana natural products inhibit black shank disease in tobacco. A. Mihaylova-Kroumova; Artiouchie, I.; Wagner, G. J., University of Kentucky, Lexington KY USA

Black shank disease, caused by the fungus *Phytophthora parasitica* var. *nicotianae*, is a major annual threat to all types of tobaccos worldwide. Thus far, the main approaches to controling this disease are the creation of resistant tobacco varieties and fungicide treatments. Some fungicides are reported to have negative effects on the environment. The goal of this work was to test the antifungal activity of several natural products that are synthesized by tobacco and other Nicotiana species, and secreted to the leaf surface. We hypothesized that cis-abienol, sclareol, labdenediol and phylloplanin can suppress race zero and race one-caused disease in burley tobaccos KY14 and KY14xL8. cis-Abienol, labdenediol and phylloplanin were collected from the tobacco leaf surface. Spores were prepared in vitro. The chemicals then spores were applied as separate drenches at the base of the stems of greenhouse potted plants. Black shank infection was monitored 6 to 13 days post infection. Sclareol was very effective in inhibiting race zero-caused disease in both tobacco lines. In KY14xL8, race one infection was completely inhibited by sclareol while in KY 14 it was reduced by 85% and delayed by six days. cis-Abienol showed high inhibitory properties toward the disease. Race zero infection was completely subdued in KY14, and race one infection was reduced by 80% and 70% in KY14 and KY14xL8, and delayed by six to ten days. Labdenediol reduced the disease by half in eight week-old KY14 plants. Tobacco phylloplanin was not as efficient as the other tested compounds in suppressing the disease. We consider sclareol to be the best candidate for future studies due to its antifungal properties and availability. cis-Abienol, despite its fungicidal action is not practical for large scale experiments. (Reprinted with permission)

Key terms: black shank, natural fungicides, sclareol, cis-abienol, phylloplanin, labdenediol.

37. Investigations into the use of stevia as a rotation crop in tobacco production systems. A. M. Koehler; Shew, H. D., North Carolina State University, Raleigh NC USA

Stevia (*Stevia rebaundiana*) is an emerging crop in the US with potential to serve as a tobacco rotation crop in organic and traditional systems. Stevia is an herbaceous perennial typically grown for 3-5 years with two harvests per season in established plantings. Stevia leaves contain multiple glycosides that are extracted for use as a nonnutritive sweetener which was approved as a food additive by the USDA in 2008. Stevia plantings in North Carolina began in 2011 and are expanding rapidly in the state. Tobacco producers have been the initial growers of stevia due to considerable infrastructure overlap. Stevia is started in tobacco greenhouses, transplanted with modified tobacco transplanters, and dried after harvest in tobacco barns or peanut trailers. The only major production difference is in harvest, where stevia is cut with a combine or similar machinery and removed for drying. Initial screening determined that common tobacco pathogens *Phytophthora nicotianae* (cause of black shank) and *Ralstonia solanacearum* (cause of Granville wilt) are not pathogens of stevia. Wilting and death of stevia plants in first and second year commercial plantings were observed in NC in 2012 and 2013. *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* were observed on diseased plants in the field and Koch's postulates were completed to verify these organisms as pathogens of stevia. TSWV has been observed in stevia in greenhouse plantings, but has not been a problem in field plantings to date. Screening is in progress to determine if stevia is a host for root-knot nematodes. At this time, there are no fungicides labeled for use on stevia, which is a critical limiting factor to expansion of commercial production. Fungicide and biocontrol field trials were initiated in 2014 and 2015 to identify and optimize products with potential to be labeled for control of *S. rolfsii* on stevia. (Reprinted with permission)

Key Terms: rotation crop, organic tobacco, disease screening

38. Multimedia tools for understanding target spot disease dynamics. A. Mendoza-Moran; Shew, H.D, North Carolina State University, Raleigh NC USA

Canceled

39. Simplifying and characterizing a blue mold resistance locus using next generation sequencing. K. Dluge; Dewey, R., North Carolina State University, Raleigh NC USA

Blue mold resistance can be very useful in more tropical areas of the world where the disease is more prevelant. However, one blue mold resistance (BMR) locus originating from *Nicotiana debneyi* has been moved to several commercial burley varieties. This source would appear in more varieties if it did not have a yield penalty associated with plants that are homozygous at this locus, and an observed reduced efficacy when the locus is heterozygous. Recent advances in high-throughput sequencing and the discovery of recombination within the fragment have allowed us to precicly characterize and begin to refine this region. RNA-seq data from near-isogenic burlery lines differing at the BMR locus, as well as an accession of *N. debneyi* and NC 2000 were analyzed to identify genes of *N. debneyi* origin that are located on the foreign introgression region in the blue mold resistant burley isoline. These genes were then used as markers that could be used to possibly find and produce a line where the introgression fragment has been reduced in size, and with reduced size, reduced associated yield drag. The results of the RNA-seq analysis will be discussed within the context of our strategy to produce superior blue mold resistant tobacco cultivars. (Reprinted with permission)

Key terms: Blue mold, Resistance, Introgression

40. Evaluation of fluensulfone for nematode management in flue-cured tobacco. A. S. Csinos; Hickey, H.; LaHue, S.; Hargett, U.; Gine, P. N., University of Georgia, Tifton GA USA

Fluensulfone (Nimitz) is a nematicide being developed by Adama for nematode management in vegetables and row crops. Fluensulfone belongs to a new class of chemistry with favorable toxicological and ecotoxicological profiles. Fluensulfone is a contact nematicide with a mode of action and classification different from other plant protection products. This presentation discusses the evaluation of different rates, and application methods of fluensulfone for management of *Melodogyne arenaria* on tobacco. In addition, several tobacco cultivars that are commercially produced in Georgia were evaluated in a nematode nursery with and without the use of fluensulfone. Trials not only evaluated rates of fluensulfone per acre but also the band width the nematicide was applied to the soil. This provided a comparison of fluensulfone not only at rates of material per acre but also concentration of the nematicide in the root zone. The application of fluensulfone in the transplant water was also evaluated at several rates. High rates resulted in phytotoxicity, but lower rates showed no stunting or phytoxicity, and may have provided nematode damage reduction. (Reprinted with permission)

Key terms: nematicide, tobacco, fluensulfone

41. Effect of fatty alcohol concentration and formulation on mechanical transmission of bacterial wilt at harvest. P. D. Peterson; Fortnum, B.A., Clemson University, Florence SC USA

Bacterial wilt disease caused by *Ralstonia solanacearum* continues to be a serious impediment to the successful production of flue-cured tobacco in the southeast U.S.A. Mechanical transmission of the bacterium during leaf removal plays a significant role in the spread and severity of the disease. As a multi-pass mechanical harvester removes infected leaves in the field, the bacterium contaminates defoliators, gleaners and leaf guides. These contaminated harvester components then transfer the bacterium to adjacent healthy plants. Previous modifications to the harvester design reduced stem injury and transmission of the bacterium. The replacement of stationary rubber leaf guides with a continuous rubber belt system showed less stem bruising and reduced mechanical transmission and infection of stalk tissues in replicated field studies. The objective of the present trial was to evaluate the use of the continuous rubber belt system in combination with C8 + C10 and C10 fatty alcohols on the mechanical transmission of *R. solanacearum* during harvest. Plants (K196) were grown under standard agronomic practices. Plots consisted of rows 15.2 m long with a 1.2 m row spacing. Experimental design was a randomized complete block with four replications and repeated in time. Inoculation was performed by misting harvester defoliators and gleaners with a bacterial suspension before harvesting individual rows. Fatty alcohol concentrations and formulations were 4, 8 and 16% of C8 + C10 (1 octanol and 1 decanol mixture, Off Shoot T EC) or C10 (N-decanol, Antak EC). The continuous belt system alone reduced mechanical transmission of *R. solanacearum* over the stationary leaf guide. Application of Off Shoot T EC and Antak EC significantly reduced the disease index and stem necrosis (P = 0.05) over the belt alone and the stationary leaf guide. (Reprinted with permission)

Key terms: bacterial wilt, fatty alcohols.

42. Novel compounds for the control of *Ralstonia solanacearum*. H. T. Tseng; Mila, A. L., North Carolina State University, Raleigh NC USA

Bacterial wilt, caused by *Ralstonia solanacearum* (Rs), is a devastating disease in tobacco growing regions of North Carolina. The only available chemical control option is the use of fumigants, whereas alternative options may require sophisticated approaches that utilize the well-studied Rs physiology system. Specifically, Rs utilizes quorum sensing, a chemical communication process bacteria use to regulate gene expression at the community level, to regulate the expression of important virulence factors. In this study, three chemical compounds that are known to interfere with quorum sensing of other pathogenic bacteria were examined: 3-indolyacetonitrile (IAN), p-benzoquinone (pBQ), and

6,7-dihydroxycoumarin (6-7-D). Specifically, their effects on bacterial growth, colony morphology, and secretion of plant-cell-wall-degrading-enzymes (CWDEs) were examined. The minimal inhibition concentration against Rs for each compound was determined to be 100µg/ml (IAN), 25µM (pBQ), and 125µM (6-7-D). Sub-inhibitory concentrations, that is 25µg/ml (IAN), 12.5µM/ml (pBQ), and 50µM/ml (6-7-D) were used to determine growth rates under broth culture condition. IAN was most effective in inhibiting bacterial growth, while pBQ and 6-7-D were less effective at sub-inhibitory concentration. No difference in colony morphology was found when Rs was grown on plate culture amended with sub-inhibitory concentrations of the three compounds. The relative activity of two CWDEs, polygalacturonase (Pgl) and endoglucanase (egl), were examined on assay plates amended with 25µg/ml (IAN), 12.5µM/ml (pBQ), and 50µM/ml (6-7-D) respectively. No differences in the relative activity of the enzymes were observed on assay plates. Tobacco cultivar K326 and Speight168 were inoculated with Rs strain W7 and treated with the compounds 48-hours before, at the same time, and 48-hours after inoculation to evaluate their effect on disease incidence. Results from this study could provide insights on potential alternative management options of bacterial wilt. (Reprinted with permission)

43. Sensitive detection of *Ralstonia solanacearum* in tobacco-planting soil with locked nucleic acid-based quantitative real-time PCR assay. Hu, L. W.; Xi, J. Q.; Liu, F.; Song, J. ZH., Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou, China; Feng, X. H.; Xiong, SH. B.; Wan, Y. F.; Zhang, R.; Zhang, H. G.; Zhou, G. W., Fuzhou Company of Jiangxi Tobacco Company, Fuzhou China

Ralstonia solanacearum is a soil-borne bacterial pathogen which causes serious yield losses in tobacco in south China, there is still no very effective method for control up to now. The detection of the pathogen in soil is essential before disease outbreak, however, the complicated composition of soil limits the efficiency and sensitivity of detection. Here, one extracellular endoglucanase gene (EG, NCBI accession number: AGH83358.1) of *R. solanacearum* FQY_4 with relatively high diversity was selected out as target for designing locked nucleic acid (LNA) primer, both forward and reverse primer possessed two centrally located LNA residues. LNA-based quantitative real-time PCR assay was developed to detect *R. solanacearum* in tobacco-planting soil. Compared with conventional DNA primer, EG LNA primer exhibited higher maximal annealing temperature, LNA-based PCR with 72℃ as annealing temperature also had efficient amplification, which is very suitable for two-step real-time PCR. In addition, real-time PCR with EG LNA primer had lower Ct value and higher amplification efficiency. A serial dilution of linearized plasmid DNA (pMD18 T vector-EG gene fragment) was used to generate a standard curve for quantitative PCR. The DNA of 12 infected soil samples were extracted and subsequently proceeded to quantification of target gene by quantitative real-time PCR using LNA primers. The concentration of *R. solanacearum* in the infected soil samples were between 2.00 x 10^5 CFU/g and 1.56x10^8 CFU/g, the results of established LNA-based real-time PCR here are reproducible. The LNA-based quantitative real-time PCR assay provided a sensitive and efficient method for quantitative analysis of *R. solanacearum* in tobacco-planting soil, which may contribute to the prevention and control of tobacco bacterial wilt. (Reprinted with permission)

Key terms: *Ralstonia solanacearum*, detection, soil, Real-time PCR, locked nucleic acid

44. Future directions for integrated pest management in tobacco. H. J. Burrack, Toennisson, T. A., North Carolina State University, Raleigh NC USA

In the ten years of the Tobacco Transition Payment Program, or the "Tobacco Buyout", flue-cured tobacco production in North Carolina has changed dramatically. The most visible among these changes have been reductions in total tobacco acreage, shifts in production centers east, and the disappearance of tobacco sale auctions in favor of direct contracts between growers and purchasers. This new production paradigm has directly and indirectly impacted pest management strategies. Four key drivers now inform our tobacco entomology research and extension activities. These are:

1. The rise in industry requirement of Good Agricultural Practices (GAP) certification. A closer relationship between grower and purchaser has resulted in additional requests being made of growers in order to obtain contracts to sell leaf. One such requirement is that growers obtain GAP certification. Among the requirements of GAPs are insect management based on economic thresholds. This increased interest and need for sound thresholds has prompted research station and on-farm experiments.

2. Concern by purchasers about pesticide residue levels has prompted research on residue management questions while US and international regulations on pesticide use to protect non target organisms have raised questions about pesticide impacts on beneficial insects in tobacco.

3. Organic tobacco production has increased, partially as response to concerns about pesticide residues, and with this increase in production has come questions about the efficacy of available organically acceptable crop protection agents and landscape scale management practices that reduce pest pressure within pesticide use.

4. Finally, alternative uses for tobacco continue to be developed. Our group has conducted projects addressing pest management concerns in tobacco grown for biofuel production and tobacco grown to produce both higher and lower nicotine concentrations than typical flue-cured leaf.
In this presentation, we will discuss these drivers as well as future potential venues for tobacco entomology research and extension. (Reprinted with permission)

45. Overcoming barriers to integrated pest management adoption in flue-cured tobacco. J. D. Slone; Burrack, H. J., North Carolina State University, Raleigh NC USA

Although established scouting recommendations are available, many growers do not follow these guidelines at a potential cost to their operation. Fewer than 40% of North Carolina flue-cured tobacco growers followed integrated pest management (IPM) practices or employed a formal scouting system in the past four years according to NC extension surveys. Additionally, growers reported an average of three foliar insecticide applications during 2013. Previous research suggests that treating only when pests exceed economic thresholds (ET) may reduce the number of chemical applications to less than two per season. Therefore, if growers were to utilize ETs based on scouting observations, it may reduce the total number of insecticide applications per season. I conducted field scale trials at three North Carolina farms in 2014 and six sites in 2015, comparing IPM practices to grower standards. Weekly scouting was conducted at all sites in which random sample of plants were evaluated for pest presence. The frequency of stops was determined based on field size, with five consecutive plants inspected at each stop. Each site included a grower standard field which was managed according to standard practice for each cooperator with insecticide applications at their discretion and an IPM field which only received insecticide applications if pest populations surpassed the ET. During the 2014 trials, IPM fields received fewer insecticide applications without an impact on yield. Although there is a cost associated with the labor of scouting, it must be considered relative to the reduction of input costs associated with fewer sprays. Additionally, fewer chemical applications is expected to result in reduced pesticide residue on dried leaf samples. Pesticide residues on cured leaf samples were higher in grower standard fields than those collected in IPM fields at farms where residues were detected. (Reprinted with permission)

Key terms: integrated pest management, economic threshold, scouting, pesticide residue

46. Understanding interactions between systemic insecticides and generalist predators in tobacco. P. N. Nelson; Burrack, H. J.; Sorenson, C.E., North Carolina State University, Raleigh NC USA

Neonicotinoid insecticides, prominently imidacloprid, are important for the management of tobacco pests including aphids, flea beetles, and thrips. These insecticides are xylem mobile and applied systemically prior to or at transplant. Key caterpillar pests including tobacco budworm, *Heliothis virescens* (Fabricius), and the tobacco hornworm,

Manduca sexta (L.), are unaffected by these insecticides. Hemipteran predators, including the spined stilt bug, *Jalysus wickhami* (Say) and *Geocoris* big-eyed bugs, significantly reduce caterpillar pests by feeding on eggs and young larvae. These predators have piercing-sucking mouthparts and feed occasionally on the plant, and may potentially be exposed to neonicotinoid insecticides.

Previous work has documented that budworm survival is higher and wasp parasitism rates are lower in tobacco treated with imidacloprid, and that imidacloprid moves from tobacco, to the caterpillar, to parasitic wasp larvae inhabiting the caterpillar. However, the effects of imidacloprid on hemipteran predators is unclear. The goal of this research is to assess the impact of imidacloprid on hemipteran predators in tobacco and develop management strategies in which insecticide applications and biological control are harmonized.

To assess population dynamics of caterpillar pests and hemipteran predators in response to imidacloprid applications under field conditions, visual counts of both groups of insects were taken during 2015 in treated and untreated plots. Sentinel caterpillar egg masses were deployed in these same plots to measure predation. Laboratory assays were performed to determine the toxicity of imidacloprid to stilt bugs, as well as document their exposure to imidacloprid via plant feeding. (Reprinted with permission)

Key terms: neonicotinoid, imidacloprid, systemic, omnivory, biological control, predator

47. Assessing the impact of flower strips on biological control of insects in organic tobacco. J. T. Klein; Burrack, H. J.; Toennisson, A., North Carolina State University, Raleigh NC USA

Experiments conducted during the past few years have shown the potential of habitat diversification in reducing insect pest density in tobacco. This reduction can be attributed to the abundance and richness of predators and parasitoids, which were preserved in this managed vegetation. The introduction of floral resources is considered a very efficient kind of management for insects that feed on pollen and nectar in some stage of their development, such as hover flies, parasitoid wasps and ladybugs. Aiming to evaluate the effect of flowering strips on biological control in organic tobacco, an experiment was conducted during the 2015 tobacco field season, in four commercial organic farms. The effect of three field border plantings on pest density and beneficial insect abundance was compared across all locations: 1) Sunflowers, 2) Buckwheat, 3) Natural field border. We established three transects, approximately 20 m apart with four scouting stops each. Transects were observed weekly from May until September, and the number of aphid infested plants, the number of tobacco flea beetles, budworms and hornworms were counted as well as natural enemies. In addition, sticky cards were placed at each observation point along transects, in the first, 8th and 16th rows. These observations will aid in determining the benefit of managed floral plantings for insect pest management and over what area potential benefits are likely to be observed. (Reprinted with permission)

Key terms: buckwheat, conservation biological control, North Carolina, sunflower

48. Insect management in tobacco grown for biofuel. T. A. Toennisson; Slone, J. D.; Burrack, H. J., North Carolina State University, Raleigh NC USA

Tobacco grown for use in biofuel production has a different set of economic constraints and agronomic practices than that grown for leaf. Lower anticipated crop value may necessitate lower production costs. With respect to pest management, this may mean less pesticide use is economically justifiable. At the same time, sucker growth to increase biomass may lead to an increase in late-season pest pressure. We replicated small-plot field experiments at two agricultural research stations in North Carolina to evaluate the effects of different insect management intensity on insect abundance and yield in biofuel and flue-cured tobacco. Biofuel plots were planted with variety CC 27 at 12" within-row spacing, and plants were topped normally but received no sucker control. The flue-cured plots were planted with variety NC 196 at 24" within-row spacing, with normal topping and sucker control practices. The

number of tobacco flea beetles (*Epitrix hiritipennis*), tobacco budworm (*Heliothis virescens*), plants infested with green peach aphids (*Myzus persicae*), and tobacco hornworms (*Manduca* spp.) were compared between biofuel and flue-cured plots that received foliar insecticide treatments when pest pressure exceeded current economic thresholds, biofuel plots that received foliar treatments at threshold plus greenhouse tray treatments of imidicloprid, and untreated biofuel and flue-cured plots. At the end of the season, all stalk and leaf biomass in the middle two rows of each plot was weighed to determine yield. Results will be discussed. (Reprinted with permission)

Key terms: biofuel, integrated pest management, North Carolina

49. The use of imidacloprid affects two parasitoids of the tobacco budworm differently. C. E. Sorenson; Taylor, S. V.; Burrack, H. J.; Roe, M. R., North Carolina State University, Raleigh NC USA

Imidacloprid is useful in tobacco for managing tobacco flea beetles, aphids, and, importantly the thrips vectors of tomato spotted wilt virus, but it has no effect on caterpillar pests of tobacco and may negatively impact the natural enemies that help suppress them. We conducted field, greenhouse, and laboratory experiments to describe the possible effects of this insecticide on *Toxonueron nigrecips* and *Campolitis sonorensis*, two parasitoids of the tobacco budworm, *Heliothis virescens*. While imidacloprid is more toxic when applied topically to *C. sonorenis* than to *T. nigriceps*, it has a greater negative impact on *T. nigriceps* parasitism in the field when the insecticide was applied systemically to tobacco plants. Parasitism rates for *T. nigriceps* are reduced in imidacloprid-treated tobacco compared to untreated tobacco, and the adult wasps have reduced longevity. No such effects were observed for field populations of *C. sonorensis*, however, and we will discuss possible reasons for these differences. We were able to establish that imidacloprid moves from treated plants to tobacco budworm caterpillars fed on treated tobacco, to *T. nigriceps* larvae and to adults emerging from these caterpillars. This study documents tri-trophic movement of a neonicotinoid insecticide and subsequent effects to beneficial insects. (Reprinted with permission)

Key terms: imidacloprid, parasitoids, tobacco budworm, tri-trophic interactions.

50. DuPont™ Verimark® and Exirel® insect control: novel insecticides for crop protection and yield optimization in tobacco. H. E. Portillo; Williams, R.W.; Smith, J.D.; Backscheider, K. A., DuPont Crop Protection, Newark DE USA

Verimark® and Exirel® insect control are novel insecticides based on the active ingredient Cyazypyr® (DPX-HGW86, cyantraniliprole) that belong to the second anthranilic diamide insecticides discovered by DuPont™. Verimark® and Exirel® are the first products in its class of chemistry that control a cross-spectrum of insect pests including *Lepidoptera*, *Dipteran* leafminers, fruit flies, beetles, whiteflies, thrips, aphids, leafhoppers, psyllids and weevils, while conserving key predators and parasitoids. Exirel® and Verimark® selectively activate the ryanodine receptor in insect muscles resulting in paralysis and rapid inhibition of feeding. Exirel® has been optimized for foliar use, demonstrating excellent translaminar movement. Verimark® has been optimized for soil applications to deliver consistent upward root systemicity. Data on the efficacy and residual control of foliar applications of Exirel® and float house and at-plant transplant water applications of Verimark® on flea beetles and tobacco budworm and their impact on crop establishment and potential crop yield benefits will be discussed. Both products have been granted reduced risk status on registered crops by the US Environmental Protection Agency (EPA), EPA approval for use on tobacco is anticipated for the 2016 growing season. (Reprinted with permission)

Key terms: Exirel®, Verimark®, Cyazypyr®, Tobacco, Feeding inhibition, crop protection

51. High efficiency genome editing in *Nicotiana tabacum* using designer nucleases. R. Dewey; Wang, Y.; Smith, W.; Yuki Matsuba; North Carolina State University, Raleigh NC USA

Emerging breakthroughs in the field of precision genome editing are enabling researchers to effectively inactivate or replace genes within the genomes of complex organisms with an unprecedented degree of accuracy and efficiency. Targeted mutagenesis via designer nucleases promises to be far superior to the classical chemical- or radiation-based methodologies traditionally employed by plant breeders, as these newer approaches are not accompanied by the plethora of undesirable secondary mutations randomly distributed throughout the genome that is characteristic of conventional mutagenesis, a feature that necessitates extensive backcrossing to eliminate unwanted secondary mutations. The most efficient and facile of the designer nuclease technologies is the CRISPR-Cas9 system. This class of designer nucleases utilizes a guide RNA component (CRISPR) to direct a nuclease component (Cas9) to the specific chromosomal location that is to be mutated. Imperfect repair of the cleaved target site, typically in the form of short deletions or insertions, leads to permanent, heritable mutations. We have used the CRISPR-Cas9 system to achieve high efficiency, targeted mutagenesis of the tobacco genome. Using CRISPR-Cas vectors designed to target specific tobacco genes, we have observed directed gene mutations in as many as 40% of the plants transformed with these vectors. By segregating the CRISPR-Cas9 transgene away in subsequent generations, one can readily obtain a final plant product that possesses the endogenous mutation(s) of interest with no transgene present. Both the advantages and implications of utilizing designer nucleases in a program of tobacco genetics and breeding will be discussed. (Reprinted with permission)

Key terms: targeted mutagenesis

52. Development of co-dominant markers for Nic1 and Nic2 in tobacco. Y. Shengming; Qin, Q.; Li, D.; Miller, R.; Jack, A., University of Kentucky, Lexington KY USA

With the impending FDA regulation, it is expected that tobacco plants with low levels of alkaloid will be more and more favored in future tobacco (*Nicotiana tabacum*) breeding. Nicotine is the predominant alkaloid in most commercial varieties of tobacco. Therefore, an understanding of the mechanisms underlying nicotine biosynthesis is particularly critical for tobacco breeders and industry to lower alkaloid content in plants and cigarettes. Nicotine is synthesized in the tobacco root under the control of two independent genes, Nic1 and Nic2. These two genes are considered as regulatory loci to control expression of nicotine synthesis-related structural genes, which encode important enzymes involved in metabolic pathways for nicotine biosynthesis. Genetic studies revealed that nic1 and nic2 mutations are semi-dominant and act synergistically, with effects of nic1 2.4 times stronger than those of nic2. Even though the nic mutants have long been available, without efficient molecular markers the introgression of nic1 and nic2 into commercial varieties has been seriously hampered. Based on the RNA-seq analysis of four near-isogenic lines with various nicotine levels, co-dominant markers closely linked to Nic1 and Nic2 genes were developed. Our results will greatly benefit the tobacco breeders and industry to generate tobacco varieties with low levels of alkaloids. (Reprinted with permission)

Key items: nicotine, Nic1, Nic2, marker, RNA-seq

53. Association mapping in a collection of tobacco reference cultivars. Step One: Variability of smoke constituents. E. Julio; SEITA; Verron, T.; Cahours, X.; Dorlhac de Borne, F.; Colard, S., Imperial Tobacco Group, Bergerac France

Recent developments of sequencing technologies and computational methods have given the opportunity to detect natural variation underlying complex traits in crops. In this purpose, association mapping can be used to identify the link between tobacco genes or molecular markers to smoke constituents. Such association can support the development of new tobacco varieties suited to the future potential regulatory constraints.

In our study, a panel of 161 tobacco varieties, composed of flue-cured, burley, dark air-cured and oriental types, was grown in open field. Because of its large genome, RNA-Seq based sequencing was chosen to capture differences of gene expression together with SNPs (Single Nucleotide Polymorphisms) variation in the 161 varieties. Three different tissues at two growth stages were used to do a comprehensive analysis of the transcriptome. After curing, cigarettes were made with each variety and were mechanically smoked according to the Canadian intense smoking conditions. The mainstream harmful or potentially harmful constituents as listed by FDA (Food and Drug Administration) were determined using in-house and internationally recommended methods.

Taking into account multiple factors linked to the growing environment and weight of tobacco actively burnt during puffing, our first investigations show significant differences of several smoke constituents between varieties, thus demonstrating the potential of association mapping for the development of future varieties. For some constituents, differences were however not significant. A description of the approach and preliminary results obtained from the smoking of a sub-group of flue-cured varieties is reported here. (Reprinted with permission)

Key terms: association mapping, RNA-Seq, smoke constituents

54. Topping and suckercide treatments trigger global changes in gene expression in tobacco. S. Pattanaik; Singh, S. K.; Fisher, C.; Yuan, L., University of Kentucky, Lexington KY USA; Lawson, D., R. J. Reynolds, Inc., Winston-Salem NC USA

Topping and control of formation of suckers are common agronomic practices that significantly impact the yield and quality of various crop plants. Application of suckercides to plants following topping is a common method for sucker control. Tobacco (*Nicotiana tabacum*), like many other crop plants, is commonly topped and treated with suckercides. We performed RNA-sequencing to study the differential gene expression in tobacco that was topped or treated after topping by two chemicals, the local-systemic suckercide, Flupro® (FP), and contact suckercide, Off-Shoot-T® (OS). Transcriptome analysis identified a large number of genes that were differentially expressed in leaves responding to topping, FP-, and OS-treatment, respectively. Among these differentially expressed genes (DEGs), some are common to all three conditions. Hierarchical cluster analysis of the DEGs revealed four groups with distinct expression patterns. DEGs, largely related to wounding and secondary metabolism, exhibited significant upregulation following topping and downregulation after suckercide treatments. DEGs related to photosynthetic processes were repressed following topping as well as suckercide treatments. Comparison of topping and FP-treatment revealed common DEGs, largely related to wound response, hormone signaling, small molecule biosynthesis, and amino acid metabolism. DEGs common to topping and OS treatment were largely related jasmonate biosynthesis and secondary metabolism. Moreover, topping and FP-treatment affect the expression of auxin and cytokinin signaling pathway genes that are probably involved in sucker formation. Our findings suggest that suckecides impact expression of genes related to a number of primary as well as secondary metabolic pathways in plants. The regulatory elements of highly upregulated DEGs can potentially be used for the development of a topping-inducible chemical-free sucker control system. (Reprinted with permission)

Key terms: RNA-sequencing, topping, suckercide, gene expression.

55. Biodesign of tobacco for high value products. J. S. Yuan; Texas A&M University/Synshark LLC, College Station TX USA

Synthetic biodesign has been employed to develop innovative routes and organelles to enable tobacco as bio-factories for high value products, like squalene. We have designed and implemented synthetic pathways and organelles to achieve record-level photosynthetic production of squalene, a triterpene with broad application as nutriceuticals, vaccine adjuvant, cosmetic product ingredient, and others. First, the synthetic pathways were designed and

implemented to redirect photorespiration by-product to terpene biosynthesis. The pathway design directly channeled a photosynthate into terpene production and has the potential to also reduce the energy consumed for recycling photorespiration products. Kinetics-based computational modeling was first carried out to evaluate how carbon re-partitioning will impact photosynthesis and terpene production. The results indicated that high terpene level via C2 redirection can be achieved without significant reduction of photosynthesis rate. The implementation of two alternative pathways led to a significant increase of terpene production. In particular, the synergistic engineering of C2 redirection with terpene biosynthesis has led to the production of over 2 mg/G FW squalene, which is over four-fold increase of the level achieved by engineering terpene pathway alone. The further carbon flux and metabolomics revealed the significant increase of squalene yield resulted from the carbon repartition from sucrose storage to terpene. Second, the synthetic organelle was designed to compartment the squalene biosynthesis and storage both in chloroplast. Both confocal and latest Raman microscopy confirmed the formation of synthetic organelle storing the squalene. The engineering strategy also increased the squalene yield by more than 4 fold to achieve 2.5 mg/G FW. Overall, the synthetic biology strategies provided novel approaches for engineering tobacco as bio-factories for various high value compounds. (Reprinted with permission)

Key word: terpene, biodesign, synthetic biolog

56. Genetic variability for upper stalk sucker growth in flue-cured tobacco. J.L. Verrier and L'Humeau, J., Bergerac Seed & Breeding, Bergerac, France

Apart from the past use of non-flowering cultivars, there has been few reports on the use of low sucker growth varieties to help reducing the sucker control agent residues in flue-cured (FC) tobacco.

Among various FC cultivars and lines with no short photoperiod requirement, the upper stalk sucker growth propensity was investigated in a three steps approach, at Bergerac (France), in 2009 - 2014:

1. Defining the trait to be assessed,
2. Studying its variability among genotypes through different environments,
3. Testing sucker control strategies using « early » and « late » sucker producer genotypes, relative to floral growth.

Fatty alcohols may control suckers smaller in size than approximately 3 cm. Therefore, the size of the biggest sucker in a given plant was considered, rather than their number or weight. Sucker control treatment dates are based on the floral growth stage of the crop. Then, for a given plant and a given observation date, the biggest sucker size and floral growth stage were recorded.

Field trial data indicate that a significant genetic variability is available for the timing of sucker growth, relative to floral growth, in flue-cured tobacco. Plant population, stalk bent due to wind and CMV infections also have dramatic impact on upper stalk sucker growth.

Selected "late" and "early" sucker producer genotypes, relative to floral growth, were tested under a sucker control schedule based on fatty alcohols only. The "late" genotype was successfully controlled up to 14 days after topping, whereas the "early" was not.

Links between the "late sucker producer" trait, alkaloid content and plant shape are to be investigated, in particular to better estimate the possibility of designing aromatic flue-cured cultivars with this trait. (Reprinted with permission)

57. Field tests for tobacco gall nematodes resistance in France. J.L. Verrier and Mazeau, B., Bergerac Seed & Breeding, Bergerac, France

Tobacco root galling due to nematodes is not widespread in France, but affects locally some farms. In 2014, two farms about 300 km distant from each other, one in Charente Maritime and one in Aveyron, were identified as having heavy root galling problems. Soil samples were sent to the ANSES nematology laboratory (35653 le Rheu, France), and in both cases *Meloidogyne arenaria* was identified. A set of flue-cured genotypes, including the resistant lines NOD8 and RL 2-1-1, formerly tested in the CORESTA nematode collaborative study, and the susceptible standard ITB 683, were installed in these farms using a randomized blocks design. Recommended practices for filler flue-cured tobacco were applied. The percent of the root system affected by galls was assessed on 8 individual plants in each plot, respectively 126 and 123 days after planting, using the following scale: 0: no galling, 1: 1 to 2% of the root system with galls, 2: 3 to 10%, 3: 11 to 30%, 4: 30 to 50%, 5: 50 to 80%, 6: 80 to 100%.

Results were highly similar in both locations. The susceptible standard ITB 683 was heavily attacked, with 70 to 100% of the root system with galls. In sharp contrast, RL 2-1-1 showed nearly no galling (average scores 0.44 and 0.38, respectively). NOD 8 had an intermediate result, with between 3 and 30% of the root system affected (mean score 2.03). In average of both locations, breeding lines had mean scores from 1.9 to 5.4, while the cultivar CETARSA 613 showed an average score equal to 4.0.

Considering that no soil fumigations are authorized for controlling gall nematodes in tobacco fields in France, breeding gall nematode resistant cultivars appears highly necessary, even when the pathogen is not widespread. These results will help in obtaining such cultivars. (Reprinted with permission)

58. Effects of three primary tillage methods on flue-cured tobacco in North Carolina. M.D. Inman, Vann, M.C. and Fisher, L.R., North Carolina State University, Raleigh NC USA

Research was conducted in 2012 and 2013 to evaluate the effects of three different primary tillage practices on overall growth and development of flue-cured tobacco; as well as soil physical properties. The tillage systems were compared across two distinctive tobacco production regions in North Carolina: the Piedmont (fall ridging, rotary tillage, and spring ridging) and Coastal Plain (fall ridging, strip-tillage, and spring ridging). Treatments were arranged in a randomized complete block design and replicated four times. Soil bulk density, porosity, water content, and penetration resistance were determined after transplanting and after final harvest. Crop yield was determined following harvest and cured leaves were assigned a USDA grade to assess quality. Crop value was determined using a combination of leaf yield and quality. As a result of high soil penetration resistance, fall ridging reduced leaf yield and value when compared to rotary tillage and spring ridging systems within the Piedmont region. Alternatively, in the Coastal Plain, primary tillage treatments did not affect agronomic aspects of production or soil physical properties. Results indicate that fall ridging and strip tillage systems were acceptable primary tillage methods for the production of flue-cured tobacco in the Coastal Plain of North Carolina. However, fall ridging in the Piedmont reduced tobacco yield and value, indicating that it is likely not an acceptable primary tillage method within the region. (Reprinted with permission)

59. An evaluation of hybrid and pure line cultivars of flue-cured tobacco for cured leaf yield and quality. G. A. Amankwa; Mishra, S.; Shearer, A.; Van Hooren, D. L., Canadian Tobacco Research Foundation, Tillsonburg ON Canada

Currently, hybrids account for about 57% of the flue-cured tobacco acreage in Canada, since the first release of a hybrid cultivar in 2006. Grower expectations for hybrids are high in terms of the potential for improved cured-leaf yield and quality which can translate into greater returns. The objective of this work was to compare the yield, grade index and grade index returns of two hybrids (CTH2 and CTH14) in relation to two standard pure line cultivars

(Delgold and CT157), using data collected from the Registered Cultivar Trial conducted in five recent years, and to determine the range of variability in traits across growing seasons. The trials were conducted in 2009 to 2014, except for 2010, at one location in the Delhi area. Results show significant differences among cultivars and seasons for all the traits. Cultivar x environment interaction was significant only for yield. The coefficient of variation for yield ranged from 9.7 to 10.8% for the hybrids and 7.9 to 9.3% for the checks, indicating the hybrids were relatively more responsive to the varying conditions. The hybrids were both superior to Delgold for grade index, but quite comparable to the other check, CT157. In terms of grade index returns, CTH2 exceeded the checks by 9.1 to 10.3% while CTH14 exceeded the checks by 8.5 to 9.7%. Thus, the two hybrids offer good options to growers in today's market. (Reprinted with permission)

Key terms: hybrid, yield, grade index.

60. Management of blank shank on burley tobacco with oxathiapiprolin. C. Ammerman; Pearce, R.; Pfeufer, E., University of Kentucky, Lexington KY USA

Black shank, caused by *Phytophthora nicotianae*, is the most damaging disease of burley tobacco in Kentucky. Along with sound cultural practices, soil and/or transplant water applications of fungicides are key management recommendations for growers. Until recently, the only fungicides labeled for use on tobacco for control of black shank were all in Fungicide Resistance Action Committee (FRAC) group 4. Although resistance to this group of fungicides has not yet been reported, there is potential for development of resistance with the repeated use of a single mode of action. In this study, a fungicide with a different mode of action is investigated.

The purpose of these studies was to evaluate the efficacy of oxathiapiprolin (OXTP) applied in transplant water and to soil after transplanting in two fields with histories of black shank. These studies compared management of black shank among OXTP, Ridomil Gold (RG), and Presidio treatments in addition to measures of plant safety.

OXTP demonstrated a level of suppression similar to RG (TPW or soil directed at transplanting, 1st cultivation, layby) when applied at 19.2 ounces/ac in TPW-only or at 19.2 ounces/ac in TPW treatment followed by applications of 9.6 ounces/ac and 3.8 ounces/ac at 1st cultivation and layby. Combinations of OXTP at 3.8 ounces/ac and RG at 8 ounces/ac in TPW and the same rates at layby were also effective and equivalent to RG. RG was effective as a single treatment in TPW in the Clark County study but not in Grant County due to a higher disease pressure where more applications for suppression would be necessary.

In conclusion, OXTP appeared to function as an alternative to RG and may be useful in a pest management program focused on alternating chemistries. (Reprinted with permission)

61. Determining black shank resistance in tobacco varieties grown in Atkinson and Coffee counties in 2013-2015. T. Barnes; von Waldner, M.; Edwards, P.; Bertrand, P.; Moore, J. M., University of Georgia, Pearson GA USA, Lewis, R., North Carolina State University, Raleigh NC USA

Black shank re-emerged as a serious problem in 2014. Varieties counted on for resistance, mainly GF-318 and NC-196 were found to show less resistance than expected even where Ridomil Gold was used. Follow-up testing found 30 isolates of *Phytophthora parasitica* were fully sensitive to Ridomil Gold. Trials were conducted in 2014 and expanded in 2015 to look for varieties that might show a higher level of resistance than growers are currently using. Each trial was a randomized complete block with four replications of each variety. All trials included K-326 (low resistance) and NC-196 (moderate resistance) for comparison. Trials done in 2014 found GL-395, CC-143 and NC-925 showed some promise. Trials conducted in 2015 found these and a few other varieties that warrant further testing. Nothing has been found that will stand alone without a chemical treatment program. All varieties tested to date have shown significant loss under very high disease pressure. (Reprinted with permission)

Key terms: black shank, varieties, resistance

62. Preemergence *Pythium* damping-off of burley tobacco cultivar TN 90. L. A. Darnell; Johnson, C. S.; Reed, T. D., Virginia Tech, Blackstone VA USA

Seedling emergence is an important consideration in tobacco transplant production. While post-emergence damping-off of tobacco seedlings can be a common problem, pre-emergence damping-off has not generally been considered as a cause of low seedling emergence. *Pythium* isolates collected from diseased tissue from tobacco greenhouses in Virginia in 2014 were maintained on PARP oomycete-selective medium and used in laboratory experiments in 2015 to investigate their effects on germinating seed of the burley tobacco cultivar TN 90. A 7.0 mm-diameter plug from an actively growing PARP culture from one of the two isolates was sub-cultured onto water agar in 60 mm-diameter petri plates at each of four infestation timings. Infested agar plugs were placed 1 cm from a surface-sterilized, uncoated seed either before adding the seed to each plate, at seeding, when a radicle was first observed emerging from the seed, or upon appearance of a cotyledon. Infestation treatments were replicated 3-5 times. Daily observations of radical emergence, radicle length, presence of a cotyledon, and discoloration of the cotyledon were made for 7-8 days after seeding for infestations performed before or at seeding and for 7-8 days after infestations performed at radical or cotyledon emergence. When infestation occurred before or at seeding, radical emergence was only 20-60%, radical growth was reduced, and cotyledon formation was completely prevented in a spring 2015 experiment. Inoculation at radical formation inhibited cotyledon formation 66-100%, while inoculation at cotyledon formation always resulted in discoloration. Additional experiments are being conducted in the fall of 2015. (Reprinted with permission)

63. Management of tomato spotted wilt virus with imidacloprid in Lowndes County from 2012-2015. J. Dawson; Kicklighter, J.; Price, J.; Moore, J. M.; and Bertrand, P., University of Georgia, Valdosta GA USA

Tomato Spotted Wilt Virus (TSWV) is a major pathogen of tobacco plants that is spread by the feeding activity of thrips. There are various symptoms of this disease. Some of the more common symptoms seen are: necrotic banding along and around the main veins, young leaves turning yellow then reddish brown, distortion of buds, concentric ringspots on leaves that merge to form larger areas of dead tissue, and death to the entire plant after a couple of days. Since TSWV is vectored by thrips, it is important to control thrips populations among the tobacco plants. Imidacloprid (Admire Pro) seedling drenches have been found to be an effective way to control thrips populations. From 1998-2014, 180 research trials conducted in Georgia found the average reduction of spotted wilt was 32 percent. The purpose of this research is to confirm that Admire Pro is controlling the occurrence of spotted wilt throughout tobacco fields in Lowndes County. Over the past years, test plots have been conducted on four different tobacco farm locations in Lowndes County for a total of 16 research trials. The results showed that 9 out of the 16 trials had control equal to or better than 32% control. (Reprinted with permission)

Key terms: tomato spotted wilt virus, TSWV, imidacloprid, thrips

64. Evaluation of fungicides for the control of blank shank and yield response on tobacco. B. A. Fortnum and P. D. Peterson, Clemson University, Pee Dee Research & Education Center, Florence SC USA

Presidio, Ridomil Gold and Orondis (Oxathiapiprolin) fungicides were tested on-farm for the past two growing seasons (2014, 2015) in Sumter County, SC, for the control of *Phytophthora parasitica* var *nicotiana* on tobacco. A randomized complete block design was used to test the selected treatments. Treatments were replicated four times each season. Tobacco seedlings were removed from a greenhouse float system and transplanted into single row plots 40 feet long on 4-foot row spacing. Fertilizer (6-6-18 N, P, K analysis) was applied at transplanting and an additional fertilizer application of 15.5-0-0 N, P, K analysis was made at first cultivation. Fertility rates followed state recommendations. Selected treatments received Presidio, Ridomil Gold and Orondis fungicides in the transplant

water applied in 100 gal/A. Transplant water plus fungicide treatments were hand applied to each plant receiving the treatment. The untreated control received only water. During 2014, soil applied fungicide treatments were made at 1st cultivation and at layby. Soil applied fungicides were sprayed over the row as a directed spray to each side of the planting bed and incorporated immediately after application with a field cultivator. During 2015, a single soil applied fungicide treatment was made at layby. In 2014 Presidio, and Orondis significantly increased yield and reduced the percent disease and disease index (P = 0.05) when applied across all application rates and application timings. Disease control was influenced by the time of application and application rate. In 2015 all Presidio + Ridomil Gold and Orondis + Ridomil Gold applications increased yields and reduced the disease index (P = 0.05) over a non-treated control. (Reprinted with Permission)

Key terms: Presidio, Ridomil Gold and Orondis (Oxathiapiprolin) fungicides

65. Greenhouse method to screen for resistance to Fusarium wilt in tobacco. N. Martinez and R. D. Miller, University of Kentucky, Lexington KY USA

Fusarium wilt is a soilborne disease caused by *Fusarium oxysporum* f.sp. *nicotianae*. This pathogen is very persistent in the soil and is effectively managed with the use of resistant cultivars. Our objective was to incorporate a reliable and inexpensive greenhouse test into our tobacco breeding program. Several Fusarium strains were isolated in Kentucky during 2014 from symptomatic tobacco stems. All isolates were purified, and subsequently tested for pathogenicity in the greenhouse with susceptible seedlings. Three pathogenic isolates were selected for species verification by observing spores grown in carnation leaf agar. Consistent results were observed for all susceptible (TN97, TN86, TN90) and partially resistant (KY14xL8, BU64, KTH2802, NC95) tobacco lines in a float bed system. The method is as follows: each unit consists of a 338-cell Styrofoam tray placed in an individual frame lined with black plastic and floated in water, using fine vermiculite as growth medium. Twelve tobacco lines are planted per tray (row of 13 cells per line). After germination, they are thinned to 1 seedling per cell and fertilizer is added to the float water. Three Fusarium isolates are grown in petri dishes of 3 different solid media: mung bean agar, acidified potato dextrose agar, and Gritton agar. Cultures are incubated for 3 weeks in the dark at room temperature and spores are scrapped into 2 Liters of sterile DI water. A new float frame is set and a heat mat is placed below the plastic and maintained at 27°C. The roots of three week-old seedlings are cut from the bottom of the tray with a metal scraper and the tray is immediately placed over the 2L spore suspension. Ten liters of tap water plus fertilizer are added after 48 hours and replenished until the disease evaluation of the seedlings is performed 2-3 weeks later. (Reprinted with permission)

Key terms: screening, resistance, greenhouse, disease, wilt, float

66. Tobacco-free campus initiative combined with carbon monoxide monitoring and counseling: quitting strategy for Kuwait College nursing student smokers. Omu, F. E.; Al-Kandari, I; Al-Marzouk, R; Paulraj, D; Rajagopal, M; John, P : College of Nursing, The Public Authority for Applied Education & Training, Safat Kuwait; Omu, A.E.: Faculty of Medicine, Kuwait University, Kuwait

Background: Smoking cessation involves changing of unhealthy smoking habit which accounts for 63% of global deaths. This study was in response to the United Nations General Assembly Global Forum for Non-communicable Disease's invitation to nurse researchers to evaluate smoking cessation interventions for their students.

Objective: To evaluate "Tobacco-free campus initiative" combined with carbon monoxide monitoring and tobacco cessation interventions as a quitting model for student nurses.

Methods: This was the second part of a multi-phase study which involved a series of 'No- smoking' campaigns, enforced tobacco-free campus initiative, mandatory weekly monitoring of biological health indicators and

biochemical feedback using expiratory carbon monoxide (CO) levels for 36 real cigarette and shisha smokers. The quasi-experiment lasted 10 weeks. Participants' data on tobacco use, quit attempts and self-efficacy (SE) were collected using a 25- item bi-lingual (Arabic -English) questionnaire. Counseling and smoking cessation aids of their choice were offered.

Results: All the participants lived with their families and 70% of the families smoked cigarettes and/or shisha. Previous quit attempts were statistically higher in males than females, (47.2% Vs 13.9 % U=76.00, P= 0.007). High SE to quit was 36% and the quit rate for the last 4 weeks was 13.9%. Biological health indicators of participants such as pulse rate and systolic blood pressure improved as a result of cessation interventions.

Conclusion: Tobacco product ban on college campus, CO monitoring followed by counseling were effective smoking cessation interventions. (Reprinted with permission)
Keywords: Tobacco-free campus, Quitting strategies for nursing students, Carbon monoxide monitoring, Self-efficacy.

67. Update on the pasture herbicide stewardship program and efforts to reduce the occurrence of off-target damage to tobacco and other sensitive, high value crops. G. Neil Rhodes, Jr.; Israel, T. D.; McIntosh, D. W., University of Tennessee, Knoxville TN USA

Off-target movement of pasture herbicides to tobacco and other high value crops continues to be an issue. Damage can result in lost productivity for growers, expensive fines and/or lawsuits, and negative publicity for the industry. We began a comprehensive educational program in 2011 that stresses the importance of proper stewardship with the use of pasture herbicides. The program was created to reduce the occurrence and impact of off-target damage to tobacco and other sensitive, high value crops; and to make available tools to help with the diagnosis of suspected cases of off-target damage. Funding was obtained via grants from Philip Morris International, Altria Client Services, Dow AgroSciences and DuPont Crop Protection. Four crops (tobacco, cotton, tomato and grape) and five herbicides (2,4-D, dicamba, aminopyralid, aminocyclopyrachlor and picloram) were selected for the development of educational materials and diagnostic tools. These include still images, time lapse videos and fact sheets that were created and made available when our initial website, herbicidestewardship.utk.edu, was launched in 2014. In 2015 the website was completely redesigned in an effort to make it more attractive and user friendly. Additionally, a shorter, less cumbersome address, herbicidestewardship.com, was created. Use of the website has steadily increased since its inception. The website has been visited over 8000 times since it was launched, and approximately half of these visits were in 2015 alone. Visits came from The United States, China, Japan, Germany, Canada, The United Kingdom, India and Brazil. (Reprinted with permission)

Key terms: herbicide stewardship, crop injury symptoms, diagnostic aids.

68. Evaluation of MCW-2 (fluensulfone) as a nematicide in flue-cured tobacco production. C. Saude; Brammall, R.A.; Shearer, A.; and Van Hooren, D. L., Canadian Tobacco Research Foundation, Tillsonburg ON Canada

Field trials were conducted in 2012-2014 to evaluate the effects of three rates (1, 2 and 4 kg a.i./ha) of MCW-2 (a.i. fluensulfone) on plant growth and yield, and populations of Root-lesion (RLN) (*Pratylenchus penetrans*) and Stunt (*Tylenchorhynchus* spp.) nematodes. The effectiveness of these treatments was compared to plants grown without soil fumigation and with soil fumigation using Chloropicrin 100 (a.i. chloropicrin 99.0%). The trials were conducted in fields with a history of high nematode populations. The flue-cured tobacco cultivar CT157 was used each year in the trials. The MCW-2 treatments were applied to the soil surface and incorporated to a 10-cm depth with a roto-tiller prior to transplanting. Precipitation occurred within 72 hours after MCW-2 incorporation. Combined data over the three years indicated that treatments did not have significant effects on topping height, leaf number at topping and eighth leaf width. The eighth leaf length, tip leaf measurements and bud percentage, were the highest for the

Chloropicrin 100 treatment and similar among the MCW-2 treatments and the non-fumigated control. The eighth leaf area for the Chloropicrin 100 and the MCW-2, 1 and 4 kg a.i./ha treatments, were also similar. Yield, as plant dry weight, of the Chloropicrin 100 treatment was significantly greater than all other treatments. Yields for all MCW-2 treatments and the non-fumigated control were similar. RLN numbers were not affected by treatments, although a slight decrease in RLN numbers was found for the Chloropicrin 100 mid-season assessment, and a slight increase in the RLN late-season counts. Chloropicrin 100 lowered mid-season numbers of combined RLN and Stunt nematodes. These results indicate that overall, the MCW-2 in the manner and rates used, was ineffective at altering numbers of soil nematodes and in enhancing plant growth and yield. (Reprinted with permission)

Key terms: Flue-cured tobacco, Root-Lesion Nematode, *Pratylenchus penetrans*, Nematicides

69. Chemical management of blank shank in Georgia. J. Shealey; von Waldner, M.; Barnes, T.; Bertrand, P.; Moore, J. M., University of Georgia, Statenville GA USA

Tobacco losses to black shank were higher in 2014 than any of the previous 20 years. Growers were left questioning the resistance of tobacco varieties and effectiveness of Ridomil Gold for management of black shank. Isolates of *Phytophthora parasitica* sent to North Carolina State university for testing were found very sensitive to mefanoxam, the active ingredient in Ridomil Gold. A new chemistry marketed as Presidio was available for use on tobacco for the first time in 2015. Trials were conducted at two locations to compare transplant water and layby treatments of Ridomil Gold and Presidio for control of black shank. At the Echols County location, disease distribution was very uneven, and may have affected the results. In this trial, plots receiving layby treatments of Ridomil Gold developed slightly less disease than the untreated plots while those receiving Presidio developed slightly more. However, final % black shank in neither Ridomil Gold treated nor Presidio treated plots differed significantly ($p=0.05$) from the untreated plots. At the Coffee County location, disease distribution was very uniform across the entire plot. In this trial, plots untreated at layby developed significantly ($p=0.05$) more black shank than plots treated with either Presidio or Ridomil Gold at layby. There was no difference in control ($p=0.05$) between Presidio and Ridomil gold. Presidio may offer growers another option for control of black shank but further testing is needed to find the best way to use it. (Reprinted with permission)

70. Evaluating tobacco varieties for resistance to black shank. J. Taylor; Shealey, J.; Hollifield, S.; Shirley, B.; Bertrand, P.; and Moore, J. M., University of Georgia, Lakeland GA USA

In 2014 growers, using what were thought to be resistant varieties, suffered major losses due to black shank even where Ridomil Gold was used. Tests conducted at North Carolina State University found the pathogen (*Phytophthora parasitica*) fully sensitive to Ridomil Gold. In 2015, trials were conducted in three South Georgia locations to evaluate varieties for black shank resistance. All trials consisted of six varieties with black shank resistance unknown or uncertain, plus K-326 and NC-196 for comparison. There were a total of 13 test varieties to be evaluated so individual trials did not have the same variety list. Differences among varieties were found in all trials. In some trials, black shank became so severe that all varieties suffered significant loss. The differences found will allow for selection of a small group of varieties that show enough promise to warrant further testing. None of the varieties tested have enough resistance for use without a two+ year rotation and chemical control program. (Reprinted with permission)

Key terms: black shank, varieties, resistance, Ridomil Gold

71. Management of spotted wilt in tobacco with imidacloprid in the northern portion of the Georgia flue-cured belt. C. Tyson; Earls, C., Griffin, C., Lanier, J., Spaid, D., Bertrand, P., Moore, J. M., University of Georgia, Reidsville GA USA

Trials were conducted from 2012 to 2015 in the Candler, Evans, and Tattnall county area to evaluate the continued effectiveness of imidacloprid for management of spotted wilt in tobacco. The trials compared spotted wilt incidence in tobacco grown from imidacloprid treated seedlings versus untreated seedlings. To facilitate comparisons, trays of untreated tobacco seedlings were transplanted at the start of 4 randomly selected rows (1 tray per row). Spotted wilt was evaluated in the untreated plants and an adjacent row of treated plants resulting in 4 reps of comparison for each trial. Over the course of this study, imidacloprid generally reduced spotted wilt at or above the average of what was seen for the state as a whole. The variation in control from place to place and year to year is consistent with what is seen in Georgia as a whole. Percent stand was generally unaffected by imidacloprid treatment. (Reprinted with permission)

Key terms: tomato spotted wilt, imidacloprid

72. UGA Extension facilitated Good Agricultural Practices (GAP) tobacco farm safety and worker training. T. Varnedore and Moore, J. M., University of Georgia, Hazlehurst GA USA

The concept of Good Agricultural Practices (GAP) aims at ensuring sustainable, economically viable production of usable tobacco.

The US Tobacco GAP program is an industrywide program supported and funded by all tobacco buying and manufacturing companies in the US. Growers are encouraged to provide on-going training and education in all elements of GAP to workers to ensure that everyone involved in the tobacco production understands the importance of working in a safe manner.

A Jeff Davis County tobacco farmer and longtime extension cooperator questioned his county agent on the availability of training materials available for training workers in farm safety and asked for help in providing the necessary training as required by the GAP program.

A training session was held at the Jeff Davis County Extension Office. Seven farm workers and the tobacco farmer participated in this 1 hour and 30 minute video training using online Spanish videos available on the web. Videos used for this training were located at the following link: http://www.nclabor.com/ash/ash.htm.

Videos were downloaded and projected for all to see in the extension office conference room. Topics included: Los Cuatro Peligros: Se Puede Evitarlos, The Top 4 Farm Hazards: How to Avoid Them; El Montacargas, Forklift Safety; Estrés Por El Calor, Heat Stress; Empacadoras de Tabaco, Tobacco Baler; Cosechadoras de Tabaco, Tobacco Harvester.

Also shown was a video (in Spanish) from the NCDOL Occupational Safety and Health Division's Agricultural Safety and Health Bureau about the prevention and treatment of Green Tobacco Sickness entitled: Prevención y Tratamiento de la Enfermedad de la Hoja de Tabaco, Preventing and Treating Green Tobacco Sickness. This video can be viewed at: https://www.youtube.com/watch?v=eOjhxAO56B4 .

This training proved to be a convenient way for Extension to assist a cooperating grower to present worker safety information to workers in their own language, document their attendance and meet some of the requirements of the US Tobacco GAP program. (Reprinted with permission)

Key terms: GAP, worker safety, training

73. Management of spotted wilt in tobacco with Actigard 50WG + imidacloprid. M. von Waldner; Barnes,T.; Beasley, E.; Curry, S.; Varnedore,T.; Bertrand, P. and Moore, J. M., University of Georgia, Douglas GA USA

Tomato Spotted Wilt Virus has been the major cause of disease loss in Georgia tobacco in all but two of the past 25 years. Losses have been particularly high in and around Coffee County, in some cases exceeding 80 percent. A program of treating seedlings in the plant house with a foliar application of Actigard 50WG 7-10 days prior to transplant followed by a spray on/rinse off treatment of imidacloprid 2-5 days later is the only spotted wilt management option proven effective thus far. Trials conducted between 2000 and 2011 established a benchmark value of 52 percent as a mean or expected level of control of spotted wilt with this program. However, during these trials, in addition to expected farm to farm variation in percent control of spotted wilt, a distinct year to year variation was also apparent. Detailed trials conducted from 2009 to 2015 have clearly documented a year to year variation in control of spotted wilt with this program. The reasons for this annual variation are unclear at present but appear to be due to an annual variation in performance of imidacloprid. In spite of documented variation in spotted wilt control with the Actigard 50WG + imidacloprid seedling treatment program, it remains the only effective management tool for reducing disease loss in tobacco. (Reprinted with permission)

Key terms: tomato spotted wilt virus, Actigard, imidacloprid

74. Evaluation of tobacco varieties for resistance to black shank disease in Berrien County. E. Beasley; von Waldner, M.; Edwards, P.; Bertrand, P.; and Moore, J. M, University of Georgia, Nashville GA USA

Black shank (*Phytophthora nicotianae*) , is a persistent soil-borne disease of tobacco in Georgia. The introduction of tobacco (*Nicotiana tabacum* L.) varieties with resistance to race 0 of *Phytophthora nicotianae* has led to an increased population of race 1 in Georgia tobacco fields. With the effect of selective pressure of resistant varieties to race 0, there has ultimately been a need for horizontal resistance, or tolerance, to race 1. The primary purpose of these trials was to evaluate variety CC 143 to other known varieties with relative resistance to black shank. Two variety trials were performed in Berrien County in 2014 and one in 2015. Fungicide recommendations for each field was made according to University of Georgia recommendations and grower preference. Black shank disease ratings were variable over the course of two years. However, in 2014, disease pressure was extremely high. The variety CC 143 was shown to provide consistent and acceptable black shank resistance. Comparisons could be made with numerous other varieties, which showed consistent, statistically similar results. (Reprinted with permission)

Key terms: black shank, varieties, resistance