2017 CORESTA JOINT STUDY GROUPS MEETING

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

ABSTRACTS

ORAL PRESENTATIONS

Presenter's name is underlined when the main author (listed first) is not presenting the paper

OPENING PRESENTATION 01

Brazilian tobacco production

SCHÜNKE I.

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SindiTabaco - Interstate Tobacco Industry Union - is an institution that represents and defends the common interests of the tobacco industry. It has interstate territorial base. The SindiTabaco comprises 15 associated companies. The main focus of the entity are regulatory issues, such as FCTC, sustainability of the tobacco production chain, including social and environmental responsibility and the visibility of the sector, aimed at economic and social aspects. The reasons that sustain the successful trajectory of the sector in the global market are directly related to the high volumes and styles produced, high level of quality, regular supply and the excellent processing facilities, customer services and ISO certification, an integrated production system and sustainability of its production chain. Brazil's Southern Region Tobacco Production, during the 2016/2017 crop, achieved the following figures: 566 growing municipalities, 600 thousand people employed in the rural areas, 299 thousand hectares planted, 686 thousand tons, R$ 6.1 billion income to growers, 40 thousand direct jobs within the industry and R$ 13.9 billion in taxes annually. The main challenges for SindiTabaco include actions such as sustainability improvements in the tobacco production chain, ongoing monitoring of FCTC issues and continuing communication efforts on various subjects.

IG 01

Comparison of intra-crop year variability in NNN in tobacco and NNN levels in smokeless tobacco products

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Although tobacco is an agricultural crop, little has been published on the variability in NNN levels in tobacco grown within a single crop year (intra-crop year variability) by multiple growers. Furthermore, the potential impact of intra-crop year variability of NNN levels in tobacco on NNN levels in smokeless tobacco products is unknown. In this work, NNN levels were measured in dark air-cured, dark fire-cured and Burley tobaccos used to produce smokeless tobacco products over either a six or ten year crop period. Additionally, NNN levels were measured in nine smokeless tobacco products manufactured over seven years. Only results from smokeless tobacco products manufactured using a single crop year of tobacco were included. NNN measurements over ten crop years for dark air-cured and dark fire-cured tobacco and over six years for Burley tobacco demonstrated a 10-fold range (lowest concentration to highest concentration) within a single crop year. Mean NNN levels exceeded 1 ppm in dark fire-cured and Burley tobacco in every year tested and six of ten years for dark air-cured tobacco. Depending on the specific smokeless tobacco product and year, the measured NNN levels generally varied from three to six fold within a single crop year. Over the seven years, NNN levels exceed 1 ppm (dry weight basis) for the vast majority of smokeless tobacco product measurements. The demonstrated variability in measured levels of NNN in smokeless tobacco products, due to natural variability in levels of NNN in tobacco, provides significant insight about measured NNN levels among smokeless tobacco products.

AP 01

Investigation and research of microbial information in tobacco-planted soil from different ecological regions in Guizhou Province

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Soil microbial characteristics can sensitively reflect soil quality and health index. In order to illustrate the difference in microbial information between different ecological regions, tobacco-planted soils were collected from 31 regions in Guizhou Province and the microbial community structure of soil was assessed and analysed by high throughput sequencing (16S V4 region). The Operational Taxonomic Units (OTUs) were significantly diverse in different ecological regions. The soil microbial population in the east was relatively higher than that in the west, while the north was higher than the south. Besides, the number of OTUs in north Guizhou Province was the highest, while that in northwest Guizhou Province was the lowest. The results showed that *Proteobacteria* and *Actinobacteria* were the top two microbial phyla in relative abundance in north Guizhou Province. In the middle area of Guizhou Province, *Proteobacteria* abundance was significantly denser than *Actinobacteria* abundance. The soil dominant genera in the southwest and middle district were much more than other regions in Guizhou Province. However, the microbial population was unbalanced in this area, raising the potential for soil-borne disease outbreaks. Furthermore, *Streptomyces*, *Thiobacillus* and *Pseudomonadaceae* accounted for a great proportion in the middle and the east region, the southeast area and Tianzhu district, respectively. Soil microbial community information will be better understood through this investigation and analysis. These results will supply a theoretical basis and build a good foundation for soil bio-remediation and sustainability in tobacco leaf production afterwards.

AP 02

Reverse logistics - receiving program for empty containers of pesticides from tobacco growers in southern Brazil

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The Brazilian legislation, through Decree 4.074/2002, has determined that users of agrochemicals and related products should return the empty containers and their covers to the commercial establishments where they were purchased. This legislation also provides for responsibilities on the part of distribution channels, growers, manufacturers and public authorities. In anticipation of this legislation and seeking solutions for the correct disposal of pesticide containers used by tobacco growers, the sector - led by SindiTabaco - has developed a roaming system for collecting empty pesticide containers used by tobacco growers. A specialised company, authorised by the environmental authorities, carries out the collection of the packaging at approximately 2,600 collection points in the rural areas of approximately 550 municipalities in the states of Rio Grande do Sul and Santa Catarina. In the Parana state the collecting system is made through of the local operations units and with the partnership of the tobacco industry. The technicians of the tobacco companies deliver invitations to all the approximately 120,000 integrated growers, stating the dates, times and places of receiving the empty containers. The Program has been constantly improving and now it has the use of software specifically developed for this purpose. The growers' data (register) and the quantities and types of delivered packages are recorded in mobile devices (tablets), allowing real-time monitoring, as well as obtaining reports with referred information. Over the last 17 years of operation, more than 13 million packages were collected and sent to reception centers accredited by the environmental authorities, where they undergo a screening process before being sent to recycling companies. The programme includes all kind of pesticide containers used by tobacco growers on other crops on their farms.

AP 03

Project SOSTAB: innovative traceability on the tobacco production chain in Italy

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The Italian tobacco production chain has evolved significantly over the last twenty years, and several different activities have converged into larger processing units, which interface themselves with international buyers. Therefore, common targets are required to focus all the growers according to stricter and sustainability-oriented production rules. The project aims to obtain a dynamic traceability model for the TTI tobacco production chain, upgrading its primary production to global market expectancies, according to controls and handling protocols already in force for vegetables for the fresh food market. SOSTAB deployment covers: i) heading of technical guidelines (GAP); ii) cultivation controls; iii) CPA residues matching CORESTA parameters; iv) crossing traceability data with industry grading results, assessing performances by farmer, growing plot, and variety. Data are managed using GIAS platform: web operated, modular and flexible. GIAS and traceability are framed on cadastral data, cultivation plan, storing units, farm resources. GIAS offers real-time response in crossing CPA/fertilizer data from label limits and guideline prescriptions, with planned field applications. Data input and assistance follow “smart” procedures (smartphone/tablet). Currently the programme manages tobacco traceability and storing all farm data. Each farmer is able to check and compare their performance by year, by each growing plot, and variety. Productivity evaluations are supported by objective data, both from the field side and quality performance, achieved at industrial grading. TTI joined the “Agricultural Labor Practices” (ALP) program by JTI, and is presently implementing ALP data management on the same platform. After three years of activity, SOSTAB managed to make farmers more conscious of their potential for improvement through a more attentive application of GAP and via technical indications by the agronomists. Industrial target commitments are now growers’ commitments, and this makes the production chain more aggressive and efficient with positive consequences on the quality of production.

AP 04

Contributions to the study of gall wasp occurrence in eucalyptus woodlots in southern Brazil

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Firewood from *Eucalyptus* spp. is the main source of energy for tobacco curing in South Brazil.

Leptocybe invasa Fisher & Lasalle, 2004 (Hymenoptera: Eulophidae) is a gall inducing wasp species that causes damage to eucalyptus plantations and nurseries and is currently spreading globally. The gall wasp can cause serious injuries to trees, affecting growth and compromising woodlot potential yield. In Brazil, the gall wasp was first recorded in Bahia state in 2008, reaching Rio Grande do Sul in 2013.

The objective of this paper is to share the occurrence of gall wasp in eucalyptus woodlots, bring attention to the potential risks and opportunities of this insect-pest to woodlot yield and consequently to the medium-long term availability of eucalyptus wood products associated with to tobacco production.

In January 2014 the gall wasp was observed at the Agronomy, Development, Extension and Training Center (ADET) of JTI Brazil in a trial with different eucalyptus species. Efforts have been made to further evaluate the biology of the gall wasp, the distribution and influence of the attack on the growth of eucalyptus species, and to identify eucalyptus susceptibility in collaboration between JTI, the University of Santa Maria and the University of Santa Cruz do Sul. Preliminary results indicate that eucalyptus has considerable variation in susceptibility to damage by *L. invasa* and that there is a relation between the degree of infestation by the gall wasp and woodlot productivity of the studied species. Since eucalyptus has many benefits for farmers, efforts put together to deepen knowledge about the gall wasp is focused on small-scale farming and can result in realistically applicable measures for prevention and management in the future.

AP 05

Effect of storage environment on nitrogen oxides formation in cured tobacco leaves

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Experiments were carried out to elucidate the mechanisms of TSNA formation during storage by confirming the participation of nitrogen oxides (NOx) produced from tobacco leaves under high temperature. The contents of NOx in an airtight environment set up by a vacuum desiccator were detected for different tobacco types, storage temperatures and duration, and moisture contents of tobacco. Results showed that NO as the main component accounted for more than 90 % of the NOx volatilized from tobacco leaves during storage. The NOx concentration produced from Burley tobacco leaves was almost nine times higher than that from flue-cured tobacco samples under the same storage conditions. When storage temperature increased from 10 °C to 50 °C, the concentration of NO and NO_2 volatilized from Burley tobacco increased gradually after treating for 48 h, and significant differences were observed between the five temperature treatments. NOx was produced from Burley tobacco samples in a short period of time (2 h) under 50 °C, and the volatilisation of NOx increased as the storage time increased. Under the same storage temperature, the NO and NOx concentrations of tobacco leaves with moisture content higher than 18 % were significantly lower than the samples with moisture content of 11 % and 12 %. Presence of activated carbon in the tobacco storage containers effectively reduced the concentration of NOx. Collectively, proper control of the storage environment is an effective way to reduce TSNA formation in stored tobacco leaves by inhibiting and reducing the production of NOx from tobacco itself.

AP 06

Dark air-cured and dark fire-cured tobacco TSNA levels in response to potassium source and rate

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Field experiments were conducted to evaluate the effect of potassium fertilizer source and application rate on yield, quality, and tobacco leaf chemistry constituents in dark air-cured (DAC) and dark fire-cured (DFC) tobacco. Field trials were located at the University of Kentucky Research and Education Center in Princeton, KY in 2016. Dark tobacco varieties used in both trials was KT D14LC. Potassium chloride (KCl) and potassium sulfate (K_2SO_4) were the potassium sources of interest. Treatments were applied utilizing a randomized complete block design with four replications. Each potassium source was broadcast-applied the day prior to transplanting at 100, 200, and 300 lbs K_2O $acre^{-1}$. An untreated control that received no potassium was also included. All plots received the same nitrogen and phosphorus according to soil test recommendations for the site. Numerical differences were observed in DFC and DAC treatments for N-nitrosonornicotine (NNN) levels, with KCl-treated tobacco having numerically higher NNN levels than K_2SO_4-treated tobacco. There was a main effect of potassium source as KCl treatments yielded higher than K_2SO_4 treatments in the DAC trial. However, this yield difference could be associated with significant differences in leaf moisture content when comparing potassium source. There were no differences in the quality grade index between KCl treatments and K_2SO_4 treatments. Preliminary observations and results from 2017 experiments will also be discussed.

AP 07

NNN levels in stable reduced converter (SRC) and low converter (LC) lines cured under conditions that favor NNN formation

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N-nitrosonornicotine (NNN), a tobacco specific nitrosamine (TSNA) found in cured leaf, filler and smoke, is formed by nornicotine nitrosation. We previously demonstrated a ~75 % reduction of cured leaf NNN content in Burley and dark tobaccos having stable reduced convertor status (SRC) compared to Low Converter plants (LC). Here we present NNN data for Burley and dark tobacco SRC and LC lines cured under normal conditions (control) and under conditions favourable for TSNA formation (experimental). SRC and LC Burley were housed in a control barn and air-cured according to recommended practices. Additionally, an experimental Burley barn was packed with about ~1/3 more SRC and LC Burley tobacco plants than the control barn and managed to retain high relative humidity during curing. For dark tobacco, SRC and LC lines were housed in a control barn and fire-cured according to recommended practices. An experimental Dark barn was packed with SRC and LC dark tobacco and cured at temperatures up to 173 °F having open flames during curing. At the end of curing, leaf samples were collected and analyzed for TSNAs. SRC plants had ~63 % and 64 % less NNN than LC plants in control barns for Burley and dark tobacco, respectively. NNN levels increased in SRC and LC tobaccos when cured in experimental barns compared to control barns. However, SRC NNN levels in experimental barns were ~76 and 61 % lower relative to LC plants cured under identical conditions for Burley and dark tobacco, respectively.

AP 08

N-nitrosonornicotine reduction in dark tobacco varieties and smokeless product prototypes

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During the past decades there has been a substantial effort to reduce the level of the tobacco specific nitrosamine N-nitrosonornicotine (NNN) and its precursor nornicotine in tobacco and tobacco products. Research on the mechanism of nornicotine formation led to the identification of three tobacco genes (CYP82E4, CYP82E5 and CYP82E10) encoding for cytochrome P450 nicotine demethylases that convert nicotine to nornicotine. Through conventional breeding, we developed dark tobacco (stable reduced converter/SRC) varieties containing the three non-functional nicotine demethylase genes. Tobacco varieties containing this new technology, named ZYVERT™, were grown on on-farm experiments and, leaf produced from these varieties, were used to make smokeless product prototypes. Control product prototypes were produced using leaf from commercial low converter (LC) varieties. Our results indicate that the presence of non-functional alleles of the three genes reduces the level of nornicotine and NNN in dark tobacco varieties by about 70 % compared to the level observed in commercial LC dark tobacco varieties. The reduced levels of NNN in the cured leaf of SRC varieties were also observed in smokeless product prototypes.

AP 09

The effect of farm vehicle exhaust emissions on TSNAs

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Even low concentrations of nitrogenous gases (NOx) produced during combustion in direct-fired flue-cured barns increase tobacco-specific nitrosamines (TSNAs) in the cured leaf. Previous work in France and Kentucky, and in our recent storage study, has demonstrated that air-cured leaf of stalk-cut plants left hanging on the stick in the barn accumulate more TSNAs than leaf stored in bales for the same amount of time. Air-cured barns are designed to allow farm equipment to drive into the barn to facilitate housing and take-down of the crop, and the NOx from the exhaust emissions from this equipment could be causing this increase in TSNAs. We did a proof of concept study to test whether this could be the case. A high converter selection of TN 90 was grown with normal crop production practices through curing. At the end of the cure, five-stick plots with six plants per stick were transferred from the air-curing barn to each of four sealed cabinets. Exhaust fumes from a 1970 model diesel tractor were ducted into each of three cabinets for 5, 15, or 45 minutes a day for nine days. The fourth cabinet was the untreated check. Leaf samples were collected from each treatment before the start of the gassing treatments, and then 24 hours after the first, third, and ninth exposure. Both 4-(methylnitrosamino)-1-(3-pyridyl)-1-(butanone) (NNK) and N'-nitrosonornicotine (NNN) increased as the cumulative exposure time increased. The rate of increase of NNN slowed after 135 minutes of exposure, but NNK appeared to increase exponentially as exposure increased. This marked increase of TSNAs, albeit under these extreme conditions, warrants further investigation in a more realistic farm situation.

AP 10

Genetic strategies for sucker control and evaluation

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Tobacco plants exhibit strong apical dominance. Molecular signals from shoot apical meristems (SAM) mediate hormonal signals that effectively inhibit axillary bud growth. Upon SAM removal, loss of hormonal signals enables axillary buds to grow into new shoots (or “suckers”) resulting in loss of yield and leaf quality. Therefore, development of tobaccos with delayed, limited or no suckering would reduce tobacco production cost, increase yield and improve quality. Different strategies were employed to identify candidate genes associated with sucker formation. Functional analysis of selected genes and their promoters were studied. GUS markers were used to evaluate the tissue and/or plant development specificity of targeted promoters. Axillary bud specific promoters were identified and fused with selected target genes for axillary bud expression. Transgenic plants were grown to layby, 8-10 leaf stage and then topped and evaluated for reduced sucker phenotypes. Over-expression of candidate gene(s) with axillary bud specific promoter(s) resulted in suppression of axillary bud growth and branch development up to four weeks after topping. The mechanisms for axillary meristem initiation and development are under investigation.

AP 11

Effects of genotype and environment on metabolite profiling of *Nicotiana tabacum*

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In order to have a better understanding of the effects of genotype and environment on the quality of tobacco, three tobacco cultivars were selected as materials and planted at three representative regions of China. Six different developmental stages were sampled for metabolomic analysis.

More than 700 metabolites were identified by metabolomic analysis and target metabolites were around 500. Around 200 metabolites, including sugar, lipid and amino acids, were found to be different among the three regions. Principal component analysis (PCA) results showed that metabolites of tobacco had obvious regional characteristics, especially for Henan. ANOVA analysis revealed the effect of environment is stronger than genotype, and developmental stage is between them. However, interaction among them had little effect. Of the many environmental factors, temperature was the most important one. Different metabolites had different genotype and environment preference. Lipids may be mostly regulated by environment. However, several secondary metabolites may be affected by genotype.

It may be concluded that for different metabolites, different strategies should be used to regulate them, according to the contributions of genotype and environment. Our findings lay a foundation for future genetic studies and provide guidance for breeding high-quality tobacco cultivars.

AP 12

Genetic and biochemical analysis of low alkaloid lines with improved leaf quality

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Nicotine is the most abundant alkaloid in cultivated tobacco (*Nicotiana tabacum*), typically constituting more than 90 % of total alkaloids. Genetic control of nicotine biosynthesis in tobacco and subsequent development of low alkaloid traits has been derived historically from nic1/nic2 mutant lines. However, the quality of cured leaf from these mutant lines is commercially undesirable. Our research showed that nicotine levels in Putrescine N-methyltransferase (PMT) RNAi and PR50-RNAi lines were reduced more than 95 % and 80-90 %, respectively. Both RNAi lines had significantly better leaf grade index after curing compared to nic1/nic2 mutant controls. Using gene editing technology, mutant PMT and PR50 lines were generated and correlations of alkaloid levels with various mutated PMT isogenes were evaluated. In addition, RNA seq and metabolomics studies were performed on the different low alkaloid lines having a range of leaf quality scores. LA Bu21, which is a nic1/nic2 deleted mutant, had a strongly differentiated alkaloid metabolic profile compared to PMT or PR50 RNAi lines. PR50 RNAi lines are relatively closer to controls in their metabolite profiles compared to PMT RNAi and LA Bu21 lines. These results are consistent with PR50 RNAi leaf grade index. In conclusion, nic1nic2 deletion impacts large number of metabolites resulting in poor leaf quality, whereas targeted gene reduction has minimal impact on metabolites and leaf quality.

AP 13

The inhibitory effects of salicylic acid on the potassium outflow from tobacco

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Potassium (K^+), as one of the most important nutrient elements, plays important roles in tobacco production, such as stress resistance and improvement of leaf combustibility and quality. After topping or during drought stress, K^+ usually outflows from tobacco, which has negative impacts on tobacco production. So, it is necessary and valuable to study the control of the K^+ outflow from tobacco. The objective of this study was to explore the effects of salicylic acid (SA) on the K^+ outflow from tobacco. Different concentrations and application methods of SA were adopted, and the K^+ content in tobacco leaf or roots culture solution were analysed before and after topping. Then, the RNAi lines with the knockout of gene TORK1, the only one known K^+ efflux channel in tobacco, were used to verify the effects of SA. The results showed that topping led to the increase of K^+ outflow from tobacco roots and decreased the K^+ content in different leaf positions. Five µM or 50 µM SA applied to culture solution could inhibit the K^+ outflow, while 500 µM SA had opposite effects. Additionally, the reduction of leaf K^+ could be alleviated when placing a cotton ball saturated with 5 µM SA onto the topped area, but SA injection into the vein had no distinguishable effect. The gene expression of TORK1 was significantly inhibited in the RNAi lines by 5 µM Estrogen, and was further inhibited by adding 50 µM SA into the culture solution. As TORK1 expression decreased, the K^+ content also decreased. However, there was no obvious effects of 500 µM SA on gene expression and K^+ content. In conclusion, SA regulates the tobacco K^+ outflow through the TORK1 channel. These results provide an original foundation for future applications of SA to tobacco.

AP 14

Chemically topping Burley tobacco

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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 series of experiments were initiated in 2015 to evaluate the efficacy of chemically topping Burley tobacco. The major objectives are to determine if Burley tobacco can be chemically topped, the appropriate product and rate of suckercide chemical, optimum timing of application, and appropriate Burley tobacco maturity group 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 suckercide 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. Our data has suggested that pre-bud (10 % button) and early-bud (50 % button) timings are best suited for chemical topping practices as treatments that target 10 % bloom stages did not completely halt inflorescence. Impacts of suckercide product rate and timing of application on leaf yield, sucker control, quality grade index, MH residues, and leaf dimensions for the agronomic portion of these experiments will be discussed.

AP 15

Effect of priming as a practice for improving yield and quality of flue-cured tobacco in Malawi

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Priming of leaves improves yield and quality of flue-cured tobacco. A field experiment was conducted at three locations in Malawi during the 2013 to 2016 cropping seasons. The aim was to investigate the effect of priming bottom leaves on yield and quality of cured leaf. The trial was laid out in a randomised complete block design with a split plot arrangement replicated three times. The main plot comprised of three priming times, namely (1) Time of priming at topdressing, (2) Priming 2 weeks after topdressing and (3) Priming at topping. The subplots had three leaf priming levels, namely by the removal of 2, 4 or 6 bottom leaves. A plant spacing of 1.2 m between rows and 0.60 m within rows was used. All recommended agronomic practices in flue-cured tobacco production were observed. The results showed that there was a significant interaction between time of priming and number of leaves to be primed on the growth, yield and quality of flue-cured tobacco. The trial findings showed that priming four leaves at topdressing recorded 40 % and 35.9 % yield and first grade leaf above the control treatments respectively. Specifically, priming four leaves at topdressing improved orange proportions by 100 % and improved overall farm income by 26.68 % over the blanket practice. This study therefore confirmed that priming bottom leaves at topdressing time formed an integral practice for improved flue-cured tobacco production. It was confirmed that farmers benefit more when priming is done at topdressing by removing four leaves for improved yield and quality of the flue-cured tobacco.

AP 16

Impact of removal of the lower stalk leaves, time of topping and leaf number on yield and quality of flue-cured Virginia

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The Brazilian tobacco is known around the word for its flavour, especially derived from upper stalk leaves. Currently, there is a market oversupply of lugs of flue-cured Virginia (FCV) and it has become an issue for the industry and farmers worldwide. The objective of this study was to evaluate the impact of removal or not harvesting the lugs on yield and quality, considering time of topping and topping height. During the 2016 crop, trials were carried out at two locations in southern Brazil in a randomised complete block design. The treatments comprised the removal or no harvesting, time of topping and number of leaves left on the stalk (topping height) and the variables evaluated were yield, grade index and revenue, as well as analysis of the percentage per stalk position. According to the results, not harvesting the bottom six leaves, topping at 65 days after transplant (DAT) with 18 leaves left on the plant decreased by 9.2 % the amount of lugs when compared with the control. Not harvesting the bottom six leaves and topping at the first extended flower decreased lugs by 10.35 % when compared to the control and the removal of the six bottom leaves at 65 DAT and topping at the first extended flower decreased by 8.75 % the amount of lugs when compared to the control. However, these treatments had a negative impact on yield and quality of cured leaves when compared to the control.

From these results, it can be inferred that not harvesting the bottom six leaves and topping at the first extended flower is a promising practice to decrease lug leaves in Southern Brazil. It also brings the benefit of not spending resources on removing the leaves from the plants, but further studies will be carried out aiming to improve yield and quality when the lower leaves are not harvested.

AP 17

Effect of bottom leaf removal and fertiliser rates on the yield and quality of flue-cured tobacco in Zimbabwe

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The leaf position on a tobacco stalk determines leaf quality. Leaf quality is lowest for the bottom prime and lug grades, and is highest for the leaf and tip grades at the upper part of the plant. Experiments on removal of lower leaves to eliminate prime (P) and lug (X) grades date back to the nineties; and results then indicated that there was no merit in this agronomic practice. Recently, there were changes in market preferences, and merchants began demanding more of the upper leaves and paying a premium for these. This necessitated the re-initiation of leaf priming studies using two new higher yielding varieties at two planting dates (September and October). In these experiments, up to ten bottom leaves were removed, and topping was done to reduce the number of remaining leaves to 14 against the standard of 18 leaves. In some treatments, additional fertilisers were applied to improve yield and quality of the remaining leaves. The highest value gain (~US$2839) was obtained when six bottom leaves were pruned. However, an additional 400 kg/ha of high analysis fertiliser was applied at pruning, resulting in a yield increase of 1104 kg/ha compared to the standard (no priming, no additional fertiliser). The same trend was obtained in the previous season, where 421 kg/ha yield increase was observed when six leaves were pruned with similar fertiliser adjustments. The cost-benefit analysis of the bottom leaf removal exercise generally resulted in economic merits in the irrigated trials, more so with the fast growing variety, KRK26. Furthermore, lower priming rates are ideal for the late planted crop as increased pruning resulted in yield losses, and fertiliser adjustments can be necessary to compensate for nutrients lost during pruning. The bottom leaf removal exercise could enhance sustainability in tobacco production and improve growers' income.

AP 18

The effect of lower leaf removal and nitrogen application to flue-cured tobacco yield, crop throw, and economic return

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Research was conducted in North Carolina to quantify the impact of the number of lower stalk leaves removed at topping and the subsequent application of four rates of liquid nitrogen. At topping, either 0, 4, or 8 leaves were removed from the lower stalk positions of flue-cured tobacco. Immediately following leaf removal, nitrogen (28 % liquid Urea-Ammonium-Nitrate) was band applied adjacent to plants at one of four rates: 0, 5.6, 11.2, or 16.9 kg N/ha in a solution volume of 187 L/ha. Leaf density, SPAD measurements, and tissue samples were collected over a five week period following treatment application to quantify changes in leaf morphology and nitrogen content. Following the conclusion of the growing season, leaf yield, quality, value, crop throw, and economic value were determined. Preliminary results demonstrate reductions in crop yield of 448 and 896 kg/ha when four and eight leaves were removed, respectively. In addition, crop value declined by $1,697 to 2,989/ha in the same leaf removal scenarios. Nitrogen application did not improve leaf yield enough to offset the financial losses incurred by leaf removal, nor did it appear to have significantly influenced leaf morphology. Despite dramatic yield and economic losses, the goal of reducing priming and lug grades was achieved in both leaf removal number treatments, with the four and eight leaf removal systems producing 19 and 3 % X grades, respectively, compared to 33 % X grades where leaf removal did not occur. Despite such unfavorable results in 2016, it is hypothesized that poor growing conditions (excessive heat and low precipitation) experienced after leaf removal and nitrogen application prohibited plants from recovering at least a portion of yield and value. Research will continue in the 2017 growing season.

AP 19

Organic fertilizer programs for tobacco seedling production

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Organic tobacco production in North Carolina has increased in recent years. Despite increasing interest, little is known regarding the appropriate management of organic nitrogen sources in a seedling float system. Research was conducted in North Carolina to evaluate three organic nitrogen programs and their effects on float water nutrition and seedling growth. Treatments evaluated consisted of Peruvian Seabird Guano (SG), sodium nitrate (SN), or a combination of SG+SN. A conventional fertilizer source (SQM 16-5-16) was included for comparison. Treatments containing SN as the sole source of nitrogen failed to produce usable seedlings due to the absence of phosphorus in the selected fertilizer program. Seedling growth and development was acceptable in treatments comprised of SG or SG+SN, and was similar to that of 16-5-16. Ammonium float water concentration was greatest in SG treatments 25 days after seeding (DAS), but declined rapidly over the following 20 to 30 days. The decline in ammonium concentration was complimented by an increase in nitrate concentration during the same period. Bicarbonate concentration was greatest in SG only (≥12.0 meq/L) and SG+SN (≥3.0 meq/L) treatments 25 DAS but was <1.0 meq/L in SN only treatments, further implicating SG as a source of bicarbonates in organic float systems. Despite the high bicarbonate concentrations documented in SG treatments, seedling growth was not impacted. Seabird Guano and SG+SN based fertility programs produced seedlings comparable to 16-5-16 and appear to be suitable for the production of organic tobacco seedlings. These fertility programs should be managed to include additional nutrients, such as phosphorus, in order to provide a complete nutrition program. Furthermore, bicarbonates should be monitored and corrected accordingly.

AP 20

Is float bed aeration beneficial to organic seedling production?

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At present, the effects of float bed aeration in the production of organic tobacco seedlings are not known. Research was conducted in North Carolina to evaluate two organic nitrogen fertilizer programs and their effects to float water nutrient concentration and seedling growth. Treatments evaluated consisted of 12-11-2 Peruvian Seabird Guano (SG) or a combination of SG + 16-0-0 Sodium Nitrate (SN). A conventional fertilizer source (SQM 16-5-16) was included for comparison. Each fertilizer source was evaluated with and without the continuous addition of oxygen from a Hydor H2Show Bubble Maker. Dissolved oxygen (DO) concentration was maintained at 5.5 to 7.5 mg O_2/L in aerated treatments. In the absence of aeration, DO concentration was commonly ≤1.0 mg O_2/L in SG treatments measured 25 or more days after seeding (DAS). Aeration reduced bicarbonate concentration by 50-83 % and promoted the oxidation of ammonium to nitrate must faster than non-aerated treatments, particularly in SG and SG+SN fertility programs. Slight differences were observed in seedling usability and vigor, with aeration and SG+SN treatments producing higher quality seedlings than SG alone. Results indicate that float bed aeration is beneficial in the production of organic tobacco seedlings and can be used to reduce bicarbonate concentration while promoting ammonium oxidation.

AP 21

Polypot system: a cost saving practice for improved tobacco seedling quality in Malawi

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This trial investigated a tobacco seedling production system known as the Polypot which is a slight variant of the Motherbed. It aimed to evaluate the effectiveness, benefits and challenges of the Polypot in relation to the other seedling production systems at three locations in Malawi from 2013 to 2016. The trial tested three seedling production systems, namely Conventional, Motherbed and Polypot. Data was collected on seedling heights, root volumes, stem diameters, number of leaves per seedling and number of over-grown seedlings, amount of water used and survival rates of each system. Results showed that seedlings from Polypots and Motherbeds were relatively taller in height, with thicker stems, had more leaves and bigger root volumes than the seedlings from the Conventional system. Seedlings raised from the Polypot system saved 71 % water compared to the Conventional seedbeds. And when planted out in the field, seedlings from the Polypot system had almost 100 % stand count compared to the ones from the Conventional beds. On yield, the Polypot, although statistically similar to Motherbed, had a 10 % yield advantage over the Motherbed system and 21 % yield advantage over the Conventional system. The Polypot also saved 69 % of watering labour compared to the Conventional system. Almost all selected farmers who tested this system claimed that the system is efficient, easy to use, secure and reliable. The partial budget analysis showed that there was a gain of USD 115.40 per hectare (1 USD = MWK 720) as in December 2016) when farmers tested the Polypot instead of the Conventional system. It was therefore the finding of this study that the Polypot system offered farmers an opportunity to reduce the cost of tobacco production in the nursery while undertaking mitigating measures against climate change in situations of water scarcity.

AP 22

Evaluation of soil solarisation as a tool for Integrated Pest Management (IPM) in small tobacco farms in southern Brazil

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Soil solarisation has a successful history as an environmentally friendly tool for IPM using solar power to control a range of soilborn plant pathogens. Therefore, this technique was evaluated in tobacco production areas in southern Brazil as a complementary practice to pest control in order to better support the sustainability of tobacco production on small farms. Seven distinct small farms with historical tobacco production were selected for this trial. Each area comprised two macro plots (8.4 × 25 meters) with treated (solarised) and untreated areas. All macro plots were uniformly prepared, over irrigated and treated ones covered with a transparent plastic sheet. Probes were installed in all plots to measure the temperature at 10 cm depth (data points collected every hour) during 46 days. Soil samples (7 per plot; treated and untreated) were collected just before and after treatment for analysis of microorganisms, nematodes, insect larvae and weed control. These plots were under crop rotation with black oat, where tobacco plants will be cultivated (200 plants per plot; 4 rows of 50 plants). These plots will be evaluated for pest incidence, yield and quality. All treated plots recorded a minimum of 577 hours above 40 °C at 10 cm depth. This solarisation treatment was effective in reducing *Ralstonia solanacearum* (all plots) and *Fusarium* sp (majority of plots) populations. This technique was also efficient for nematode populations reduction (*Helicotylenchus* sp, *Meloidogyne* sp and *Pratylencus* sp). Insect larvae were not observed in these experimental areas. Weeds were not observed in treated plots (100 % control), except for *Cyperus rotundus*. All results summarised above suggest that solarisation is a potential IPM tool to pest control in tobacco production areas in southern Brazil. The technique could be used as a complementary support towards sustainable production on small tobacco farms.

AP 23

Resistance and control of yellow stunt disease in flue-cured tobacco in southern Brazil

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Yellow stunt disease, also known as field root-rot, is primarily caused by *Pythium dissotocum* and has been detected over all the flue-cured tobacco (FCV) production regions in southern Brazil over recent years, prevailing in the state of Rio Grande do Sul (RS). It is estimated that farmers supplying FCV tobacco to Alliance One Brazil (AOB) suffer losses of more than 6 % due to the incidence of the disease, depending on the year. The objective of this study was to find an integrated solution to minimise losses caused by yellow stunt. Two research focuses were developed. Field trials on infested fields were carried out for testing several active ingredients of fungicides and times of application. Experiments to evaluate the resistance of commercially grown FCV hybrids (AOV 405, AOV 2103, AOV 413) with and without application of fungicide in the field (Fluopicolid + Propamocarb) were also conducted. The AOB breeding program started screening, selecting and crossing resistant genotypes on nurseries, which resulted in releasing a FCV hybrid highly resistant to yellow stunt in 2013, the hybrid AOV 413. Regarding the fungicide trials, Azoxystrobin and Fluopicolid + Propamocarb showed efficient disease control in susceptible cultivars. There were no significant differences for yield and quality of the AOV 413 with and without fungicide spraying, showing that the hybrid is highly resistant to this disease.

AP 25

Tobacco genomic resources for gene discovery

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As one of the most widely cultivated non-food crops grown in around 120 countries worldwide, *Nicotiana tabacum* holds significance as both an economically important crop and also as a model organism. Historically it has played a key role in the development of tissue culture and transformation techniques now widely used across all branches of plant molecular biology. As an allotetraploid formed by the recent interspecific hybridisation of diploid parents *N. sylvestris* and *N. tomentosiformis*, its genome is large and complex: approximately 4.5 Gb in size and comprised of around 70 % repeat elements. As a result of such genomic complexity, production of a genome sequence with sufficient quality to anchor to chromosomes has been a challenge.

We have recently published an assembly of the K326 cultivar that has been produced through the combination of optical mapping and next generation sequencing technologies. This assembly achieves an N_{50} size of 2.17 Mb and enables anchoring of 64 % of the genome to chromosomal locations, dramatically increasing the utility of the genome to the community.

In support of the public release of the sequence, a number of tools have been made available via the Solanaceae Genomics Network to enable the use of the genome as a resource for gene discovery (www.sgn.cornell.edu/organism/Nicotiana_tabacum/genome). These include a JBrowse genome browser, a BLAST facility and a VIGS design tool. In this talk, we will demonstrate the utility of these resources in the discovery and characterisation of gene function in the tobacco plant.

AP 26

Combining gene expression, metabolomics, and conventional breeding to increase the nitrogen use efficiency of Burley tobacco

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High rates of nitrogen fertilization are required currently for cultivation of Burley tobacco to achieve the desired leaf yields and qualities. However, Maryland type tobaccos are grown using a nitrogen fertilization rate that is ~60-70 % lower than that used typically for Burley. Increased nitrogen use efficiency (NUE) can help increase productivity, reduce environmental impact and potentially limit undesired nitrogen containing constituents. The objective of this study was to understand the NUE potential of Maryland tobacco by identifying possible gene targets that differentiate nitrogen utilization of Maryland tobacco from Burley tobacco. Maryland and Burley tobacco plants were grown in the greenhouse using either 100 ppm nitrogen or 25 ppm nitrogen and were fed continuously in an ebb and flow system. Gene expression was analyzed at a whole genome level by RNA-seq. Metabolite profiling was performed using multiple approaches. Twenty-seven diagnostic metabolites were found that discriminate between the Maryland and Burley phenotypes. Correlation analysis was used to determine which genes had expression levels that correlated with 27 metabolites. Whole genome SNP analysis of breeding populations coupled with the gene expression correlations revealed genomic loci with concentrated numbers of differentially expressed genes. This study not only revealed possible gene targets that could be modified to improve nitrogen utilization in Burley tobacco, but also identified genetic loci that could be used for selecting nitrogen use efficient lines from breeding programs in the future.

AP 27

Insights of whole genomic studies in Burley recurrent selection program

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The whole genomic prediction (WGP) and Genome Wide Association Study (GWAS) is gaining every day more space in breeding programs in different species. To use the WGP, a “big” number of molecular markers covering the genome of the specie is required and by the statistical models it is possible to associate them with the phenotype in order to increase the efficiency of the program. In tobacco breeding these methodologies are not applied like in corn and soybean. So there are many questions about the use and the efficiency of these methods. In this way the objective of this study is to evaluate the possibility of the usage of WGP and GWAS in tobacco recurrent breeding programs. For this 193 S0:1 progeny from the Cycle “0” of Burley recurrent selection program were evaluated in lattice design with three replications in two environments. The characteristics evaluated were yield (kg of leaves per plot) and total alkaloid content. DNA samples were collected from all the progenies to obtain genotyping-by-sequencing (GBS) data. The Genomic Best Linear Unbiased Prediction (GBLUP) additive model was used with the cross validation to access the prediction accuracy (phenotype × genotype) of the model. After the data imputation and filter process, 3.185 markers were used to run the model. The prediction accuracy in the cross validation using 70 % of the genotypes to fit the model were below 20 %. The GWAS study showed peaks around reported genes in the literature. These preliminary results shows that to incorporate the WGP and GWAS in the breeding program more studies should be done.

AP 28

Identification of the genes underlying the yellow Burley phenotype in *Nicotiana tabacum*

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The primary distinguishing characteristic of cultivars of the Burley tobacco market class is their high degree of chlorophyll deficiency, particularly on stems, stalks, and midveins, which becomes accentuated during plant maturation. This 'yellow Burley' phenotype is conferred by a double homozygous recessive genotype (yb_1yb_1 yb_2yb_2) at the *Yellow Burley 1* (Yb_1) and *Yellow Burley 2* (Yb_2) loci. This genetic deficiency affects the physiology of Burley tobacco cultivars and translates into, among other things, reduced N utilization efficiency and increased potential for accumulation of tobacco-specific nitrosamines, a potent class of carcinogens that exists in greater amounts in tobacco products containing cured Burley tobacco leaves. Through map-based gene discovery and transgene complementation, we have shown that Yb_1 and Yb_2 encode for proteins with a very high degree of similarity to a characterized gene from *Arabidopsis* involved in chloroplast maintenance. Genetic analysis of a historical set of tobacco cultivars indicates that Burley tobacco evolved via a two-step process: an 8 bp deletion in Yb_2, followed by human selection of a spontaneous homozygous 1 bp insertion in Yb_1, whereby both mutations lead to truncated protein products. The identification of the genes at the Yb_1 and Yb_2 loci represents the first reported example of map-based gene discovery in *N. tabacum*, and their identification sheds light on the physiological differences between Burley tobacco and other tobacco market classes.

AP 29

Comparative proteomic analysis reveals differential protein and energy metabolisms from two tobacco cultivars in response to cold stress

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Given the development of tobacco is sensitive to low temperature, cold stress is a critical problem for tobacco production. Uncovering of the mechanisms for cold response in tobacco would contribute to improving its cold tolerance, but the precise molecular mechanism in this process remains unclear. In our novel screening for cold sensitivity in different tobacco cultivars, NC567 displayed a cold tolerance phenotype, while Taiyan8 is hypersensitive to low temperatures. In the current study, the samples of NC567 and Taiyan8, with or without cold treatment, were used in iTRAQ based proteomics to uncover their different mechanisms in response to cold stress. A total of 4317 distinct proteins were identified and the differential proteins in four comparison sets were used for further analysis. The GO analysis indicated that the majority of differentially expressed proteins were involved in metabolic and cellular processes. 55 proteins decreased in NC567 but increased in Taiyan8 under low temperature, while the levels of 42 proteins were lower in Taiyan8 at normal temperature but higher in Taiyan8 than NC567 under cold treatment, suggesting the variations for cold response in these cultivars. The levels of components involved in protein synthesis/degradation, photosynthesis/respiration, as well as ROS scavenging, were significantly different in these comparison sets, providing evidence for a model that protein metabolism and energy balance are essential for creation of an intracellular environment in response to low temperature in tobacco cells. In conclusion, our study identified several novel pathways associated with low temperature stress and provides hints for the further improvement of cold tolerance in tobacco plants.

AP 30

Feasibility to develop ultra-low nicotine tobacco leaf complying with proposed future nicotine regulation - a review of genetic approaches

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One of the objectives of the WHO Framework Convention on Tobacco Control (FCTC) is to reduce the addictiveness of the tobacco product. To achieve this objective, the WHO Tobacco Regulation study group issued a recommendation to mandate a reduction in cigarette blend nicotine levels to 0.04 %. But is this technically feasible? Blend nicotine content could be significantly reduced using chemical nicotine extraction methods, however essential flavour compounds were also extracted resulting in poor quality tobacco. Cigarettes made using this process were met with low consumer acceptance and were a commercial failure. The focus is now on identifying genetic approaches to reduce nicotine content without affecting tobacco leaf quality. This review will examine the various genetic approaches being explored to reduce nicotine content, with respect to their ability to reduce nicotine and the effects on toxicant levels (e.g. TSNAs) and leaf quality (where such data is available). Some of these include the knockout or silencing of the main nicotine metabolic pathway genes, modulation of transcription factor regulatory genes, the application of non-coding RNAs, and transporter genes. Nicotine is known to affect the flavour amplitude and the impact of cigarette smoke. Complying with potential ultra-low cigarette blend nicotine ceilings creates a challenge for the tobacco industry to develop cigarettes that are acceptable to consumers.

AP 31

Growth and nutrient absorption by flue-cured tobacco in field conditions

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Knowledge of nutrient absorption during the tobacco plants development cycle is essential for the specific fertilisation programmes for long cycle varieties and its growing conditions, to fine-tune the management and fertiliser use, as well as reduce costs and environmental risks. It is therefore possible that the nutritional requirements of long cycle varieties are not the same as short cycle in which nutrient absorption was studied and described in the literature. The objective of this study was to evaluate the vegetative growth and nutrient uptake by tobacco plants in field conditions. Field experiments with flue-cured tobacco were conducted at six locations with different soil and climatic conditions. Tobacco plants were harvested every seven days and separated into leaves and stalks for plant dry matter and nutrient content determinations. The results indicated that the dry matter accumulation rate was similar to the nutrients curve uptake shape but it was not synchronous, the maximum dry matter rate occurred at 81 days after transplanting. The amounts of nutrient uptaken by tobacco plants decreased following the order K, N, Ca, Mg, S, P, Mn, Fe, B, Zn, Cu e Mo. Flue-cured tobacco absorbed very little macro and micronutrients in the first 40 days after transplanting, increasing the uptake sharply after that and achieving the maximum uptake rate from 68 to 78 days after transplanting. The greater percentage of N, P and K absorption occur from 49 to 91 days after transplanting achieving 75,2 %, 72,4 % and 79,1 % of total uptake, respectively. These results can be used for optimising the sidedressing timing and clearly indicated that the nutrient absorption of long cycle varieties is not the same of that of short cycle.

AP 32

Evaluation of various NPK fertilizer formulations applied as side dressing to tobacco in Santa Cruz do Sul region, RS, Brazil

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The objective of this research was to evaluate the qualitative and quantitative yield of tobacco (*Nicotiana tabacum* L.) comparing various formulations of nitrogen-potassium fertilizers applied as sidedress treatments. Six trials were conducted in distinct flue-cured tobacco production areas in Santa Cruz do Sul region, RS, Brazil. Normal base fertilization of 640 kg/ha of the formula 10-16-10 was applied. Later, sidedress fertilization was applied, which varied between two and three applications according to the need for (N) replacement in each area. The experimental design was a randomized complete block. Management and cultural treatment followed the recommendations of the companies to which the producers were commercially linked. Tobacco was harvested four times. Sidedress applications of experimental treatments (Trt. 1. 15-0-15 contains KNO_3 [potassium nitrate] / 15-0-15 contains KNO_3 / 15-0-15 contains KNO_3; Trt. 2. 15-3-15 contains $NaNO_4$ [sodium nitrate] and SOP [sulfate of potash] / 15-0-15 contains KNO_3 / 15 -0-15 contains KNO_3; Trt. 3. 15-3-15 contains SOP / 15-3-15 contains SOP / 15-3-15 contains SOP; Trt. 4. 15-3-15 contains SOP / 14-0-15 contains $CaNO_3$ [calcium nitrate] and KNO_3 / 14-0-15 contains $CaNO_3$ and KNO_3) of 320 kg/ha were applied twice in two years with a third application of 160 kg/ha in the third year to compensate for leaching. The qualitative and quantitative yield data were statistically analyzed using the SASM-Agri system for the analysis of variance and the Duncan test at 5 %. Cured leaf yield was similar for all treatments. However, higher quality of the leaf from treatments 1, 2 and 3 resulted in greater income for the cured leaf amounting to 7, 8 and 6 percent, respectively, when compared to the income from treatment 4. These trials suggest an advantage in leaf value and income to the grower when KNO_3 is used as the primary source of sidedress fertilizer.

AP 33

The effect of chloride application rate to the yield, quality, and chemical constituents of flue-cured tobacco

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Chloride (Cl^-) application rates that exceed 33.6 kg/ha are generally discouraged in the production of flue-cured tobacco due to the negative effects the micronutrient can impart to yield, quality, and smoke flavor/aroma. Despite the negative effects associated with Cl^- application, unintentional applications of the nutrient sometimes occur. To quantify the season long effects of Cl^- application, research was conducted in North Carolina. In 2016, four application rates of Cl^- (0, 33.6, 67.3, and 100.9 kg Cl^-/ha) were evaluated in flue-cured tobacco fertilized with liquid 28 % urea-ammonium-nitrate. Tissue samples were collected from the fourth leaf below the apical meristem at five growth stages: two weeks after fertilizer application, at layby, two weeks after layby, at topping, and after curing. Leaf Cl^- concentration increased with Cl^- application rate, with the 67.3 and 100.9 kg rates typically being greater than the one percent threshold established by industry. In addition, cured leaf Cl^- concentration was greater than one percent in all treatments where Cl^- was applied. Despite high Cl^- concentration, toxicity symptoms were not observed in either growing environment, indicating that visual symptoms of excessive Cl^- cannot be solely relied upon to determine if excessive application of Cl^- has occurred. Leaf yield, quality, and value were not affected by Cl^- application; although, it is probable that smoke flavor and aroma would be negatively impacted in treatments receiving >67.3 kg Cl^-/ha. The concentration of other nutrients (N, P, K, Mg, S, and NO_3^-) was generally not affected by Cl^- application rate within each sampling interval. Research will continue in three additional North Carolina environments in 2017.

AP 34

Humic and fulvic acid as biostimulants in tobacco production

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Humic (HS) and fulvic (FS) substances are formed by chemical and biological transformations of plant and animal matter and from microbial metabolism, and represent the major pool of organic carbon at the earth's surface. Due to the stabilization of HS in humic-clay aggregates and their intrinsically slow rate of mineralization in soil they are not however considered appropriate for a direct source of nutrients to plants (i.e. as a replacement for N and P based fertilisers). Three field experiments were conducted in 2015 to evaluate the effect of humic and fulvic acid application associated with the reduction of base fertiliser on tobacco yield and nutrient uptake. The experiment had a 4×3 factorial in a completely randomized block design with four replications, with four treatments of humic and fulvic acid (0, 3, 6 and 9 L ha^{-1}) associated with three base fertilisation rates (soil test recommendation, 0.75 × the soil test recommendation and 0.5 × the soil test recommendation). Humic and fulvic acid was applied at 30 days after transplanting via drench. The results showed that there is no response on tobacco yield to HS and HS application. Decreases of 6.2 % and 11.1 % in yield were seen as base fertilisation reduced from the 0.75× and 0.5× to the soil test recommendation, respectively. There is no effect on foliar content of N, P and K with HS and FS application. The results showed that there is no effect of HS and FS on tobacco production and the base fertilisation cannot be replaced by the usage of HS and FS.

AP 35

Spotted wilt control update

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Spotted wilt continues to cause losses in Georgia tobacco. The only effective means of reducing spotted wilt loss is a foliar spray of acibenzolar-S-methyl followed by a spray on/rinse off tray drench of imidacloprid. In 76 trials conducted from 2000 to 2009 this program was found to deliver a mean reduction in spotted wilt incidence of about 52 %. Some variation in results was seen during these tests but it could not be defined beyond application errors. With the onset of contract production in 2005 most transplant production consolidated into a few commercial plant growers. Currently about 75 % of the tobacco transplants used in Georgia are grown and treated by 3-4 growers. This consolidation should eliminate, or magnify application errors. Since 2009 we have monitored spotted wilt incidence and control at 10 or more locations. A marked annual variation in spotted wilt control has been seen. The reasons are not fully understood but evidence suggests much of this variation is due to variable effectiveness of imidacloprid. Late season breakdowns in aphid and spotted wilt control support this conclusion. Imidacloprid insensitive tobacco thrips have been found in treated peanut and cotton fields but the insensitivity has not persisted in greenhouse colonies. Tobacco thrips inhabiting late winter/early spring weeds have not been tested. Area environmental conditions cannot account for annual variation in spotted wilt control. Transplant date trials conducted at the UGA research farm, where specific weather data is recorded, have shown marked variation in spotted wilt control from one date to the next. In these trials planting into cooler/wetter soil has tended to result in reduced spotted wilt control (R^2=0.46; p=0.05). these same conditions delay or slow early plant growth which has been suspected to reduce spotted wilt control in farm trials.

AP 36

Study on the anti-TMV activity of the alkaloids from *Nicotiana glutinosa*

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Tobacco mosaic virus (TMV) is one of the main diseases of tobacco. This study aims to investigate the anti-TMV activity of chemical compounds from tobacco by using the bioassay-guided method and provide the basis for anti-viral biological pesticides from tobacco. The constituent of anti-TMV activity was isolated from *Nicotiana glutinosa* in Yuxi by bioassay. Alkaloids that include three new compounds (1-3) and nine known ones (4-12), were isolated from the whole-plant of *N. glutinosa* by various chromatographies such as silica gel, Sephadex LH-20, RP-18 column chromatography and HPLC. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D NMR techniques. These compounds were also evaluated for their anti-tobacco mosaic virus (anti-TMV) activity. The results revealed that the compounds 1, 2 and 3 exhibited high anti-TMV activity with inhibition rates of 93.1 %, 81.6 % and 71.5 %, respectively. The rates are higher than those of the positive control. The other compounds also showed potential anti-TMV activity. This is the first report on the defense chemicals in tobacco, and provides the scientific basis for future systemic research.

AP 37

Loss-of-function of a tobacco eukaryotic translation initiation factor confers resistance to tobacco bushy top virus

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Tobacco bushy top disease causes severe damage to tobacco (*Nicotiana tabacum*) in Sub-Saharan African countries such as Malawi, Zambia and Tanzania. Reportedly this disease is caused by a complex of tobacco bushy top virus (TBTV, the genus *Umbravirus*) and its helper virus (a member of the genus *Polerovirus*) for aphid transmission. To control this disease, the utilisation of resistant varieties is efficient and economical, although there are currently no resistance varieties for commercial production. One breeding strategy for resistance to viral diseases is inactivation of a recessive gene such as eukaryotic translation initiation factors (eIFs). Various viruses selectively use their host eIFs for replication and cell-to-cell movement. A number of studies have demonstrated that an impaired eIF confers recessive resistance to potyviruses in various host plants. This study was conducted to identify an eIF for which loss-of-function leads to resistance to TBTV, and to obtain its mutant tobacco plants for development of resistant varieties. First, transgenic tobacco plants with suppressed expression of eIFs gene by RNAi were produced. Then these progenies were inoculated mechanically with TBTV. Results show that *eIF(iso)4E*-silenced lines exhibited reduced susceptibility to TBTV. Next, each mutant tobacco plant harboring nonsense mutation within *eIF(iso)4E-S* encoded in the S-genome derived from *N. sylvestris* and *eIF(iso)4E-T* encoded in the T-genome derived from *N. tomentosiformis* was screened from ethyl methanesulfonate-mutagenized tobacco library. Subsequently, double mutant (sstt) and wild type (SSTT) for *eIF(iso)4E* were generated by crossing between single mutants of *eIF(iso)4E-S* (ssTT) and *eIF(iso)4E-T* (SStt). Results of TBTV inoculation assay of mutant lines showed that loss-of-function of *eIF(iso)4E-S* conferred resistance to TBTV.

AP 38

Agronomic performance of doubled haploid lines and their use as parental lines of hybrids

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Production of doubled haploid lines (DH) is often used to significantly reduce the amount of time required to achieve homozygosity in tobacco breeding populations. The most prominent reported disadvantage with regard to using DH techniques in tobacco is their unsatisfactory agronomic performance and, in some cases, the difficulties in inducing haploids. The objective of this study was to compare methods for generation of DH and determine whether any loss of vigor, which may be detected in the DH lines, would carry over to their use in hybrid varieties. Ten androgenic-derived doubled haploid (ADDH) experimental lines of the Burley inbred TN 90LC, ten ADDH of the Burley inbred GR 149LC, ten maternally-derived doubled haploids (MDDH) of TN 90LC and four MDDH of GR 149LC were produced. Split-plots trials were carried out at three locations to evaluate agronomic traits. Pollen was collected from all ADDH and MDDH lines and used to fertilize the diploid female lines to generate the DH-derived hybrids KT 204LC and TN 97LC, which were compared to their respective diploid versions under the same experimental design previously mentioned. The ADDH TN 90LC population yielded 111 Kg/ha less than the inbred and was the only DH population significantly different from the inbred sources. The overall agronomic performance of the DH-derived hybrid lines was superior to their respective ADDH and MDDH parental lines, as well as displaying significant differences between lines for fewer agronomic traits. DH populations were equal to or inferior to the inbred diploids but it displayed individual lines that performed better than their source varieties. The unsatisfactory performance of some DH lines was not detected in the hybrids that it originated, which appears that any eventual loss of vigor is recovered by hybridization.

AP 39

Identification of genetic determinants controlling cadmium accumulation in tobacco

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Cadmium is a toxic heavy metal naturally absorbed and accumulated by plants. It is present in tobacco (*Nicotiana tabacum*) leaves and potentially in the smoke of cigarettes. Reducing the cadmium content in leaves of tobacco plants requires a better understanding of the mechanisms controlling the accumulation of this element. Our project aims at identifying genetic loci controlling cadmium accumulation using two approaches, a genome wide association study (GWAS) and a quantitative trait *loci* (QTL) approach. The GWAS approach was developed on a panel of ~160 ancient varieties, using ~6000 single-nucleotide polymorphisms (SNPs). The QTL approach was developed on a F2 progeny issued from a cross between two lines showing a two-fold variation in leaf cadmium content. This QTL approach was done using a Bulk Segregant Analysis (BSA) with ~8000 SNP markers generated by double-digest Restriction Associated DNA (ddRAD) sequencing. For both approaches the phenotyping was done in the field. The GWAS approach revealed different markers associated to the change in cadmium accumulation but still remain limited by the number of markers. In contrast the QTL approach revealed ~70 SNPs arranged at least three loci controlling cadmium content in tobacco leaves. These *loci* are under further examination. Our results may help breeders to create new tobacco varieties with reduced cadmium contents in their leaves.

AP 40

Genetic screening for tobacco breeders

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New breeding techniques could be very useful in the near future to create new tobacco cultivars. However, conventional breeding remains widely used by the tobacco breeders and will stay relevant and complementary. The development of high throughput sequencing has paved the way to the identification of several genes of interest and many molecular markers (microsatellites or SNPs). These markers are mostly related to resistance against pathogens and to the chemical composition of leaves. They can also be used to speed up the breeding process and give better accuracy for breeders. On the other hand, molecular markers can be used to do fingerprinting or differentiate tobacco types.

The major issue in the coming years will be to integrate molecular markers in the breeding process. Different techniques to identify these markers are available. PCR or hybridization systems are the most popular, but the miniaturisation of high throughput sequencing systems could be a new revolution for genetic screening. Our experience will be shared in an attempt to assist tobacco breeders with deciding which method to adopt.

AP 41

Greenhouse method to screen for resistance to Granville wilt in tobacco

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Granville wilt is a soilborne disease caused by *Ralstonia solanacearum* race 1 biovar 1. This bacterial 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 screening into our tobacco breeding program for year-long testing. Several *Ralstonia* strains were obtained from North Carolina State University with the required permit. All isolates were tested for pathogenicity in the greenhouse with susceptible seedlings. The method is as follows: each unit consists of a 338A-cell Speedling's Styrofoam tray placed in an individual frame lined with black plastic and floated in water, using fine vermiculite as growth medium, and set at 30 °C degrees in the greenhouse. Twelve tobacco lines are planted per tray and after germination, they are thinned to one seedling per cell, and plants are allowed to grow for five additional weeks. In the laboratory, the *Ralstonia* isolate is grown in casamino acid-peptone-glucose (CPG) solid medium. Cultures are incubated for 48 hours at 30 °C and bacterial cells are scrapped into sterile water and brought to the greenhouse. A new float frame is set and a heat mat is placed below the plastic and maintained at 30 °C. The roots of six week-old healthy seedlings are injured, by cutting them from the bottom with a metal scraper and the tray is immediately placed over a 5 liter bacterial cell suspension (~108 CFU/ml). After 48 hrs, water plus fertilizer is replenished as needed until seedlings are evaluated two weeks later. Consistent results were observed for all susceptible (Hicks, TN86, TN90) and partially resistant (NC95, Speight 168, K346) tobacco check lines tested. Several other lines such as TKF7002, Speight 225, Speight 227 and Speight 236 were found to be highly resistant to bacterial wilt in this float bed system.

AP 42

Progress in development of a high-throughput phenotyping test as breeding tool for broomrape resistance in tobacco: an overview of results

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Tobacco broomrape (*Phelipanche ramosa*) is a widespread parasitic plant that causes serious damage in some countries. Selection of resistant varieties is an essential pillar of an integrated broomrape control strategy but research performed on broomrape resistance until now is tedious and time consuming because phenotyping, through germination rate measurement, is usually evaluated by counting germinated seeds under a binocular microscope. The objective of this study was to adapt to tobacco a method developed on rapeseed by the team at the French University of Nantes. Their publication describes a fast and easy technique for broomrape germination rate determination based on a standardised 96 well plate test coupled with spectrophotometric reading. Twenty tobacco and related species genotypes with tolerance potential were selected for this study. The two controls, tolerant vs susceptible, were Wika and ITB 31612. Root exudates were sampled from individual plants during six weeks and deposited on broomrape non-dormant seeds. GR24 was used as the positive germination control. After staining treatment with methylthiazolyldiphenyl-tetrazolium bromide (MTT), spectrophotometric reading results were collected. After four weeks of exudates collection, significant average germination rates of 8 % on the reference tolerant genotype and 40 % on the reference of susceptible genotypes were obtained. Initial work on these two control genotypes has been key in defining the best test conditions for tobacco. Evaluation of the 20 genotypes allowed detection of a potential in other resistance sources that are explored more largely in current programmes. A high-throughput phenotyping tool, such as this test, could support our resistance breeding strategy; the higher the resolution of the phenotype analysis, the more likely that new genes and complex interactions will be revealed.

AP 43

Evaluation of dark tobacco transplanting intervals following 2,4-D and saflufenacil herbicide applications

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Marestail (*Conyza canadensis* L.) has become a major weed problem in dark tobacco production in western Kentucky due to its persistence in conservation tillage production systems and its tolerance to glyphosate herbicide that is typically used in burndown applications prior to transplanting in conservation tillage systems. 2,4-D and saflufenacil herbicides are effective burndown options for marestail control prior to planting other crops such as corn and soybean, but are not viable alternatives prior to tobacco transplanting due to long rotational intervals given on product labels. In discussion with chemical manufacturers of these herbicides, the primary reason for the long rotational intervals is lack of field research data showing tolerances in a crop such as tobacco that occupies relatively minor acreage in the U.S. Field experiments were conducted in 2015 and 2016 to evaluate tobacco response to 2,4-D and saflufenacil applications made at various intervals prior to transplanting dark tobacco in a strip-tillage system. Treatments in the 2,4-D experiment included two rates of 2,4-D (1.17 L/Ha and 2.33L/Ha of 2,4-D amine product [Shredder®]) applied at five timings prior transplanting. Timings were 50, 40, 30, 20, and 10 days prior to transplanting. An untreated check was included that received no 2,4-D. No injury was seen from either rate at any timing. There were no significant differences in yield of treatments. In a separate experiment, saflufenacil was applied at 0.06 L/Ha of product (Sharpen®) at the same timings as in the 2,4-D experiment. In the saflufenacil experiment, substantial injury and stand reduction was seen at 10 and 20 days prior to transplanting, but no injury or yield reduction was seen at 30 or more days prior to transplanting.

AP 44

Plant related factors impacting MH residues in flue-cured tobacco

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Maleic hydrazide (MH) is the only truly systemic compound for tobacco sucker control and is thus an important component in a cost effective sucker control program with minimal worker exposure to crop protection agents (CPAs). However, among other CPAs commonly used on tobacco, application of MH results in comparatively high residues in cured tobacco and remains a significant industry concern. While U.S. growers have made significant progress in reducing MH residues, levels found in cured tobacco vary substantially due to weather conditions, application technique, and plant related factors. A four-year study was conducted at the Virginia Tech Southern Piedmont Center to evaluate factors impacting the weathering of MH residues on green tobacco and the ultimate residue level present in the cured leaf. Applications of MH were made on three dates (before 1st harvest, after 1st harvest, and late after 1st harvest) and at three times of day on each application date (8 a.m., noon, and 4 p.m.). Green leaf samples were collected the day after MH applications and following any significant rainfall event. The green leaf sampling data reveal the weathering of MH residues following application and differences between applications made throughout the day. Applications made before the first harvest were subject to more total rainfall and thus tended to have lower cured leaf MH residues. Applications made at 8 a.m. resulted in significantly lower MH residues in both green and cured leaf samples compared to applications at noon and 4 p.m.. Results of this study illustrate how application timing, rainfall, and plant related factors impact MH residues and how grower management practices can have a positive impact on MH residues.

AP 45

Preliminary study on the metabolites of Spirotetramat in tobacco for possible residue definition

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Residue definitions are determined for enforcement/risk assessment purposes when legislative Maximum Residue Levels for food/feed are established. Spirotetramat (SP) is an insecticide and has been selected as one of the Guidance Residue Level (GRL) candidates for CORESTA Guide No. 1. Although it has been reported that SP is mainly metabolized/degraded to four compounds, SP-enol, -ketohydroxy, -monohydroxy, and -enol-Glc the metabolism of SP in tobacco has never been reported. The purpose of this study was to identify the metabolic behavior of SP in artificially spiked and field treated (incurred) tobacco. One spiked sample was prepared by mixing SP (2.0 mg/kg) into ground flue-cured (FCV) tobacco. A time course analyses of the mother compound and four metabolites in the spiked sample were performed at 0, 1, 7, 14, 28, and 90 days. For incurred sample preparation, SP was applied three times (totally 225 g/ha) to FCV tobacco in a field after topping. The incurred samples were also analyzed after curing of tobacco leaves. In the spiked sample, the level of SP was gradually decreased and almost vanished after 90 days, while those of SP-enol and -ketohydroxy were gradually increased. The two other metabolites were not detected. The residual level of SP and the metabolite SP-monohydroxy was below the limit of quantification in the incurred samples. Alternatively, the three remaining metabolites were above the limit of quantification. It should be noted that SP-enol-Glc was detected in the incurred samples despite no detection in the spiked sample, presumably because SP-enol-Glc is generated by living tobacco cells. It is crucial to investigate the behavior of SP in incurred samples for monitoring Good Agricultural Practices. The results of these studies could be useful information for determining the residue definition and GRL of SP.

AP 46

Pesticide residues in and on tobacco – a mystery?! Part I

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Over the past years the residue levels for the registered fungicide Luna Sensation (250 g/l Trifloxistrobin, 250 g/l Fluopyram) differ widely between the results of residue trials from the years 2011 and 2012 and the levels detected on the grower side. Therefore, in 2015 pesticide residue studies with a particular focus on Luna Sensation were conducted to a wider extent by dividing the samples partly into leaves, lamina and midribs.

Tobacco was cultivated at two locations, Forchheim and Ettlingen. Four fungicides were applied within the regular pest management programme, with Luna Sensation being only applied in Ettlingen. Both locations were harvested five times each. The leaf samples for residue analysis were taken from the reapings of Ettlingen and analysed mass spectrometrically. Completely untreated tobacco served as control. Due to a mistake in collecting leaf samples, two stalk positions from Forchheim were also analysed and revealed quantifiable residues of fluopyram and trifloxystrobin, although Forchheim was not treated with Luna Sensation and was irrigated during the season.

In summary the results show that residue levels can be influenced by the sample constitution as well as by the cultivation method and that active ingredients (a.i.) differ in their residue behaviour. The a.i. fluopyram especially showed persistent residue levels in all leaf parts. Considerable residue levels could also be observed in the untreated tobacco samples that were cured with Luna Sensation treated tobacco as well as on untreated samples cured together with tobacco that was not treated with Luna Sensation.

Further analyses are needed to determine (1) the residue behaviour of the a.i.s from different pesticides; (2) if these residue levels are influenced by irrigation or leaf sample constitution, and (3) if tobacco can be contaminated during the curing process in the barn.

AP 47

Pesticide residues in and on tobacco – a mystery?! Part II

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In the past years big differences in the levels of pesticide residues for the registered fungicide Luna Sensation (250 g/l Trifloxistrobin, 250 g/l Fluopyram) were observed between the results of residue trials from the years 2011 and 2012 and the levels detected at grower sites. Therefore, in 2015 pesticide residue evaluation, especially with Luna Sensation, were conducted to a more substantial extent, i.e. analysing whole leaves, lamina and midribs. By reason of the results of the 2015 evaluation (Declercq, 2017) in 2016 this study was continued by analysing all applied pesticides used at the NiCoTa tobacco production site. The objective of these studies was to evaluate the residue behaviour of different pesticides, depending on the part of the leaves, the location and irrigation. Ten different active materials, representing fungicides, insecticides and suckericides, were under investigation. Flue-cured tobacco leaves from five to six reapings and two locations were separated into whole leaves, lamina and midrib samples. Besides sampling from treated tobacco, completely untreated tobacco was collected in the same way and was analysed for all materials, too. The ten materials differed regarding the absolute levels of the residues, the ratios between whole leaves, lamina and midribs, the locations and the contamination of complete untreated tobacco. In general the levels in midribs were much less compared to whole leaves and lamina and the levels in the first harvest were higher than in the subsequent ones. As a conclusion of these two years investigations, when talking about pesticide residues it is necessary to give as much information as possible, i.e. for example, which parts of the plants were analysed or a description of the climatic conditions. But nevertheless, in some cases, the residue levels will still not be explainable.

AP 48

High performance amino acid application and its relationship with flumetralin residues in flue-cured tobacco: impact on sucker control and tobacco sustainability in Brazil

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Sucker control in flue-cured tobacco planted in Brazil is based on use of the active ingredient flumetralin, whose dosage varies from 3.0 to 4.0 L/hectare, and is classified as toxicological class I in Brazil.

Not only is the tobacco industry concerned about tobacco growers' health and environmental protection, but there is also a trend in searching for alternatives to lower the residues of any active ingredient, including flumetralin, with the objective of delivering tobacco to the industry almost free of residues. Based on this objective, high performance amino acids were tested in a field trial performed in Santa Cruz do Sul during the crop season of 2016/2017. The objective of this trial was to evaluate the efficacy of amino acids (Cutcontrol product) applied in conjunction with reduced dosages of active ingredient flumetralin for sucker control in flue-cured tobacco.

The following treatments were applied right after topping tobacco in the trial: 1- Check (water); 2- Standard product (PrimePlus BR 3.0 L/ha); 3- Cutcontrol (3.0 L/ha); 4- Cutcontrol (3.0 L/ha) + 0.6 L/ha PrimePlus BR; 5- Cutcontrol (3.0 L/ha) + 0.3 L/ha PrimePlus BR; and 6- Cutcontrol (3.0 L/ha) + 0.15 L/ha PrimePlus BR. The variety was PVH2343, transplanted on 25 August 2016.

None of the tested treatments caused phytotoxicity in the plants. The most efficient dosages, which caused the highest sucker control indexes and the highest tobacco yield were Cutcontrol 3.0 L/ha + PrimePlus BR 0.6 L/ha (treatment 4) and Cutcontrol (3.0 L/ha) + 0.3 L/ha PrimePlus BR (treatment 5). Also, the flumetralin residue content in the tobacco from these treatments were <0.02 ppm in both treatment (leaf B position) against 0.21 ppm in the standard treatment.

Cutcontrol is a product that contains high performance amino acids, and thus can be used in combination with the sucker control products that are commonly used in Brazil. Based on the observed data from this field trial, our conclusion is that Cutcontrol can be used to produce tobacco with significantly reduced flumetralin residues.

AP 49

Potential alternative control for *Ephestia* spp. using parasitoid wasps

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Different practices are already used to control lepidopteran pests in stored tobacco, mostly including chemical or physical practices, preventive or disinfesting. Biological methods, used more frequently in tobacco field sites, are not used yet in stored tobacco in Brazil. Natural enemy *Habrobracon hebetor* (Say, 1857) (Hymenoptera: Braconidae) is a parasitic wasp that attacks the last larvae stages of *Ephestia* ssp. (Lepidoptera: Pyralidae), paralysing and feeding on the larvae. The species is non-toxic, very small (1 mm), high reproductive and leaves no residue in tobacco processing. The objective of this study was to evaluate a biological sustainable method to reduce the occurrence of *Ephestia* spp. in farmers' and tobacco industry warehouses. Individuals of *H. hebetor* were bred and multiplied at Universidade de Santa Cruz do Sul (UNISC) and released in green tobacco in different Japan Tobacco International (JTI) warehouses and various tobacco farmers in different regions, with weekly monitoring of *Ephestia*'s population by Gachon traps. At farmers and warehouses the control of the *Ephestia*'s population was approximately 75 %; and at JTI warehouses the number of lepidopterans (adults and larvae) reduced significantly, replacing periodically the use of chemical disinfesting practices, reducing costs and labour. Data from 2015-2016 and 2016-2017 tobacco crops show the efficiency of this method for controlling infestations with *Ephestia* spp. Releasing the parasitoid wasp *H. hebetor* could potentially be used in different warehouses as biological control of *Ephestia* spp., enriching the natural population by mass-release.

AP 50

Trichome extracts from *Nicotiana* hybrids - a resource for testing disease/insect resistance

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Glandular trichomes on the surface of species of the *Solanaceae* family produce sugar esters (SEs) that are species and cultivar-specific. SEs have insecticidal, antibiotic, and hormone-like properties, i.e. SE with medium-chain acyl groups (C_7-C_{12}) are more toxic to budworms, hornworms, aphids, whitefly, and bacteria. Sugar esters have great potential as a class of naturally occurring pesticides and antibiotics that degrade rapidly and are not known to be harmful to wildlife and other non-target organisms. Greater knowledge of the biological function of chemically-diverse sugar esters could assist the breeding programs and genetic engineering aiming to enhance crop protection using fewer synthetic pesticides.

Objective: The objective of this work was to analyze the acyl content of sugar esters from Nicotiana hybrids in search of unusual acyl compositions resulting from joint parental inheritance, and availability of abundant medium-chain length groups.

Methods: Nicotiana hybrids generated at KTRDC, Lexington, KY, USA, and a few commercial hybrids were grown in the green house. Trichome exudates from these were collected, and analyzed via GC-MS to identify their SE acyl profiles.

Results: Most of the hybrids inherited the profile of one parent but in one case a new composition was observed. Acyl profiles of two Turkish Samsun × *N. benthamiana* hybrids were very similar to the paternal profile (mostly 6- and 5-methylheptanoic acids). Production of SEs, however, was four fold higher than that of *N. benthamiana*. Additionally, certain commercial hybrids tested produced mainly octanoate and 2-methylbutyrate, similar to *N. alata* and *N. sanderae*, but in six fold higher quantity. Trichome extracts from micropropagated plants had a uniform qualitative profile.

Future work: Micropropagated plants will ensure sufficient accumulation of chemically-stable extracts for further tests against tobacco pests and diseases.

AP 51

Change of microbial community composition in bacterial wilt infected tobacco-planting soils and regulation of the functional bio-organic fertiliser

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Bacterial wilt caused by *Ralstonia solanacearum* is one of main soil-borne diseases during the growth of tobacco. Soil microbes play important roles in tobacco growth and health. Little is known about the differences of soil microbes between healthy and bacterial wilt infected soils. It was found the soil microbial composition and diversity was distinct between healthy and bacterial wilt infected tobacco-planting soils by Illumina-MiSeq sequencing technology. Healthy soils exhibited higher microbial diversity than the bacterial wilt infected soils. Abundance of 36 bacterial genera were significantly different between healthy and bacterial wilt infected soils. 19 bacterial genera were more abundant in the healthy soils, while the rest 17 genera were more abundant in the bacterial wilt infected soils. Interestingly, the beneficial microorganisms (e.g. *Bacillus*, *Bradyrhizobium*, *Nocardioides* and *Micromonospora*) were more abundant in the healthy soils compared to the bacterial wilt infected soils. Based on the results of Illumina-MiSeq sequencing, three Bacillus strains with strong activity against *R. solanacearum*, were isolated from the soil planted with tobacco, and then added into organic fertiliser to form functional bio-organic fertiliser (BOF) with resistance to tobacco bacterial wilt. The results showed BOF could reduce the disease index of tobacco bacterial wilt and effectively control the bacterial wilt. After application of BOF the soil microbial community altered dramatically. Abundance of *Sphingomonas*, *Paenibacillus*, *Thermobacillus* and *Clostridium* increased obviously, which can degrade organic materials and promote growth of benerifical microbes and tobacco. Abundance of *Streptomyces* and *Bacillus* also increased obviously in soil treated with BOF, which are antagonists to *R. solanacearum* by producing antibiotics. The results indicate that BOF positively impacts on the composition and abundance of soil microbial community that are beneficial for control of tobacco bacterial wilt.

AP 52

Biological control of soil borne diseases in tobacco

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Biotic stress components are one of the limiting factors for productivity in tobacco in India. Fusarium wilt caused by *Fusarium oxysporum* f.sp *nicotianae* (Mysore region) and Black shank caused by *Phytophthora parasitica* var. *nicotianae* (Northern Light Soils/NLS region) are two major soil borne diseases in flue-cured tobacco production. The control of these soil borne diseases in a single season is very difficult as these organisms are persistent in the soil for long periods. Chemical control measures are the *ad hoc* options to manage the disease during the crop season. But the non-chemical methods of pest and disease management are gaining importance in the current pesticide residue free tobacco production.

The objective of the study was to develop a non-chemical sustainable long term control option for soil borne disease management in tobacco. Successful bio-control options and deployment mechanism were worked out for the permanent control of these diseases. *Trichoderma* species were isolated from 42 tobacco soil samples and screened *in vitro* for the antagonistic activity against the pathogen. The best isolate of *Trichoderma viride* (ITC-3) was multiplied through a technique developed for the farm scale mass multiplication and used in the suppression of the pathogens. The *Trichoderma viride* strain, ITC-3, suppressed the pathogen and recorded control of 62-84 % in different fusarium wilt experimental trials (two random block designs [RBD] in two seasons). Application of 625 ml/ha (spore load of 2×10^{-9}/ml) of *Trichoderma* liquid formulation was able to control black shank by 59-61 % over the control in two RBD trials conducted over two seasons. In bulk trials (three locations and two seasons), on the same piece of land, 98 % control of black shank was recorded by the second year in the NLS region. This practical and farmer friendly technique is effective in controlling fusarium wilt and black shank while addressing the pesticide residue problems.

AP 53

Interactions between tobacco bushy top virus and its satellite RNA

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Tobacco bushy top disease (TBTD) has caused significant yield and economic losses in Yunnan Province, China. The disease is caused by an unique virus complex consisting of tobacco bushy top virus (TBTV), TBTV satellite RNA, tobacco vein distorting virus, tobacco vein distorting virus-associated RNA, and an unidentified viral RNA. The objectives of this study were to determine the complete genome sequence of TBTV satellite RNA, and to investigate the interactions between TBTV and its satellite RNA, which are the two important components in the TBTD complex. The complete genome sequences of seven TBTV satellite RNA isolates were determined and their molecular variations were evaluated. Agrobacterium-mediated infectious clones of TBTV and its satellite RNA were constructed to facilitate the research. The results showed that TBTV satellite RNA was a new satellite RNA and the identities shared among these seven TBTV satellite RNA isolates ranged from 72.0 % to 99.4 %, with the lowest identities between Yongren isolate and the others. TBTV alone was able to replicate and move systematically and cause mild symptoms in plants (*Nicotiana tabacum* and *N. benthamiana*). While the satellite RNA was dependent on TBTV for its replication and systematic movement, it boosted the symptom severity of the disease when co-infected with TBTV. TBTV satellite RNA stimulated mildly the accumulation of TBTV genomic RNA in *N. benthamiana*, while in *N. tabacum*, it downregulated the accumulation of TBTV genomic RNA slightly, indicating host specificity in the association of TBTV and its satellite RNA. This study would broaden the understanding of TBTD complex and the mechanism of plant virus interactions, and lead to develop new stratigies in the control of TBTD.

AP 54

Cucumber mosaic virus-IB induced endoplasmic reticular stress in *Nicotiana benthamiana*

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The cellular translation machinery is hijacked by large amounts of viral proteins upon virus infection. The unfolded proteins accumulated in the endoplasmic reticulum (ER) results in ER stress (ERs), which triggers the unfolded protein response (UPR) or programmed cell death (PCD). ERs is implicated in some plant virus infections that replicated in the ER. To investigate if cucumber mosaic virus (CMV) that replicated in the tonoplast induced ERs, UPR genes by qRT-PCR, ER morphology by confocal microscopy and ultrastructure of cytopathology by electron microscope in CMV inoculated *Nicotiana benthamiana* leaves were compared with PBS buffer inoculated control. The results showed that the ERs sensor *BiP*, UPR genes (*bZIP28*, *bZIP60*, *PDI*, and *CAM*) were upregulated significantly at 12 hours post inoculation and PCD gene *NAC089* was upregulated at 48 h, with 1.23–4.21 fold higher levels in CMV inoculated leaves than in PBS controls ($P<0.05$). Especially, the ER morphology was hyperplasia and rearrangement was significant at 5-10 days post CMV inoculation. More disintegration of organelle membranes, invagination of tonoplast into double membranous complex structures of microautophagy, and macroautophagic structures in the cytoplasm appeared in diseased cells than in the controls. This indicated CMV infection induces ERs including activating ER genes and causes ER proliferation. The host may activate autophagy, organelle degradation or even cell death, to suppress viral infection.

AP 55

Genetic and functional analysis of *va* resistance durability to Potato virus Y in tobacco

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Many recessive resistances against potyviruses are mediated by eukaryotic translation initiation factors (eIF4E). In tobacco, the *va* resistance gene commonly used to control PVY corresponds to a large (almost 1Mb) deletion affecting a copy of eIF4E (S10760)[1].

We compared the resistance durability conferred by various types of mutations affecting the S10760 gene (large deletions, Frameshift, or EMS nonsense mutations). Thirteen tobacco genotypes were challenged with ten PVY isolates according to their biological and molecular characteristics[2]. The "large deletion" genotypes display the largest resistance spectrum and durability towards the ten PVY isolates, whereas, the "Frameshift" and "EMS nonsense" mutants display the less durable resistance, with rapid and frequent apparition of resistance-breaking PVY isolates.

Upon further investigations, genetic and transcriptomic analyses revealed that resistance durability is strongly influenced by a complex genetic locus which contains two eIF4E copies, one of which is truncated in the most stable genotypes, while the other is differentially expressed between the tested varieties. RNASeq transcriptomic data and Q-PCR experiments demonstrate that the expression level of this second copy, T021658, is positively correlated with resistance durability. This suggests that T021658 might act as a decoy, in a virus-plant non-productive interaction and limiting the ability of PVY to evolve towards resistance-breaking. Taken together these results show that *va* durability can be explained by redundancy/competition effects in the eIF4E gene family, similar to what was recently shown in tomato[3].

[1] Julio, E., et al., 2014. Plant Molecular Biology Reporter 32 (4), 781-942.
[2] Janzac, B., et al., 2014. Plant Disease 98, 1521-1529.
[3] Gauffier, C., et al., 2016. The Plant Journal 85, 717–729.

AP 56

Cloning and expression induced by TMV of a pathogenesis related protein gene *NtPR10* in tobacco (*Nicotiana tabacum*)

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In order to investigate the biological function of tobacco pathogenesis-related protein PR10, *NtPR10* was obtained from the tobacco G28, which is 483bp in length, encoding 160 amino acids. This *NtPR10* belongs to pathogenesis related protein family containing the "Bet_v_I" and "P-loop" domain, related with nuclease activity. The bioinformatics analysis indicated *NtPR10* did not contain transmembrane region, no signal peptide, and exhibited intracellular localisation features by TMHMM, SignalP and PrositeScan. The expression pattern of the gene in the resistant and susceptible cultivars was studied by RT-qPCR. The results showed that the *NtPR10* gene was significantly up-regulated in the susceptible cultivar G28 in TMV treatment. While in resistant cultivar *Sumsun* NN, The *NtPR10* was up-regulated at six hours after TMV infection, and down-regulated between 12 hours and the eighth day, and then increased gradually to the pre-infection level after 16th day. From above analysis, *NtPR10* responses to TMV infection, and the expression trend is opposite in resistant and susceptible cultivars, suggesting that the *NtPR10* has an important function in the process of TMV infection, that provides the foundation for further analysis of the biological function of *NtPR10*. It also provides a new method of tobacco disease control.

2017 CORESTA JOINT STUDY GROUPS MEETING

WORKSHOP PRESENTATIONS

WORKSHOP SUSTAINABLE TOBACCO PRODUCTION

APW 01

Delivering positive change through the Sustainable Tobacco Programme

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AB Sustain has a wealth of tobacco farming and processing sustainability data gathered over the last 16 years on a global scale. The latest iteration of our sustainability service, the Sustainable Tobacco Programme (STP), was launched in 2016. This collaborative, industry wide initiative is supported and operated in the supply chains of six leading tobacco brand owners. The programme operates in over 52 countries and gathers data on 180+ suppliers of tobacco across 5 million smallholders. Our specialist assessors work with field technicians and supply chain business owners to verify the data collected on farms. We gather data on over 1000 sustainability indicators across five pillars; Governance, Crop, People, Facilities and Environment, to give a holistic and verified report into practices at both tobacco processing sites and their supplying farms. At the programme's core is continuous improvement, with a strong focus on providing supply chains the information/data needed to help drive positive change.

The next step for STP is to further validate this approach, and provide ongoing tangible evidence that it is delivering change for the industry in a sustainable way. There is also the opportunity to add value to the wider sustainability landscape through leveraging the dataset that we have amassed to provide further insight into global practices and the impact of change.

APW 02

Agronomic data collection, management, and analysis: an overview of the MobiLeaf system

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The collection of farm-level agronomic information is not new to the tobacco industry, however over the last 10 years the amount and level of detail being collected has significantly increased. Many programs designed to measure farmer adherence to sustainable farming practices require on-farm monitoring on numerous aspects of the farm and farmer – from training to seedling production to CPA applications to curing fuel, and more. Capturing hundreds of data points on each farmer requires a system that can facilitate data collection in a timely manner, allows for the management of large data sets, and provides analytic tools to show status and progress on each measurable. The tobacco industry is now in the era of Big Data.

With the large volume of agronomic data being collected in many tobacco operations today, utilizing electronic based systems and tools proves to be the most effective and efficient means to capture and manage data. There are various systems in place around the world to capture agronomic data, however this presentation will provide an overview of MobiLeaf, a global system specifically designed to capture tobacco agronomic related data at the farm level. The presentation will outline some of the hardware, software, and supporting tools utilized in the MobiLeaf system.

APW 03

Actions against child labour and alternatives to rural youth

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For more than 15 years, the tobacco sector has developed actions to raise the awareness of the growers about the importance of education and the fight against child labour, as well as to comply with current Brazilian legislation, which since 2008 has determined that children under 18 cannot work in tobacco fields. According to the latest census by the Brazilian Institute of Geography and Statistics – IBGE, in 2010, it was the small tobacco-producing farms that had the highest rate of child labour reduction in Brazil. Looking for the reduction of child labour and to give to the young rural people new opportunities without leaving the countryside, the Growing Up Right Institute took up the challenge of creating a pioneer pilot course, with methodology and pedagogy, especially focused on rural youth. In 2016, the Rural Professional Learning Program was implemented in five municipalities in of Rio Grande do Sul - Candelária, Santa Cruz do Sul, Vale do Sol, Venâncio Aires and Vera Cruz. Aimed at adolescents aged 14 to 18, from families of small rural growers, the programme's purpose is to provide education alternatives to the rural youth so that they can be prepared for the future. The big difference is that young people receive proportional wages and benefits according to the rules of the Young Apprentice Program, but they do not work in the fields or companies that are the contractors. The activities, aimed at rural management, are carried out in the school counterparty in partner schools and in their communities. The main objectives of the Growing Up Right Institute are related to offering learning alternatives, professionalisation and opportunities for qualifying the adolescents in the rural areas with the purpose of developing rural management and entrepreneurship and generating possibilities for the professionalisation of boys and girls.

APW 04

Reshaping pest management in the tobacco agro system: biological control and judicious use of pesticides

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The tobacco agro-system is a pesticide intensive enterprise which consumes various insecticides, herbicides, fungicides and nematicides every year in Zimbabwe. In light of hazards caused by excessive use of pesticides worldwide, there have been calls to phase out highly toxic pesticides, and use less toxic ones, judiciously and embrace Integrated Pest Management (IPM). In a bid to promote Good Agricultural Practices in the tobacco agro-system in Zimbabwe, the Tobacco Research Board (TRB) is involved in research to develop and recommend pest management strategies that are friendly to both the grower and the environment. The objective of this study was, therefore to evaluate in separate experiments, the mating disruption and the mass trapping techniques in managing budworm (*Helicoverpa armigera*) (field), the use of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* as well as a botanical extract azadirachtin in the management of the tobacco aphid *Myzus persicae nicotianae* and budworm (laboratory and micro plots). *Beauveria bassiana* was also evaluated for the control of the storage pest *Lasioderma serricone* (laboratory). The use of mating disruption and mass trapping reduced budworm damage by 50 % in tobacco lands. The use of entomopathogenic fungi resulted in 60 % tobacco aphid mortality and upwards of 70 % tobacco beetle mortality. The use of azadirachtin had a repellent effect on budworm causing them to leave the plants. These findings are adequate evidence for mass traping, mating disruption and biological control using entomopathogenic fungi to be adopted by the tobacco agro-system as effective components of IPM. In the event that most effective pesticides currently in use in the tobacco agro-system in Zimbabwe are phased out or banned, the Tobacco Research Board has enough arsenal in the form of insect pest management alternatives that could be recommended to the industry for the continued sustainable production of the golden leaf.

2017 CORESTA JOINT STUDY GROUPS MEETING

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

ABSTRACTS

POSTER PRESENTATIONS

Presenter's name is underlined when the main author (listed first) is not presenting the paper

APPOST 01

Nicotine reduction: use of modelling approach to evaluate unintended consequences, a focus on illicit trade

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In 2015, the WHO TobReg issued an advisory note recommending a strategy for reducing nicotine in tobacco to substantially lower levels. The authors considered that regulation of nicotine levels would lead to a decrease of smoking prevalence. In their review, they assessed the feasibility and relevance of the strategy using a range of different research activities, from tobacco plant genetics to consumer sensorial perception. However, what is also readily apparent is a number of unintended consequences that arise from such a strategy. For example, reducing nicotine raises multiple issues from an agronomy perspective, which would threaten the livelihood of millions of farmers. Additionally, market disruption would foreseeably benefit and increase in illicit trade.

Although the use of simulation models to predict the impact of a new policy is recognised as extremely valuable, only a few publications have simulated the impact to a population. The use of simulations would allow regulators to evaluate options and make informed decisions that do not have unintended consequences that undermine the original policy aim.

The objective of our study was to develop a simulation model enabling the assessment of the impact of nicotine reduction policy on illicit trade. Both the baseline and counterfactual case were considered. The baseline corresponds to the status-quo and predicts population status in future if regulation does not change. The counterfactual case predicts the population status if the current conventional cigarette (CC) becomes illicit once replaced by the reduced nicotine cigarette (RNC).

Each individual of the population was classified in one of four groups: non-smoker, smoker of licit products (RNC), smoker of illicit products (CC) and former smoker. For the baseline, status transitions were derived from published data. For the counterfactual case, scenarios were tested considering the reported negative impact of RNC on smoker's satisfaction and on the probability to switch to more satisfying illicit products. Trends were assessed under various scenarios and demonstrate in some conditions the risk associated with a nicotine reduction strategy.

APPOST 03

Sponged tobacco: meteorological and altitude influence on curing methods in flue-cured systems in South Africa

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Flue-cured tobacco in South Africa has over the years produced high amounts of sponged leaf, although the reaping of leaf was done at the correct physiological time. We asked if this was due to incorrect curing methods and due to meteorological influence and altitude, if so how do we correct it. To substantiate our claims we implemented data loggers, these were placed in three locations inside the barn.

Our data showed that the results correlated with our statement, finding that the fast loss of moisture was due to the curing areas being situated 1000 meters above sea level. Each data sample was analysed focusing on one hour intervals at the crucial stages of curing. Analyzing the data from our weather station we found that there was a correlation between loss off moisture from the barns and meteorological conditions outside the barn. A new curing method and system was devised to maintain the wet bulb at 38 °C. This system connects the wet bulb directly to the thermostat, using only the wet bulb as control method.

Our conclusion after three seasons of comparing data and leaf quality, the difference in sponged percentage of tobacco between manual curing and Dyna-Thermo-Sync (wet bulb control) is stark. With farmers showing results of 0 % sponged tobacco from barns on this system, compared to barns that produce on average 20 % and more sponged leaf with conventional methods. We also found that it reduced curing time and had a reduction on the overall cost. The meteorological and altitude influence on curing was found to be definite and not negligible as was the original perception.

APPOST 04

Developing flue-cured tobacco hybrids with combined resistance to granville wilt and root-knot nematodes and angular leaf spot in Malawi

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Flue-cured tobacco production in Malawi is constrained by both soil-borne (root-knot nematodes and Granville wilt) and aerial borne (angular leaf spot and alternaria brown spot) diseases. To address these multiple challenges, a breeding programme was initiated in 2011/2012 season to develop F_1 hybrids possessing multiple disease resistant and high yielding traits. In 2014/2015 and 2015/2016 season, six F_1 hybrids and three checks were evaluated for disease resistance, cured leaf yield (kg/ha) and quality at five sites in Malawi. A randomised complete block design with three replications was used. Data was collected on disease reaction, yield and quality. Highly significant differences were observed for root-knot nematodes reaction across sites. The test hybrids, HMR09-24 and HMR09-27 were comparable to a nematode resistant check, KRK 26, in terms of nematode resistance but better than the local check, AFH4. The hybrids showed susceptibility to Granville wilt with variable levels as compared to the resistant check, PVH 2110. No significant differences were observed for angular leaf spot, all test lines gave low scores. HMR09-25, HMR09-29 and HMR09-28 produced higher cured leaf yields and compared well with the recommended local checks. No significant variations were observed among the treatments on quality as reflected in the cured leaf colour distribution. Based on two year results, HMR09-29, HMR09-27 and HMR09-26 emerged as better hybrids in terms of combined disease resistance to root-knot nematodes and angular leaf spot while giving better yield and cured leaf quality.

APPOST 05

Effect of FMC Crop+ on reduction of oxidative stress in tobacco seedlings exposed to low and high temperatures

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Tobacco production in Southern Brazil is challenged by low temperature stress at early stages of crop development. Stress causes overproduction of ROS (Reactive Oxygen Species), which leads to oxidative stress in plant tissue. ROS molecules damage cell membranes and proteins disrupting plant metabolism and limiting crop production. FMC Crop+, an organomineral foliar fertilizer manufactured by Cytozyme Laboratories, Inc., USA, was previously shown to reduce ROS overproduction and improve plant tolerance to abiotic stress in tomato and *Arabidopsis thaliana*.

The objective of this study was to evaluate the effect of FMC Crop+ on tobacco (*Nicotiana tabacum*, var. PVH 2343 flue-cured) seedlings exposed to low and high temperatures. The seedlings were grown in controlled conditions for 15 days. Fifteen-day old seedlings were divided into two groups: 1) untreated control, and 2) treated with FMC Crop+. The control and treated seedlings were exposed to either low temperature (5 °C), room temperature (25 °C), or high temperature (35 °C) for 48 hours. Oxidative stress status of the treated tobacco seedlings versus control was evaluated 12 hours after treatments by measuring antioxidant activity and membrane lipid peroxidation in the leaf tissue. FMC Crop+-treated seedlings had significantly higher antioxidant activity in plants exposed to low temperature (37.1 % vs. 0.24 % in control) as well as high temperature (30.0 % vs. 0.86 % in control). Additionally, membrane lipid peroxidation in seedlings exposed to low temperature was reduced from 49.8 µM TBARS/mg in the non-treated to 2.26 µM TBARS/mg in the treated plants. In summary, high antioxidant activity and reduced membrane peroxidation indicated lower oxidative stress and better stress tolerance of treated tobacco seedlings. The potential benefits of these findings on utilization of FMC Crop+ in tobacco production is discussed.

APPOST 06

Soil applications of maleic hydrazide do not control tobacco axillary bud growth

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Increased focus on residues of the plant growth regulator Maleic Hydrazide (MH) have prohibited some U.S. producers from applying the suckercide to control tobacco axillary buds. As such, an alternative to the conventional foliar application of the material might prove beneficial to producers. Given that MH has systemic activity, reasoning might infer that soil application and root uptake might provide sufficient sucker control. To test this hypothesis, soil applications of MH at two rates (2.52 and 5.04 kg a.i./ha) were evaluated in four North Carolina environments. Soil applications of MH were preceded by two fatty alcohol (FA) applications and one flumetralin application or three fatty alcohol applications without flumetralin. A conventional treatment of foliar applied MH preceded by two applications of FA and one application of flumetralin was included for comparison. Foliar applications of all suckercides was delivered at 467 L/ha. Soil application volumes were similar to foliar and were delivered in two band applications to the soil surface adjacent to plants. Sucker control was similar between MH application rates and was greatest in the conventional foliar application method. In two Coastal Plain environments, the inclusion of flumetralin provided >89 % control. When the same treatment was evaluated in two Piedmont environments, efficacy was reduced by 30 to 50 %. In the absence of flumetralin, sucker control was further reduced in soil applied MH treatments, indicating that MH contribution to sucker control was minimal. Differences between the two MH application rates were not observed. Results indicate that soil applications of MH are unlikely to inhibit sucker growth as efficiently as conventional foliar applications and that the recommended foliar application rate of MH (2.52 kg a.i./ha) is sufficient for sucker suppression.

APPOST 07

Effects of fertigation and micro-spraying on growth of flue-cured tobacco, soil properties and water use efficiency

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The use of water saving irrigation systems and technology is of great significance in tobacco production in central China due to the high frequency of drought in the early tobacco growth stages. In recent years, micro-spraying irrigation and fertigation systems were introduced in tobacco production, and field experiments were set up separately to investigate the effects of these techniques on plant growth, soil properties, tobacco productivity and quality, and water use efficiency. The treatment of micro-spraying experiments included three micro-spraying amounts (12 mm, 24 mm, 36 mm each irrigation, two irrigations total for each treatment) with conventional furrow irrigation (60 mm each irrigation, also two irrigations total) as control. The fertigation experiment included three nitrogen applications (54 kg-N, 46 kg-N, 39 kg-N per hectare) with conventional furrow irrigation and 54 kg-N per hectare as control. The results of the micro-spraying experiment showed that micro-spraying two times with 24 mm each time promoted tobacco growth, resulting in best performances of agronomic traits. With the increase of irrigation quota, the depth of moistened soil and soil volume weight increased, and furrow irrigation resulted in too deep moistened soil that lead to lower available nitrogen content in the layers of 0-30 cm depth. Irrigation significantly increased tobacco yield and value, with micro-spraying 24 mm each time having the highest yield and value for three consecutive years. Total water productivity for micro-spraying 24 mm per time was 23.24 % higher than that of furrow irrigation, followed by micro-spraying 36 mm per time. The results of the fertigation experiment showed that fertigation by drip pipe significantly increased water use efficiency and nitrogen use efficiency, so the amount of nitrogen applied should be decreased by 15-20 % compared with the conventional furrow irrigation method to ensure timely mature, high maturity and high quality tobacco production. Fertigation and micro-spraying proved to be two ideal alternatives to replace traditional furrow irrigation by showing significantly high water and nitrogen use efficiency, improved leaf quality and value.

APPOST 09

Sustaining low TSNA levels in Malawi Burley tobacco using the LC Protocol

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Burley tobacco has variable levels of conversion of nicotine to nornicotine, the precursor for the main Burley tobacco specific nitrosamine (TSNA), N'-nitrosonornicotine (NNN). Malawian tobacco is inherently low in TSNA levels, affording a competitive advantage over tobacco from elsewhere around the world, particularly if legislation is introduced limiting TSNA levels in cigarettes. Because of global concerns about TSNAs in the international tobacco trade, Agricultural Research and Extension Trust (ARET) considers that an opportunity exists to continue reducing TSNA levels in Malawi tobacco, eventually improving its competitiveness by introducing low converter (LC) technology. The study aimed at introducing LC methodology and establishing the conversion status of ARET's varieties. Activities included technical and analytical support in seed production planning, field sampling and alkaloid analysis by partners from the University of Kentucky (UKY) funded by Phillip Morris International. Seven previously unscreened varieties were grown alongside a high converter check in the 2016/17 season. Individual plants were sampled according to the LC protocol and analysed for alkaloids. Results revealed variable, but generally low, rates of conversion in Malawi Burley varieties. Conversion was 1.0-99.2 % in male fertile lines and 1.0-79.9 % in male sterile lines compared to 90.5-99.1 % for the high converter check, TN 90H, indicating satisfactory sampling and curing. The varieties are now LC screened and available for certified seed production. Integrating such best practices in seed provision with promotion of other seed and leaf integrity and traceability initiatives will help enhance compliance, competitiveness and maintain Malawi as a desirable origin for reduced-risk tobacco for sustainability in the global marketplace.

APPOST 10

Degradable characterisation and risk assessment of suckercides residues in tobacco leaf

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A modified gas chromatography technology was developed to detect suckercide residues of dlibutalin, flumetralin and pendimethalin with a series of good agricultural practice (GAP) samples at planting stage, and during the storage process and smoking process in tobacco leaf. The results showed that the standard curve, the quantitative limit and the recoveries conformed to the requirements of the pesticide residue detection method. The half-life of pendimethalin, butralin and flumetralin in fresh leaf was 2.15~5.89 days, 5.17~12.2 days, 3.26~5.16 days, respectively, and in the cured tobacco leaf in storage process was 173~495 days, 192~462 days, 216~533 days, with the characteristic of the higher original concentration and the longer half-life. In the process of combustion and absorption, the migration rate of suckercides from tobacco leaf to main stream smoke was 8.52 %~14.58 %, 16.73 %~21.04 %, 13.30 %~13.71 %, with the higher transfer rate in the lower concentration. Moreover, at the recommended dose of rational application of suckercide in tobacco planting, the suckercide residues are lower than GRLs of CORESTA. Standardising the rational application and lengthening the safety interval are the key measures to control the suckercide residues.

APPOST 12

Di@gnoPlant® Tobacco: a mobile tool to identify tobacco diseases

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In order to reduce the use of crop protection agents in the field, the early and reliable identification of diseases and the detection of emergent pests have proven to be crucial stages in plant protection. An early diagnosis enables implementation of the most appropriate protection method(s). An INRA (French National Institute for Agronomic Research) team in Bordeaux has developed several plant protection applications, including tobacco, for smartphones and tablets to help identify diseases on the plant.

What disease causes the symptoms? And what control methods can be used? Tobacco farmers and technicians can now gain immediate access via smartphone or tablet to research knowledge and expertise in plant protection. With Di@gnoPlant® Tobacco, the user is able to identify diseases by means of an image database. Fact sheets detail the symptoms and biology of the incriminated pest or disease and the optimised protection methods adapted to the context.

This development has two aims:

- to build a continuum of diagnostic/advice tools already accessible over the internet via the INRA e-Phytia® website;
- to make it available in the field using the new information and communication opportunities provided by smartphones and tablets (App store and Google play).

Developed in French by INRA, Di@gnoPlant® Tobacco has been translated into English by CORESTA to make this tool accessible worldwide free of charge. Data from other parts of the world are being entered.

The application will be demonstrated during the poster session.

APPOST 13

Fungal diversity analysis of yellow sun-cured tobacco leaves during curing

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Yellow sun-cured tobacco is a Chinese tobacco species that has been cultivated for several hundred years in China. Due to the special quality traits, yellow sun-cured tobacco is of great potential for developing Chinese style cigarettes with unique aroma and taste characteristics. On the surface or in the tissue of tobacco leaves, there are a large number of microbes, the metabolic activities of which may influence the transformation of chemical components throughout the curing process and thus the quality of tobacco leaves. The present study analysed the composition and dynamics of fungal communities of yellow sun-cured tobacco during curing by a combination of pure cultivation and molecular identification techniques. Cultivable fungi were preliminarily isolated from the early, middle and late stages of curing, using potato dextrose agar and sabouraud dextrose agar medium. The representative strains were then phylogenetically analyzed based on ITS sequences. A total of 126 fungi strains were isolated. The strains isolated from the middle leaves were relatively less compared with those from the bottom and upper leaves. In addition, the tobacco leaves seemed to contain more fungi in the early and late stages compared to those in the middle stage of curing. The 126 fungi strains were distributed in 2 phyla, 5 classes, 10 orders, 14 families and 17 genera. *Ascomycota* was the dominant phylum, with 93.65 % of the isolates belonging to this phylum, and *Epicoccum*, *Arthrinium* and *Fusarium* were the dominant genera, accounting for 23.02 %, 13.49 % and 11.90 % of the isolates, respectively. It was found that the fungal diversity of yellow sun-cured tobacco leaves during curing was relatively abundant, and the fungal composition varied in different leaf positions and dynamically changed as the curing process proceeded. The results of this study will shed light on improving tobacco quality traits by artificial regulation of the fungi community during the curing process.

APPOST 15

Effect of Azact CE on fungus gnats larvae (*Bradysia impatiens*) control in tobacco seedlings production in floating systems

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Fungus gnats (*Bradysia impatiens*) are major insect pests of tobacco seedlings mainly due to the direct feeding damage caused by the larvae and adults, and the ability of larvae to transmit certain soil-borne plant pathogens to the tobacco roots. Active ingredient azadirachtin is found in commercial insect growth regulators and can be part of potential insecticides for fungus gnats larvae control. Azact CE is a product that contains azadirachtin A and B in its composition, and it is the first azadirachtin based product that shows insecticide and fungicide activity registered in Brazil. The objective of this study was to investigate the ability of Azact CE to control *Bradysia impatiens* larvae in K326 tobacco seedlings in the floating system. The seedlings were cultivated in styrofoam trays and received the treatments starting at 15 days after emergence. The treatments were the following: 1- Check (water); 2- Azact CE 8.5g a.i./ha; 3- Azact CE 9.0 g a.i./ha; 4- Azact CE 9.6 g a.i./ha; 5- Azact CE 10.0 g a.i./ha and the standard product (Azamax, 9.6 g a.i./ha). Phytotoxicity effects were evaluated, and there was no phytotoxicity symptoms in any of the tested dosages. Treatments with Azact CE 9.6 g a.i./ha and Azact CE 10.0 g a.i./ha showed the highest *Bradysia impatiens* control efficacy, which was 77 % and 80 % respectively, when compared to the control treatment. These treatments also resulted in the highest seedlings yield, with 96 and 97 % of seedlings being considered good for transplanting. The seedlings were healthier than the non-treated ones, and resulted in seedlings with 24.0 and 23.7 grams/seedling compared to 12.5 grams/seedling (dry weight). The results indicated that Azact CE is a safe product for *Bradysia impatiens* larvae control in tobacco seedlings.