2019 CORESTA JOINT STUDY GROUPS CONFERENCE

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

AP 01

The history of tobacco production in Zimbabwe - a synopsis

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Tobacco is an extremely important crop in the economy of Zimbabwe and in the 2017-18 season, a record 252.5 million kg of tobacco with a value of $737.2 million was sold. This paper will consider the history of tobacco production in Zimbabwe and some pertinent factors that have led to the success of tobacco as a cash crop in the country. Organised cultivation of the golden leaf in the country dates back to the late 17th century although there are documented and folklore reports of *Nicotiana rustica* growing and consumption by the indigenous people prior to this. In the 1905-06 season, a Tobacco Tender Board was established and by 1924, the first tobacco research station had been established. In 1936, the Tobacco Marketing Act which provided for the formation of the Tobacco Marketing Board and the compulsory selling of tobacco through Auction Floors was promulgated. The Tobacco Research Board (TRB) was reconstituted under the Tobacco Research Act of 1950, with a mandate to direct, control and carry out tobacco research. This resulted in a vast improvement in the quality of the country's tobacco and in 1963 the industry hosted the CORESTA Third World Tobacco Scientific Congress. TRB also hosted the 1994 CORESTA Congress. Research is continually being carried out to further improve the viability of tobacco production and the Zimbabwean industry is consistently making efforts to inculcate good agricultural practices at grower level to ensure continuity well into the future.

AP 02

Tobacco production and marketing trends in Zimbabwe in the last two decades

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An overview of trends and challenges in flue-cured tobacco production in Zimbabwe is given. Following the country's independence in 1980 there was an initial drop in flue-cured tobacco production and number of farmers. This was reversed quickly such that by the late 1980s annual production was over 120 million kg, hectarage was over 57 000 and the number of growers had stabilised at around 1 500 large-scale growers. During the 1990s a slow land resettlement programme saw the number of growers rising to around 6 500, with about 1 700 large-scale commercial growers by 2000. The tobacco area planted (107 000 ha) in 2014 surpassed the peak of 1998 of 92 000 ha. Annual sales reached a record of 237 million kg in 2000. The period 2001-2008 was largely dominated by the land reform programme. The number of growers increased rapidly thereby increasing the production potential. Prior to land reform, more than 95 % of the total flue-cured tobacco production came from the large-scale growers who raised production finances on their own. During this period, production fell to a 30-year low of 48 million kg. In 2003 the introduction of the dual selling system where auction sales would operate alongside contract production and purchasing systems were recommended to and approved by government. The period 2009-2018 was characterised by the introduction of new policies, monetary deregulation and an improvement in financing of production. A record 145 725 farmers registered to grow tobacco resulting in a new record crop of 252 million kg in 2018. Challenges that were faced in dealing with the resurgence of production included the large number of farmers, funding of production, compliance, need for increased training and extension services, deforestation, and lack of marketing skills.

AP 04

United Nations sustainable development goals and impacts of the tobacco and alternative product sectors

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In 2015, the United Nations published 17 sustainable development goals (UN SDGs) and their corresponding targets to be achieved by 2030. The third goal "Good health and wellbeing" specifically refers to the tobacco activities with the target to "*strengthen the implementation of the WHO/FCTC in all countries*".

The tobacco and alternative product sectors are likely to impact most of the UN SDGs even if some goals are more particularly relevant than others from a social perspective. In 2018, CORESTA decided to support a project with the objectives to i) elaborate a scientific framework for assessing environmental and social impacts of these sectors, ii) identify scientific tools for performance assessment, and iii) measure the gaps between required and available tools.

During the period December 2018 – February 2019, a survey was conducted among and beyond the CORESTA members to better understand the positive and negative impacts of the tobacco and alternative product sectors with regards to the UN SDGs, to collate examples of actions undertaken for enhancing the positive impacts and mitigating or suppressing the negative ones, and finally, to identify the tools and KPIs used to measure and monitor the consequences of these actions. A workshop was organised subsequently in May 2019 to consolidate the contributions to the survey, to identify the needs and to set priorities for a possible work programme.

The conclusions of this project will be presented along with sets of tools, assessment frameworks, recommendations and possible implications for future activities of CORESTA and/or other organisations.

AP 05

Afforestation: toward sustainable tobacco production in Zimbabwe

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The Food and Agricultural Organisation (FAO) estimates that Zimbabwe loses 300 000 hectares of indigenous forest per annum, an area the size of three Harare metropolitan areas, of which 45 000 is attributed to tobacco. The Sustainable Afforestation Association (SAA) was established by tobacco merchants of Zimbabwe in 2013 with the aim to mitigate the effects of deforestation attributed to tobacco curing. To achieve this goal, the industry must plant trees, use them more efficiently, protect them from pests, veld fires and poachers, and find alternatives to trees for curing. To this end SAA is planting trees, educating communities on the value of trees and promoting the enforcement of the laws related to fires and theft. It is however estimated that approximately 25 000 ha of commercially planted forest is needed per annum to allow the curing of a fully sustainable crop. To date, SAA has planted approx. 17 500 ha, at a rate of 3 500 ha per annum, while other tobacco companies have also their tree planting regimes. SAA's footprint in Zimbabwe is now quite visible on most main roads, as trees are getting closer to maturity and harvest. Additionally, research is being carried out into alternative fuels by SAA, the Tobacco Research Board (TRB) Kutsaga and the merchants, as well as training farmers to become more efficient. The achievements to date and the challenges being faced in this project will be discussed in this paper.

AP 06

How the tobacco merchants in Zimbabwe address sustainability in leaf production

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The major tobacco merchants in Zimbabwe have worked for several years to ensure sustainability and traceability of contracted tobacco. Due to the market organisation, this covers approximately 60 % of total domestic production.

Companies have adopted policies, codes of practice, recording and monitoring systems and external verification systems which ensure full traceability of the leaf and of the practices followed at each contracted farm. Companies have also invested in the necessary structure to fulfil the objectives of their sustainability and traceability programmes. The main programmes adopted, somehow overlapping, address governance, productivity, environmental and social issues. While some of the programmes are competitive, some activities have been addressed as a sector to magnify the impact and the results.

Responsible curing practices address the improvement of curing barns (research by the Tobacco Research Board [TRB] and other independent bodies) and the availability of sustainable fuel which does not affect natural forests. With this purpose in mind, the merchants set up the Sustainable Afforestation Association (SAA) recognizing that earlier efforts to reverse deforestation were not effective enough, and that a different approach was needed. SAA provides a sustainable source of timber for use in the tobacco industry; it investigates and implements strategies for the conservation and rejuvenation of existing indigenous and commercial forests and it undertakes activities and projects directly or indirectly relating to the provision of sustainable sources of timber and the conservation and rejuvenation of existing timber resources. SAA complements the individual company activities of tree planting with their contracted farmers and communities.

Social programmes need to be tailored to the situation in the field. The Tobacco Industry and Marketing Board (TIMB) worked closely with the Ministry of Labour to carry out a farmer survey to identify issues such as child labour and unsafe working conditions. The Tobacco Industry and Marketing Board (TIMB) is working on guidelines to be followed by the sector.

AP 07

Evaluation of a biodegradable plastic greenhouse float tray

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The Trilogy tray was introduced in 2015 as the first commercially available plastic greenhouse float tray for tobacco transplant production in the United States. Modifications to the original design have been made in subsequent years. More recently, for research purposes, an additive has been included in the plastic to promote biodegradability of the tray. The additive allows photodegradation of the plastic through oxidation or the addition of carbonyl groups to the polypropylene plastic molecule when exposed to sunlight. This oxidation is necessary to facilitate the biodegradation of the plastic once the trays are exposed to an active microbial environment. The objectives of this study were to quantify the degradation of Trilogy float trays under laboratory and greenhouse conditions and to propose how such tray technology may be incorporated into sustainable greenhouse tobacco transplant production.

Laboratory testing was conducted to measure plastic degradation under accelerated ultraviolet (UV) light exposure conditions of plastic tray samples to which three rates of a proprietary additive had been added. The physical integrity of the plastic was evaluated as well as laboratory quantification of the addition of carbonyl groups to the plastic. Results suggest a useable life for the plastic of 5 to 6 years of outdoor exposure. The response in degradation was dependent on the rate of the additive in the tray. The lifespan of trays in actual use would be expected to be longer since UV exposure is significantly reduced once the seedlings grow to cover the trays and trays would typically be stored out of the sun when not in use. Greenhouse trials are currently under way to evaluate the degradation of the plastic under actual greenhouse conditions.

AP 08

Isolation, screening and characterisation of plant growth promoting *rhizobacteria* isolates for their ability to induce drought tolerance on tobacco (*Nicotiana tabacum*)

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Water availability is one of the major challenges limiting agricultural production in Zimbabwe. Recently, the country has been experiencing notable shift in seasons, uneven rainfall distribution, mid-season droughts and decreases in annual rainfall which are issues of great concern. Elsewhere, the use of drought tolerant varieties and genetic engineering are amongst the mitigation strategies in use. Additionally, the use of plant growth promoting rhizobacteria (PGPR) has been reported to be effective in enabling plants to withstand drought effects. This study aimed to isolate, screen and characterise native PGPR for their ability to induce drought tolerance on tobacco seedlings. Drought tolerant weed species were collected from hill tops, arid and semi-arid regions of Zimbabwe and the roots were cut out, placed into test tubes and then heated under a water bath to eliminate the non-heat tolerant species. Thereafter roots were discarded and the solution subjected to serial dilutions, after which plating was done on amended nutrient agar (NA).

Over 200 bacterial isolates grew and were collected from petri dishes for further evaluation. These isolates were then evaluated in five *in vitro* tests namely, bacterial growth under reduced water availability, exopolysaccharide production, phosphate solubilisation, ammonium and cellulase production. From these tests the ten best isolates were selected for identification and further evaluation. Molecular characterisation using 16s rDNA sequencing showed that these isolates fell into six species namely *Bacillus subtilis*, *Bacillus firmus*, *Agrobacterium tumefaciens*, *Pseudomonas fluorescens*, *Pseudomonas putida* and *Pantoea agglomerans*. Greenhouse trials then followed to evaluate the ability of these isolates to induce drought tolerance on tobacco seedlings. Parameters such as their ability to improve chlorophyll content, biomass weights and nutrient status of tobacco seedlings were also evaluated. Further tests will be conducted under field conditions; however preliminary results showed that these isolates have great potential in inducing drought tolerance on tobacco seedlings.

AP 09

Extracting value from tobacco waste through activating the carbon in tobacco stalks

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Tobacco is an economically important crop accounting for over 20 % of export receipts in Zimbabwe. This crop is grown by well over 173 000 growers on a hectarage of over 100 000. However, in Zimbabwe only the leaf is currently valuable as stalks have no economic use. Elsewhere, due to the high lignin and cellulose content, tobacco stalks are being used for the production of energy briquettes among other uses, giving the crop additional value. The objective of this trial was, therefore, to investigate the possibility of using tobacco stalk material as a precursor for activated carbon preparation as a new means of enhancing value. The preparation of activated carbon using tobacco stalks and microwave heating was studied and optimised. The prepared activated carbon was further applied as an adsorbent for methylene blue (MB) and lead removal from water. The optimised conditions for activated carbon preparation were a radiation power of 280 W for a period of six minutes having impregnated the precursor material with 30 % $ZnCl_2$ for 24 hours. The activated carbon yield was 49.43 % with an iodine number of 1264.51 mg/g and a point of zero charge of 5.81. The adsorption kinetics for both MB and lead followed the pseudo second order kinetic model with the intra-particle diffusion model suggesting a two-step adsorption mechanism. Experimental adsorption data for both MB and lead also fitted well within the Langmuir adsorption isotherm model. It can, therefore, be concluded that tobacco stalks can be turned into an economically important product through the production of activated carbon which compares favourably with commercial products on the market.

AP 10

Study on pollution control and thermal efficiency optimisation of tobacco bulk curing barn

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Coal is still used as a fuel to provide the necessary heat for most tobacco curing barns in China. Due to the lack of corresponding equipment and the measures to reduce emissions, the gas pollutants from curing barns, such as SO_2, smoke, etc., are often discharged into the atmosphere without any processing, and it causes serious pollution to the environment. To solve the pollution problem of SO_2 and particulate matter (PM) during fuel combustion in tobacco barns, this study investigated the pollutant emission properties and mechanisms of tobacco barns in five provinces, i.e. Hu Bei, Fu Jian, Gui Zhou, Yu Nan and He Nan. Equipment was designed to control the emission of SO_2 and PM according to emission properties, and the effect of the equipment was verified. The results showed that desulfurisation and dedusting efficiency were in the range of 75-90 % and the concentration of pollutants was lower than the corresponding emission standards with the use of the equipment. There was a large heat loss in the tobacco barn, i.e. the heat loss of exhaust gas was 10-20 % and the heat loss of incomplete combustion was 4-11 %. The thermal efficiency of the curing room was calculated by the indirect balance method, and the calculation results showed that the thermal efficiency of the tobacco barn at the five demonstration sites was 25-45 %. Moreover, the fuel-rich region with a high thickness of coal seam in furnace caused a high pollutant emission and a large heat loss. However, the use of automatic continuous coal feeding equipment could effectively reduce the emission of pollutants and the loss of heat, and also improve the thermal efficiency of the tobacco curing room. Therefore, the use of desulfurisation dedusting facilities and automatic coal filling techniques could significantly reduce pollutant emissions in bulk curing barns.

AP 11

Tobacco mutants with fewer suckers

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In tobacco, depending on strong shoot apical dominance, suckers grow vigorously after topping. Tobacco plants produce up to a third sucker sequentially at each leaf axil during the cultivation period. Suckers adversely affect tobacco leaf yield and quality. Therefore, sucker control is a key process to achieve tobacco production with high yields and good quality. We earlier isolated five genes involved in axial meristem formation. RNAi knock-down tobaccos of these five genes exhibited normal growth of the first sucker, but the second sucker was suppressed. The objective of this study was to evaluate the effects of these genes on sucker growth during cultivation in the field. First, knock-out (KO) tobacco mutants of each isolated gene were screened from an ethyl ethanesulfonate-mutagenized tobacco library (var. Tsukuba 1: flue-cured tobacco). Subsequently, the suckers at each leaf axil in the screened mutants were counted before and after topping in the field at the Leaf Tobacco Research Center (LTRC) in Japan. In this presentation, we present results of KO mutants of three out of five genes. The KO mutants showed normal growth of the first sucker but showed significantly suppressed second sucker formation compared with wild type. These results were similar to earlier results obtained from the RNAi knock-down tobaccos in a greenhouse. The findings imply that screened mutants may be useful for developing new varieties with fewer suckers.

AP 12

Molecular mechanism involved in maleic hydrazide-mediated sucker control

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Molecular signals from the shoot apical meristem (SAM) mediate a hormonal signal that effectively inhibits axillary bud growth. Upon removal of the SAM, the hormonal signal is lost and enables axillary buds to grow into new shoots (or “suckers”) resulting in yield loss and poor leaf quality. Sucker control chemical applications are critical for managing sucker growth following plant topping. Predominantly, maleic hydrazide (MH) has been used to manage sucker growth as a systemic sucker control reagent. After application, maleic hydrazide is absorbed by leaves and translocated through the plant to small sucker buds. The inhibitory effect of MH on sucker growth is considered to be the result of the suppression of plant metabolism (inhibition of enzymatic activity) and interference of the compound with plant hormones and growth regulators. MH also acts as an inhibitor of the synthesis of nucleic acids and proteins. Understanding the mechanism of MH on sucker suppression may provide additional insights for the development of non-chemical sucker control. In this study, transcriptome profiles were carried out before and after MH treatments. Differential expression analysis showed that several cell cycle genes were inhibited from MH treated plants compared to controls. Experiments are in progress to evaluate these cell cycle genes with sucker specific promoters.

AP 13

Development of codominant markers related to powdery mildew resistance from *N. tomentosiformis* origin

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Powdery mildew is a common tobacco plant fungal disease which can reduce growers' crop yields, particularly in South Africa, Asia and Europe. Three different resistance sources are known, coming from wild tobacco species *N. tomentosiformis* and *N. glutinosa*, or from *N. tabacum*. The latter has recently been identified as a recessive resistance controlled by two closely linked *mlo* genes.

In this study, *N. tomentosiformis* dominant resistance was investigated in a F2 flue-cured population segregating for powdery mildew resistance. This resistance was first introgressed into the Zimbabwean variety TB 22, a flue-cured line obtained from a hybrid between *N. tabacum* and *N. tomentosiformis*. The resistance was then introduced into modern breeding tobacco lines in Bergerac, France.

RNA-Seq analysis was used to compare resistant and susceptible F2 plants, but also to identify single nucleotide polymorphisms (SNPs) between them. Polymorphic contigs were compared to the reference genome available on Solgenomics, showing that the polymorphism characterising resistant genotypes is spanned on a large portion of chromosome 19. Linkage mapping using SNPs and SSR markers confirmed this finding. SNPs were confirmed on a larger set of tobacco varieties using RNA-seq data mapped to Nt19. From these results, KASP™ markers were designed, enriching the panel of tools available for efficient breeding of powdery mildew resistant lines.

AP 14

Field tests of loss-of-function tobacco lines of eIF(iso)4E for resistance to tobacco bushy top disease and agronomic traits

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Tobacco bushy top disease (TBTD) is one of the serious diseases affecting tobacco (*Nicotiana tabacum* L.) production in African countries such as Malawi, Zambia, Tanzania and Ethiopia. This disease is caused by a mixed infection of tobacco bushy top virus (TBTV, genus *Umbravirus*) and its helper virus for aphid transmission (a member of the genus *Polerovirus*). In a recent study, we demonstrated that loss-of-function of eukaryotic translation initiation factor eIF(iso)4E in tobacco conferred resistance to TBTV by sap inoculation in a greenhouse. The objective of this study was to evaluate the loss-of-function tobacco lines of eIF(iso)4E for resistance to TBTD by aphid transmission under a natural environment and for agronomic traits in fields. Field tests for resistance to TBTD were conducted for three seasons in fields of the Agricultural Research and Extension Trust in Malawi. Agronomic traits were examined at fields of the JT Leaf Tobacco Research Center in Japan. The loss-of-function tobacco lines of eIF(iso)4E of both flue-cured and Burley types were used for this study. Wild type lines for eIF(iso)4E were used as a control. Regarding the incidence of TBTD, the loss-of-function lines of eIF(iso)4E were significantly lower than the control lines in almost all field tests. Regarding plant height and total leaf numbers at the flowering period and yield of cured-leaves, the loss-of-function lines of eIF(iso)4E were not significantly different from the control lines. These results suggest that loss-of-function of eIF(iso)4E in tobacco confers resistance to TBTD under a natural environment and that it does not adversely affect agronomic traits. Future cultivation of tobacco varieties with loss-of-function of eIF(iso)4E will contribute to stable leaf tobacco production in regions adversely affected by economic losses from TBTD.

AP 15

Augmenting available soil nutrients through the use of growth promoting microbes in tobacco production

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Sustainable agriculture on a limited space of land is a huge problem for most tobacco growers in Zimbabwe. The pressure on land resources continuously depletes soil productivity leading to over use of synthetic fertilisers to compensate for the loss in soil quality. This practice is not sustainable and poses an environmental risk as tobacco is grown on sandy to sandy loam soils that are prone to leaching, leading to the contamination of water bodies. In addition, the overuse of synthetic fertiliser compromises soil biodiversity thus impeding the sustainable function soil ecosystems. Thus research on alternative ways of improving soil productivity is imperative. A two-year study was carried out at Kutsaga Research Station, to evaluate the effect of Micro-plus, an organic bio-nutrition based product with over 30 soil micro-organisms incorporated, for its effectiveness in tobacco production. Micro-plus contains among other microbials, multiple friendly strains of *Bacillus*, *Enterobacter*, *Pseudomonas*, *Stenotromonas*, *Rhizobium* and *Trichoderma* selected for their ability to rehabilitate and enhance soil fertility. The trial was set up in a randomised complete block design with four treatments which included a sole Micro-plus treatment and in combination with 100 %, 50 % and 25 % of the standard fertiliser programme. Results showed that this product, used with 50 % of the standard fertilizer treatment, gave the best yield and quality of the treated crops. Applying Micro-plus to plots treated with 100 % of the standard fertilisers gave no additional benefits to the grower while, on the other hand, Micro-plus as a stand-alone treatment was not effective. This paper will explore the implications of reducing the reliance on chemical fertilisers and adoption of bio-nutrition based products, such as Micro-plus, not only on tobacco yield and quality but also on soil health.

AP 16

Greenhouse and field applications of various organic nitrogen fertilizer sources

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Current nitrogen fertilizer programs for organic flue-cured tobacco production have proven successful but are not absent of agronomic concern. In the current evaluation, different organic nitrogen sources were evaluated for their usability in seedling and field production. Sources evaluated for seedling production were Seabird Guano, Feather Meal, Blood Meal, Liquid Chicken Litter + Sodium Nitrate, Sodium Nitrate, Corn Gluten, Soy Protein, Dry Chicken Litter, and Fish Emulsion. A conventional control was included for comparison (SQM Ultrasol Premium 16-5-16). Analysis of float water samples over a 60-day timespan revealed that treatments containing Liquid Chicken Litter + Sodium Nitrate, Sodium Nitrate, and SQM Ultrasol Premium 16-5-16 contained the highest nitrate-N and lowest ammonium-N concentrations. The same treatments contained the lowest bicarbonate concentration between 10 and 40 days after seeding (≤ 4 meq HCO_3-/L). At the conclusion of the study SQM Ultrasol Premium 16-5-16, Seabird Guano, Liquid Chicken Litter + Sodium Nitrate, and Fish Emulsion produced the highest number of usable seedlings. Treatments comprised of Blood Meal, Sodium Nitrate, Corn Gluten, and Soy Protein produced the fewest usable seedlings due to severe phosphorus deficiency. In the field evaluation, cured leaf yield was as follows: Sodium Nitrate > Soy Protein ≥ Blood Meal ≥ Seabird Guano > Corn Gluten ≥ Feather Meal ≥ Dry Chicken Litter > Non-treated Control. Cured leaf value followed a similar trend. Liquid Chicken Litter + Sodium Nitrate and Fish Emulsion were excluded from the field study. Preliminary results suggest that Liquid Chicken Litter + Sodium Nitrate and Fish Emulsion might be suitable alternatives to Seabird Guano in the float system and that Soy Protein may serve as another option for field production.

AP 17

Variation of soil potassium quantity-intensity relationship in flue-cured tobacco-rape rotation system under long-term located potassium application

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The aim of this study was to explore the effect of long-term potassium application on soil potassium supply capacity, so as to provide certain scientific basis for rationalising potassium application and continuing research on the development of agricultural soil.

A long term potassium fertilisation experiment on a tobacco-rape rotation system was carried out. It was designed to have 0,187.5 or 375 $kg \cdot hm^{-2}$ K_2O applied to flue-cured tobacco and 36 $kg \cdot hm^{-2}$ to rape. Soil samples were collected for analysis of apparent balance and quantity-intensity (Q/I) of soil potassium, and relevant parameters, i.e. K equilibrium activity ratio (AR_e), soil labile K (K_L), non-specific labile K ($-\Delta K_0$) and specific labile K (Kx), potential buffering capacity (PBC), K and Ca+Mg exchange free energy change (-ΔG), and soil properties, like readily available K (Kav), slowly available K (Knex), K saturation degree (K_S), clay mineral components were analysed. Moreover, relationships between Q/I parameters and soil properties in the flue-cured tobacco-rape cropping system were studied.

Results show that after 20 years of the stationary experiment, AR_e varied in the range of 0.38×10^{-3}~$8.91\times10^{-3}(mol \cdot L^{-1})^{0.5}$, $-\Delta K_0$ of 0.02~0.55 $cmol \cdot kg^{-1}$, K_L of 1.61~2.16 $cmol \cdot kg^{-1}$, KX of 1.06-2.14 $cmol \cdot kg^{-1}$, PBC of 6.20~7.76 $cmol \cdot kg^{-1}(mol \cdot L^{-1})^{-0.5}$, and -ΔG of 11.70~19.72 $kJ \cdot mol^{-1}$. Obviously K application enhanced both AR_e and $-\Delta K_0$, but reduced K_L, Kx, PBC, and -ΔG. Kav, Knex, K_S and Kab were very significantly and positively correlated with AR_e and $-\Delta K_0$, but negatively with K_L, Kx, PBC and -ΔG. Significant relationships were observed between the parameters of the Q/I curve of soil K, and these parameters could be integrated into a principal component index, which was significantly lower in K application treatments than in no K application treatments. Soil potassium was retained mainly interlaminarly in clay mineral crystals and lower in bio-availability in the soils with no K applied, but on edges of the clay mineral crystals and higher in bio-availability in the soils applied with K. Hence, the soils with no K applied were higher in vermiculite content, but lower in illite content than the soils with K applied.

In conclusion, all the Q/I curve parameters, such as AR_e, ΔK_0, K_L, Kx, PBC, -ΔG, can be used as indicators for evaluation of soil potassium supply capacity. Apparent balance of soil potassium is a major factor driving changes in K Q/I curve shape and in the differentiation of its parameters, variation of potassium supply capacity and evolution of soil minerals. The higher the K application rate, the higher the soil potassium surplus and the higher the soil supply capacity. In the soils under long term potassium deficiency, mineral potassium transforms into specific labile K at a higher rate than specific labile K does into non-specific labile K. Long-term potassium deficiency causes transformation of illite into vermiculite.

AP 18

Establishing application protocols for poultry litter and feather meal in organic Burley tobacco production

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Information on nutrient management in organic Burley tobacco is limited. Unlike conventional nitrogen (N) sources, which are readily available for plant use upon application, organic N fertilizers require microbial mineralization to become plant available. As such, optimizing the synchrony of plant N demand and N fertilizer mineralization can be challenging. Two studies were conducted at the Mountain Research Station in Waynesville (W), North Carolina and the Upper Mountain Research Station in Laurel Springs (LS), North Carolina in 2018. Study one investigated the effects of application methods (broadcast, split application, and side dress) of pelleted, hydrolyzed feather meal (Nature Safe® 13-0-0) and composted chicken poultry manure (Harmony 5-4-3). Study two compared application rates (168, 224, 280, and 336 $kg \cdot ha^{-1}$) of both organic fertilizers. A conventional control (SQM 12-0-46 + 28-0-0 liquid UAN) was split applied (112 $kg \cdot ha^{-1}$ broadcast during field preparation and 112 $kg \cdot ha^{-1}$ side-dressed ten days after transplanting). Yields, quality, total alkaloid content, and leaf nitrate levels increased linearly with increasing application rates at LS. Leaf nitrate concentrations were highest in feather meal regardless of rate at LS. At both sites feather meal significantly increased total alkaloid content compared to poultry manure. In study two, application methods did not affect yield at LS; however, split application resulted in higher yields compared to broadcast application at W (4350 $kg \cdot ha^{-1}$ vs 3760 $kg \cdot ha^{-1}$, respectively). Similar to study one, alkaloids where higher in feather meal treatments (4.3 %) than poultry litter (3.8 %). The linear trend in application rates suggests that higher rates may be required to maximize yield. Furthermore, split application of fertilizer may benefit growers as it resulted in higher yields at LS and may protect against potential N leaching in case of significant early season rain events. Finally, feather meal may be a better option as it resulted in higher total alkaloids across both studies.

AP 19

Effects of rotation and organic fertilisers on soil water-stable aggregate stability and their associated carbon and nitrogen in flue-cured tobacco production

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Long-term mono-cropping and over application of chemical fertilisers will result in reduced water-stable soil aggregate stability and soil nitrogen (N) and carbon (C) stocks. To understand how agronomic practices affect soil structure and organic matter storage, this study investigated the effect of rotation and fertilisation type on the proportion of water-stable aggregates and aggregate-associated soil organic carbon (SOC) and total soil nitrogen (TSN) concentrations and stocks. Two tobacco rotation systems (tobacco monoculture and tobacco-corn rotation) with five fertilizer treatments (0 kg N/ha, and 75 kg N/ha, and 450 kg oil cake + 75 kg N/ha, and 15000 kg manure + 60 kg N/ha, and 3000 kg straw + 75 kg N/ha) were established in 2007. After ten years, soil aggregation and aggregate-associated SOC and TSN were significantly affected by rotation and fertiliser types at different soil depths. Rotation, fertilisation type and soil depth rotation significantly affected soil bulk density, mean weight diameter (MWD) and geometric mean diameter (GMD) of soil aggregate. Compared with tobacco mono-cropping, tobacco-corn rotation can significantly increase MWD and GMD, and GMD of soil aggregates will also significantly increase with the increment of soil depth. Compared with mono-cropping and sole-application of chemical fertiliser, rotation and organic fertiliser can effectively improve the proportion of large macro-aggregate components (> 2000 µm) and MWD and GMD, but the proportion of micro-aggregate components (250-53 µm) is significantly reduced. The effect of fertiliser type and rotation on the soil organic carbon (SOC) content of large macro-aggregate components (> 2000 µm) was more obvious than that of the soluble total soil nitrogen (TSN) of the aggregates under this particle size. To sum up, rotation and organic fertiliser application in tobacco production can stabilise the proportion of large-size aggregates in the surface soil and promote the formation of agglomerates, to better maintain the SOC and TSN.

AP 20

Implications of chloride application rate and nitrogen fertilizer source on flue-cured tobacco

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Chloride (Cl^-) assimilation by flue-cured tobacco can be toxic and may negatively impact leaf quality and combustion when tissue concentration exceeds 1 %. Uptake is influenced by exposure to NH_4^+ and Cl^-, particularly that coming from fertilizer. The impact of these factors has not been fully described in modern systems utilizing reduced-cost or custom-blend fertility programs. Research was conducted to test the interaction of four Cl^- application rates (0, 34, 67, and 101 kg ha^{-1}) and four nitrogen (N) fertilizer sources (calcium nitrate, ammonium nitrate, urea-ammonium nitrate, and ammonium sulfate) on the growth and development of flue-cured tobacco. The impact of N source was minimal in green tissue; however, ammonium sulfate reduced cured leaf quality relative to other N sources. Chloride rates ≥ 34 kg ha^{-1} reduced foliar total N and NO_3 measurements by 0.12 to 0.42 % and 789 to 1,348 mg kg^{-1}, respectively, two weeks after application. Nitrate concentration was also reduced at layby, while P, K, and Mg responded positively to Cl^- application between in late-season measurements. After curing, total N and alkaloids were reduced while reducing sugars were maximized as Cl^- application increased from 0 to 34 kg ha^{-1}. Chloride concentration exceeded 1 % in application rates ≥ 34 kg ha^{-1} in early-season and post-curing measurements, although toxic symptoms were not observed nor were yield, quality, or value affected. In order to maintain cured leaf quality producers should utilize N sources containing ≥ 25 % NO_3. Likewise, Cl^- application should not exceed 34 kg ha^{-1} in order to maintain the integrity of manufactured products.

AP 21

Evaluations of rimsulfuron application timing and rate in flue-cured tobacco

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Field studies were conducted from 2016 through 2018 to evaluate flue-cured tobacco response to rimsulfuron application timing and rate (17 and 34 g a.i. ha^{-1}). Additionally, weed control was evaluated in combination with current recommended herbicide programs for flue-cured tobacco production. Depending on rate, visual injury ranged from 6 to 33 % across environments when rimsulfuron was applied postemergence over-the-top (POT) three weeks after transplanting (WAT). By six WAT no visual injury was observed and there were no differences in yield, quality, or value of cured-leaf when compared to tobacco receiving only clomazone plus sulfentrazone pre-transplant (PRE-T). No injury was observed for any PRE-T or post-directed (PD) at lay-by application containing rimsulfuron. When applied PD, rimsulfuron performed similar to pendimethlain and napropamide for Palmer amaranth (*Amaranthus palmerii*) and grass control (*Digitaria sanguinalis*, *Eleusine indica*, and *Echinochloa crus-galli*), while providing slightly greater control of yellow nutsedge (*Cyperus esculentus*). These results indicate rimsulfuron did not adversely affect yield components of flue-cured tobacco. However, due to potential early season injury, POT applications of rimsulfuron will not be recommended. The addition of rimsufluron, PRE-T and PD, would provide flue-cured tobacco growers with additional options for weed control.

AP 22

Organic suckercides: screening various compounds for efficacy and injury potential

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Organically derived fatty alcohol products are useful for the control of tobacco axillary buds (suckers) and are greatly needed by commercial tobacco farmers. Recently, USDA-National Organic Program approval of these products has been scrutinized, leaving farmers, certifying agencies, and tobacco buyers with questions about the efficacy and injury potential of alternative products. The objective of this research was to evaluate a variety of materials that might be used for chemical sucker control. Materials investigated included pelargonic acid, vegetable oil, canola oil, and peppermint + spearmint oil using two different application methods: a standard 3-nozzle boom or a dropline. Fatty alcohol was included for comparison and evaluated using both application methods. Chemical injury was minimal following each treatment except for those containing pelargonic acid. Injury was greatest when applied with the 3-nozzle boom and was reduced by nearly 50 % with the dropline; however, injury following the dropline application was still greater than that resulting from other treatment combinations. Sucker control was acceptable with pelargonic acid (83 to 98 %) and was similar to that resulting from fatty alcohol (99-100 %). Sucker control was <40 % among all other treatments, with peppermint + spearmint oil (32 to 34 %) providing better efficacy than canola (9 to 15 %) or vegetable oil (-3 to 7 %). Cured leaf yield, quality, and value were likewise greatest in fatty alcohol treatments due to maximized sucker control and minimized chemical injury. Treatments containing the alternative products resulted in lower post-harvest measurements due to extreme injury (pelargonic acid) or poor sucker control. Producers are encouraged to utilize fatty alcohol until the alternative products can be re-formulated and re-evaluated.

AP 23

Evaluation of contact and local-systemic sucker control products on flue-cured tobacco in Canada

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In Canada, control of axillary buds (suckers) for flue-cured tobacco is currently achieved through the use of long chain fatty alcohols, more specifically n-decanol (C10). Fatty alcohols are contact-type suckercides that must be applied in a way that contacts each leaf axil to effectively control sucker growth. Recently, research has shown that pelargonic acid (PA) has contact activity on suckers but can result in phytotoxicity. However, sucker control without phytotoxicity using multiple applications at lower rates (0.5 % to 1.0 %) has been reported. The Canadian Tobacco Research Foundation initiated a field experiment in 2019 with the objective to evaluate other sucker control products in comparison with the only registered active ingredient (n-decanol) on agronomic performance of flue-cured tobacco. The biocontrol product Beloukha (a.i. 680 g/L PA) from BELCHIM Crop Protection was applied in five applications with 0.75 % v/v for the first application and 1.0 % v/v for all remaining applications at a volume of 467 L/ha. In addition, the local-systemic products, Drexalin Plus (a.i. 13.7 % flumetralin, Drexel Chemical Company) and Tak-Plus (a.i. 56 % 1-decanol + 4 % flumetralin, Drexel Chemical Company) were also evaluated. Preliminary results including sucker control, plant growth, leaf drop and phytotoxicity data from the field experiment will be discussed.

AP 24

Effects of early removal of lower leaf on ripening patterns, yield and quality in tobacco

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With the global over-supply of filler styles tobacco (*Nicotiana tabacum* L.), especially leaf from lower plant positions, growers are seeking methods to increase economic returns. One such approach that Zimbabwe growers' practice is the early removal of the lowermost leaves. These are considered to hold little economic value in the current global markets. This practice is also used by growers on later planted crops to reduce leaf ripening clashes with earlier planted crops. The aim of the research was to determine the following effects of the early removal of lower leaves: days to topping after transplanting, diseases, leaf ripening rates, yield and quality. Research was conducted in 2018/19 season using three sets of trials (each having three subsets) to determine the outcome of not removing leaves and removing 5 and 10 leaves at six weeks after planting. All three sets were topped to 19 leaves. All agronomic practices were carried out equally between the trial sets (no additional fertilizer was applied). During the trial period precipitation levels were supplemented with irrigation over unusually lengthy dry spells. It was found that the removal of five leaves and ten leaves delayed topping by one week and two weeks respectively. Notable differences were found between the ripening rates of each trial set. The effects on quality, yield and economic merit will be discussed further in the body of this paper.

AP 25

Lower leaf removal and nitrogen application programs for flue-cured tobacco production

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With a current global over-supply of flue-cured tobacco, tobacco producers in North Carolina have been encouraged to remove the lowermost leaves prior to harvest due to their low value in manufactured products. The objective of this research was to compare lower-leaf removal programs. Research was conducted in 2016 and 2017 to quantify the agronomic effects of three lower-leaf removal programs (0, 4, and 8 leaves plant^{-1}) and the subsequent delivery of four nitrogen (N) application rates (0, 5.6, 11.2, and 16.9 kg N ha^{-1} above base recommendation). All treatment combinations were applied during the early flowering stage of growth (8-10 weeks after transplanting). Programs absent of leaf removal generally produced the highest cured leaf yield. The addition of 16.9 kg N ha^{-1} increased yield when compared to lower N application rates within the 4 leaf removal program. Nitrogen application did not affect yield in the 8 leaf removal program. Cured leaf value was greatest in the 0 leaf removal program ($US 10,131 ha^{-1}) and was reduced in the 4 and 8 leaf programs by $US 1,611 and 2,645 ha^{-1}, respectively. Lower-stalk positions were nearly eliminated in the 8 leaf removal program, while the 4 leaf removal program reduced their presence by more than 50 %. Ultimately, if these programs are to be encouraged or required by industry, the removal of 4 leaves per plant proved to be more practical when paired with additional N, due to moderate yield reduction and lower-stalk leaf production.

AP 26

Cured leaf residues following applications of fluopicolide, indoxacarb, and oxathiapiprolin to flue-cured tobacco

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Pesticide residues found on cured tobacco remain a large concern to the allied tobacco industry. To better quantify cured leaf residues, three active ingredients (fluopicolide, indoxacarb, and oxathiapiprolin) were applied to flue-cured tobacco grown in six North Carolina environments from 2016 to 2018. Fluopicolide residues were consistently among the highest documented in this evaluation (7.25 mg/kg maximum), which was most likely a result of the compound having the shortest PHI (7 days) among the products tested. The highest indoxacarb residue was 2.15 mg/kg, which was identified in lower-stalk position samples collected from one environment in 2018. Additional data suggests that indoxacarb residues are likely to be < 2.0 mg/kg. Oxathiapiprolin was below the limit of quantification (0.09 mg kg^{-1}) in 98.6 % of the samples analyzed and averaged 0.10 mg/kg in the lower-stalk position of one environment in 2017. Results from this study indicate that cured leaf residues from fluopicolide are moderate relative to indoxacarb and oxathiapiprolin, but are not as great as other pesticides for which Guidance Residue Limits are currently established. Furthermore, it is plausible that residues from commercial farming operations would be lower than those reported due to Integrated Pest Management practices. Further investigations are warranted in order to better identify residues resulting from applications delivered using recommendations put forth by Cooperative Extension Services in the southern U.S.

AP 27

A low cost, efficient, multi-fuel heat exchange system availed to Zimbabwean small holder growers

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Zimbabwe currently has some 165,000 registered tobacco growers, 85 % of which are small holders who utilise less than two hectares of land for growing the crop. This group of growers relies mostly on indigenous fuel-wood for curing their crop, a practise that is unsustainable as it leads to deforestation. While coal is readily available, the current heat exchange systems for most of the barns utilised by small holders require electricity-powered blowers for it to burn efficiently. Unfortunately, electricity is not readily available in the rural areas where most of these growers are located. Thus, it became necessary to develop alternative heat exchange systems that obviate the requirement for an electrical energy source. Recently, a new heat exchange system named the Twin Turbo Design (TTD) was developed to fulfil this requirement. The main objective of this study was, therefore, to evaluate the curing efficiency of the Twin Turbo heat exchange system. Three treatments, namely the Twin Turbo Barn, a Retrofit barn (Rocket barn retrofitted with the Twin Turbo heat exchange system design) and the Standard Rocket barn were evaluated. Coal cobbles were used as the curing fuel. Results showed that the Twin Turbo barn had the highest fuel use efficiency within the acceptable barn turnaround time of seven days. Additionally it had the highest combustion efficiency despite the absence of a fan and also produced high quality leaf. It was also found that this heat exchange is not only more efficient but also has the added advantage that other sustainable fuel sources such as biomass briquettes and LP gas can be utilised. While there are concerted efforts to find more environmentally friendly and sustainable substitutes for coal, it is currently the only readily available alternative to the use of indigenous fuel wood sources and the small holder growers can now also adopt its use.

AP 28

Latest innovations in improving efficiencies in tobacco curing in Zimbabwe: the Super Eco Curer

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As ROC Systems is on a continuous development drive, the search for new novel ways to improve quality and efficiency of curers has led us to the design of the super eco curer. Using different wet bulb temperatures on each day of the curing process brings about the optimum conditions for the three basic stages of curing, eliminating fungal barn rot, sponge in lower reapings and slate in higher reapings. Using the different wet bulb temperature we determine which enzyme we want to work in the leaf at the different stages. With a traditional 6-day cure, Stage 1 which is day 1 and 2 we run the curer at a web bulb of 33 °C to 35 °C which is the optimum temperature for chlorophyllase, the enzyme that metabolises chlorophyll, at this stage sufficient wilting is also expected to concentrate the chlorophyllase enzyme and speed up colouring. Stage 2 which is day 3 and 4 is lamina drying and starch breakdown. The key enzyme for the breakdown of starch is amylase and its optimum operating wet bulb temperature is 38 °C and above. Having sufficiently wilted our leaf on Stage 1 ensures that we have increased the concentration of our amylase in the leaf and this speeds up the breakdown of starch. Stage 3, which is our traditional day 5 and 6, is our mid-rib drying where we are aiming at energy saving with the most efficient wet bulb temperatures being 40-43 °C. Curing the super eco way has ensured our clientele with up to 30 % saving on curing fuel and up to 30 % more capacity for the same capital outlay.

AP 29

Construction of green flue-cured tobacco strips aging technology based on four-stage controlled atmosphere method

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Aging of flue-cured tobacco strips plays an important role in improving the quality and use value of tobacco. Natural aging is the most widely used storage method at present, but there are still some problems, such as shorter suitable aging duration, easy occurrence of mildew and storage pests and using chemical pesticides for fumigation. In this study, we established a new controlled-atmosphere (CA) aging technology which divided the aging period into four stages according to the different purposes. The first stage is based on natural aging, and the latter three CA stages were CA pests and mildew control, CA aging and CA quality maintenance stage, respectively. The sensory quality, appearance quality, carotenoid content and pH value were compared between natural aging and four-stage CA aging using flue-cured tobacco strips from different tobacco production areas. The results showed that the tobacco beetles and mildew can be effectively controlled using low oxygen concentration (≤ 2 %) for 90 days. After this, the oxygen concentration was adjusted to 7 % ~ 9 % for more than 120 days. This CA aging stage can significantly improve the sensory quality and delay the over-darkening of tobacco strips. Lastly, reducing the oxygen concentration below 2 % and maintaining it until the tobacco stack was opened. The technology not only improved the tobacco aging quality, but also extended the suitable aging duration by at least 15 months compared to the natural aging. The decrease rate of carotenoids and pH values were also delayed. The key periods of tobacco aging duration were subdivided and chemical pesticides were avoided in our study. The four-stage CA aging technology supplied a green, high-quality and environmentally friendly system for tobacco strips storage.

AP 30

Lethal effect of low pressure on the cigarette beetle

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Low-oxygen (O_2) controlled atmosphere (CA) is becoming used more commonly for disinfestation of stored tobacco. Generally, low-O_2 atmospheres are achieved by nitrogen-purge. Practical CA chambers require approximately 24 h to reduce the partial pressure of O_2 to lethal levels. Decompression is another means of reducing the O_2 partial pressure. Lethal low pressures are achievable in much shorter periods of time using a vacuum pump. This study examined the lethality of low pressures on the cigarette beetle (*Lasioderma serricorne*) to verify the equivalence of low O_2 and low pressures in terms of lethality. Tests were conducted in a temperature-controlled room (15-35 °C). Eggs, larvae, pupae, and adults were exposed to reduced pressure in vacuum desiccators (0.85 L). Desiccators were serially connected to a vacuum pump by polyurethane tubing. The air in the desiccators was evacuated to the target pressures (2.5, 5, or 10 kPa) with a vacuum pump. The desiccators were disconnected sequentially from the distal end and were vented at predetermined intervals. After treatment, viability of the insects was assessed. Results demonstrated that the highest lethality was achieved at 5 kPa. At this pressure level, the time necessary to achieve 99 % lethality in all growing stages (9.8 d at 20 °C; 7.0 d at 25 °C) was much shorter than low-O_2 (0.5 %) treatment (35.6 d at 20 °C; 25.2 d at 25 °C). Susceptibility to low pressure varied among growing stages: eggs and pupae were the most tolerant; adults and larvae were less tolerant. Low-pressure treatment is not equivalent to low-O_2 treatment because the most tolerant stages against low-O_2 are opposite: adults and larvae. These results suggest that low-pressure and low-O_2 treatment can be mutually complementary and that synergies can be created by combining these two treatments.

AP 31

Agronomic performance of some exotic tobacco cultivars under the Zimbabwean environmental conditions

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Tobacco production is an activity of high economic importance in many countries and variety development and selection of appropriate varieties is key to productivity. Breeding and deployment of novel and suitable varieties is a business which spans continental barriers and it is, therefore, prudent to routinely screen cultivars developed from other places (exotic) for novelty, broad adaptation and resistance to commonly occurring local tobacco diseases. A three-year study (2016-2018) was conducted at Kutsaga Research Station in Zimbabwe with the objective of evaluating the agronomic performance of 13 exotic flue-cured cultivars [Brazil (6), China (2), South Africa (2) and United States of America (3)] against the performance of five locally developed varieties. The experiment was setup as a randomised complete block design with 17 entries, three blocks and two replications. Full agronomic data including days to 50 % topping, leaf expansion measurements, cured leaf yields and styles, grade indices and stalk heights will be presented and discussed along with the performance of each exotic cultivar and the implications.

AP 32

Development of short season tobacco varieties for marginal tobacco growing regions of Zimbabwe

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The advent of climate change has brought about a need for tobacco varieties capable of adapting to marginal climatic conditions. Also, the perceived profitability of the tobacco enterprise by small scale growers in Zimbabwe has led to a staggering increase in the number of tobacco farmers, with the consequence that tobacco is now cultivated in marginal and non-traditional regions. These regions receive significantly lower rainfall, which is often poorly distributed and also experience consistently higher seasonal temperatures compared to traditional growing regions. An ideotype of varieties to ameliorate such debilitating challenges is fast growing to escape drought, capable of producing acceptable yields, multi disease resistant particularly to root-knot nematodes (*Meloidogyne javanica*) and tobacco mosaic virus. Such an ideotype variety would be capable of quick growth and development that would allow it to accumulate biomass relatively quickly before the full effects of drought conditions limit productivity. A breeding programme was, therefore, designed which generated 12 experimental tobacco hybrids that were subjected to two years of full agronomic testing in a very marginal flue-cured tobacco production area (Makoholi Research Station, Masvingo, Zimbabwe). Results of the trial will be presented and discussed. The economic implications of deploying short season tobacco varieties to farmers and contracting merchants will also be highlighted.

AP 33

Cigar wrapper production in Zimbabwe: an overview of past, present and future prospects

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The cigar wrapper tobacco type was once grown in Zimbabwe in the early 1950s in a project that was championed by British American Tobacco (BAT). However, production was stifled owing to viability challenges coupled with loss of interest from growers. There has been renewed interest in the growing of cigar wrappers in Zimbabwe spurred by a variety of reasons such as worldwide demand and forestry conservation. To serve this niche market, the Tobacco Research Board has for the past five years focalised agronomic research and the development of new varieties with improved yields, enhanced foliar disease resistance and excellent cigar wrapper cured leaf quality. However, to resuscitate cigar wrapper production in Zimbabwe, a review of the past is critical. A thorough scrutiny of the bottlenecks, challenges faced and current and prevailing opportunities is necessary. This presentation will provide perspectives and insights into the significance of cigar wrapper production in Zimbabwe, historical background, current status, major limitations and opportunities. The paper will offer strategic information to be utilised by researchers, investors, leaf merchants, policymakers and other stakeholders on cigar wrapper production in Zimbabwe.

AP 34

Alkaloid content of wide tobacco germplasm: the search for high and low nicotine content accumulating genotypes

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Tobacco alkaloids are essential for the flavour, addictiveness, carcinogenicity and utilisation of blended flue-cured tobacco. Although there are strict limits on nicotine and nornicotine content permitted, there is renewed impetus to determine the possible extremes of alkaloid content in tobacco, especially for nicotine. Information on the nicotine content potential of tobacco varieties is specifically important for nicotine extraction and nicotine blending purposes. A two-year field study was conducted at Kutsaga Research Station in Zimbabwe to determine the alkaloid content (nicotine, nornicotine, anatabine and anabasine) of 57 lines of wide tobacco germplasm annotated as having either high or low nicotine content but without the specific content having been determined. Two experiments were set up, the first with 40 lines designated high nicotine content and the second with 17 lines designated low nicotine content. A Split-plot statistical design with two blocks for each trial was used for both experiments with variety and nitrogen fertiliser (34.5 % N) level being the main and subplots, respectively. Two fertiliser levels were used, for the first experiment; 5 g (normal) and 7.5 g (+50 %) per plant while for the second experiment, 5 g (normal) and 2.5 g (-50 %) per plant. Agronomic management of axillary bud topping and nitrogen rate manipulation were employed to enhance or subdue nicotine production as required for the experiment. Cured leaf was subjected to alkaloid content analysis. The results demonstrated the plasticity of nicotine accumulation across wide genotypes and a full range of alkaloid composition in each genotype will be presented. High or low nicotine content genotypes will act as models for in-depth genetics and biochemistry studies of nicotine and other alkaloid biosynthesis and ultimately inform breeding projects of strategies on lowering or increasing the nicotine content of varieties.

AP 35

Genetic mapping and characterization of the pale yellow (*Py*) locus responsible for accelerated senescence in dark tobacco

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In commercial dark tobacco varieties (*Nicotiana tabacum* L.), accelerated senescence trait not only helps reduce TSNAs that are otherwise accumulated during the yellowing process associated with traditional dark varieties but also, could benefit growers in managing their double crop fire-curing barns. Previous work has shown the effect of two loci (*Yb1* and *Yb2*) on accelerated senescence in Burley tobacco. The pale yellow (*Py*) locus, that is distinct from the *Yb1* and *Yb2* loci, is also known to accelerate senescence in Burley, flue-cured and dark cultivars. Incorporating the *Py* locus into a breeding program till date entailed a time consuming and subjective selection process, as the locus was uncharacterized. In this study, we mapped the *Py* locus on the tobacco genome, and identified SNP markers to facilitate accelerated breeding of the trait.

The mapping population used in this study is an F2 generation resulting from a cross between dark tobacco variety Narrow Leaf Madole LC (NLM LC, non *Py* variety) and TI1372 (source of the *Py* locus). A total of 192 F2 individuals were phenotyped using multiple methods for the *Py* trait, and a total of 93 F2 individuals and the parental lines were genotyped using a custom tobacco axiom array consisting of nearly 170,000 SNPs spread across the tobacco genome. The genotypic and phenotypic data were used in a Quantitative Trait Loci (QTL) mapping experiment to identify the genetic control of the *Py* trait. A QTL explaining 75 % of variance in the *Py* trait was identified from this analysis. We further designed high throughput KASP® markers for six SNPs within this region, to enable rapid identification of the *Py* trait in breeding populations. Also, putative candidate genes within this QTL region were identified using RNASeq differential gene expression analysis. Further efforts to characterize the mode of action and function of these candidate genes are currently underway.

AP 36

Mutations of phytochelatin synthase (PCS) genes by CRISPR/Cas9 reduce accumulation of cadmium in tobacco leaves

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Cadmium (Cd) is classified as a Class 1 “known human carcinogen” by the International Agency for Research on Cancer (IARC). Cd accumulation in crop plants including tobacco (*Nicotiana tabacum*) can lead to human exposure. Cd is a non-essential heavy metal and may cause oxidative stress and proteolytic degradation. Phytochelatins (PCs) have a major role in Cd detoxification. PCs form stable complexes with heavy metals and are subsequently transported into the vacuoles. The objective of this study was to determine whether cadmium in the plant leaves is reduced by inactivation of the phytochelatin synthase (PCS). In this research, two types of *PCS* genes in *N. tabacum* derived from *Nicotiana silvestris* and *Nicotiana tomentosiformis* named *NtPCSs* and *NtPCSt* were identified. Using the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system, we created mutants of *N. tabacum* with both genes inactivated. Plants in which the *NtPCSs* and *NtPCSt* genes were both mutated had 85 % less leaf cadmium concentration than wild-type plants. When these mutants were grown in the greenhouse, they did not show any significant difference compared with wild-type plants except for height. *NtPCSs* and *NtPCSt* mutants were 15 % lower in height than wild-type plants, but the number of leaves and the weight of leaves did not show any significant difference. Mutations in the *PCS* genes caused a slight growth disorder, but the concentration of cadmium in the leaves of *N. tabacum* was greatly reduced.

AP 37

Metabolic and transcriptomic shifts during tobacco leaf post-harvest senescence

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Tobacco is harvested after plant flowering, when the leaf senescence is more or less visible depending on cultivars. Before the drying phase, current practices allow the senescence to proceed until the detached leaf becomes yellow. This yellowing phase is known to be critical for the final properties of the leaf. This phase was analysed for chemical and expression changes in leaves of cultivars belonging to the three main tobacco types, Virginia, Burley, and Oriental, grown according to their respective practices. In line with important metabolic changes, we observed a significant transition in the genetic programme, including chlorophyll degradation, amino acids, and reducing sugar production within hours following the field harvest. We found that in Burley and Oriental tobacco varieties, certain amino acids, such as asparagine, are rapidly synthesised upon detachment, whereas the Virginia tobacco variety mainly produces reducing sugars. Genes differentially expressed after two days of leaf senescence were identified. Downregulated genes mostly belong to metabolic pathways, such as oxidation reduction, carbohydrate and lipid metabolism, and photosynthesis (e.g. RuBisCO subunits). On the other hand, upregulated genes are related to redox reactions, transcription regulation, proteolysis, hydrolase activity, and other catabolic processes. This finding is consistent with previous studies depicting leaf senescence processes. Go-terms analysis performed after 48 hours allowed identification of key transcripts involved in sugar and amino acid metabolism pathways, which may explain some major observed biochemical changes linked to the redistribution of carbon and nitrogen resources during tobacco leaf senescence.

AP 38

Metabolic profiling of mutant tobaccos with distinctive aroma based on gas chromatography-mass spectrometry and multivariate analysis

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A large number of mutant materials were obtained by chemical mutagenesis of tobacco with ethyl methylsulfonate (EMS) and new materials with distinctive aroma characteristics were achieved by field screening, identification and sensory evaluation. To investigate the chemical natures in mutant tobaccos with distinctive aroma (rose and lemon), principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) were applied to analyse the differences of volatile composition and metabolites in tobacco buds at bud stage by a non-target detection method based on gas chromatography-mass spectrometry (GC-MS). The results showed that 27 volatile components were detected in the three materials, including 8 alcohols, 2 aldehydes, 3 ketones, 11 olefins and 3 others. Among them, the content of trans-beta-ocimene in rose material was significantly higher than that in the other two materials, while the content of linalool in lemon material was higher. A total of 60 metabolites, including amino acids, sugars, alcohols, organic acids and fatty acids were detected in the three materials. Fourteen metabolite markers, including 5 amino acids, 4 organic acids, 3 carbohydrates and 2 other metabolites, were screened from the metabolites of the three materials by VIP analysis. The results indicated that the metabolic pathways of terpenoids, amino acid metabolism, organic acid metabolism, sugar metabolism and KREBS cycle of the two mutant materials were altered. This study can provide ideas for the molecular breeding and genetic improvement of mutant tobacco materials, as well as some suggestions for the industrial utilisation of these new tobacco materials aimed at the development of new tobacco products.

AP 39

First report of a pepper potato virus Y isolate affecting tobacco in Zimbabwe

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Potato virus Y (PVY) is an economically important disease, capable of causing 100 % yield losses particularly in late planted tobacco. This is so because the early growth stages coincide with a peak in aphid vector populations. In December 2017, severe necrosis and bronzing symptoms were widely reported particularly on young tobacco plants throughout the tobacco growing regions. In most of these cases, symptoms were reported to be most severe just after a rainfall event. This study was, therefore, initiated to establish the identity of the PVY strain that caused the outbreak. Thirty-one tobacco leaf samples of PVY symptomatic plants were collected from Banket and Kutsaga lands. Total RNA was extracted and reverse transcribed to cDNA. The cDNA was converted to DNA using primers targeting the coat protein, polymerase (N1b) and 3'untranslated regions. The DNA amplicons were cleaned and sequenced. Results of sequence analysis showed eight isolates identified with *PVY JVW-186* Genbank accession KF770835.1 (a recombinant isolate identified on pepper in Kwazulu Natal, South Africa) at 97 % to 99 % nucleotide identity and zero E value. Two isolates were identified as PVY^{N} and PVY^{O} whereas, the remaining 21 sequences had sequence peaks indicating co-infection and were discarded. The sequences of the isolates were deposited in the GenBank database (accession numbers MH157082 to MH157091). Phylogenetic construction and molecular evolutionary analyses showed study isolates clustered with the PVY^{C} and PVY^{O} reference isolates from various geographic sites and obtained from the Genbank. This study demonstrates continued recombination of PVY strains as contributing to host adaptation, further highlighting the risk in solanaceous crops. To the best of our knowledge this is the first report of a pepper *PVY JVW-186* strain affecting tobacco in Zimbabwe. This forms the basis for further studies.

AP 40

Progress in breeding and biocontrol dual approaches for tobacco broomrape management

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Tobacco broomrape (*Phelipanche ramosa*, *Phelipanche aegyptiaca* and *Orobanche Cernua*) is a widespread parasitic plant from the Orobanchaceae family, that causes important damage in tobacco crops. Broomrape is mainly present in Europe, Middle East and Asia and no satisfactory chemical control strategy is available for growers. A project combining breeding and agronomical efforts was initiated in France in 2016 involving research and technical partners to evaluate and implement an integrated broomrape control strategy. The objectives of this multidisciplinary approach were to improve the understanding of the genetic broomrape tolerances in connection to different crop strategies. After several years of herbicide treatment evaluation bringing no satisfactory results for growers, the key objective of this project was to develop a new approach based on the use of broomrape pathogens as biocontrol agents.

The breeding part of the project, led by Bergerac Seed & Breeding (BSB), focused on evaluating identified sources of tolerance to different broomrape populations and species. Testing Wika and two new tolerance sources on populations of broomrape collected in different countries revealed that the Wika recessive gene brings interesting tolerances to *P. ramosa* and *P. aegyptiaca* populations while none to *O. cernua* ones.

The biocontrol part of the project, led by Agroécologie and Arvalis, focused on hundreds of fungi isolated from symptomatic broomrape in 2017 and 2018. Based on morphotypes, a coarse visual identification and original plot locations, one hundred of them were evaluated for their pathogenicity to broomrape. This screening revealed a dozen fungi strains as promising mycoherbicide candidates but whose *in situ* efficacy on tobacco broomrape has yet to be validated.

Combining breeding efforts and new biocontrol strategies brings some hope to tobacco growers for an integrated control solution to broomrape parasitic activity.

AP 41

Evaluation of the bio-efficacy of the biological control agents, *Trichoderma*, *Paecilomyces*, *Mycorrhiza* and *Bacillus* species against root-knot nematodes

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The recent phasing-out of fumigant nematicides, coupled with limited land for long-term rotations in Zimbabwe, brought to the fore the need for new technologies for the management of root-knot nematodes (RKNs) in Zimbabwe. The use of biological control agents is one such method, which is not only environmentally friendly but also sustainable. The objectives of this trial were, therefore, to evaluate the efficacy of biological control agents for root-knot nematode control on tobacco in Zimbabwe. Five biocontrol agents namely *Trichoderma harzianum* (T 77), *Mychorrhiza* spp. + *Trichoderma harzianum*, *Paecilomyces lilacinus* + *Bacillus firmus*, *Mychorrhiza* spp. and *Paecilomyces lilacinus* were evaluated in a pot experiment in the greenhouse. Root-knot nematode susceptible (KM10) and RKN-resistant (K RK64) cultivars were used. The trial was repeated for three seasons. The experimental design was a factorial replicated five times. Plants were individually inoculated with 0, 2000 and 5000 *M. javanica* eggs. Thereafter, the test biological control agents were applied at the following rates; *Mycorrhiza* spp. at 40 kg/ha and 60 kg/ha, *Mycorrhiza* spp. + *Trichoderma harzianum* at 200 kg/ha and 300 kg/ha and *Paecilomyces lilacinus* at inoculation. A chemical treatment, fluopyram was also included as a standard. Root galling assessments were conducted at seven weeks after inoculation using a scale of 0-8 (where 0 = no galling and 8 plant dead). The results showed that *Paecilomyces lilacinus* gave some RKN control (galling =1.44) at the lower initial egg population of 2000 eggs while the *Trichoderma* isolate was effective even at 5000 eggs. Used in combination with resistant varieties, good rotations and recommended cultural control practices, these two biological control agents provide a promising sustainable control option for RKNs on tobacco in Zimbabwe.

AP 42

Study of the control effect of preceding crops on tobacco black shank

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The aim of this study was to investigate the control effect of preceding crops on tobacco black shank and the effect on diversity of soil microorganism.

Ten crops, e.g. rape, wheat, barley, broad bean, pea, garlic and ryegrass, were selected as preceding plants and grown in the fields with aggravated tobacco black shank. The effectiveness of crop rotation on tobacco black shank disease control was measured by the density assay of *Phytophthora parasitica* var. *nicotianae* using fluorescence quantitative polymerase chain reaction (qPCR) assay, tobacco leaf disk assay and soil dilution plating. More importantly, the diversity of rhizosphere microbial community structure of different preceding crops was analysed by terminal restriction fragment length polymorphism (T-RFLP).

Our results showed that three preceding crops, ryegrass, garlic and barley, can effectively reduce the levels of *Phytophthora parasitica* var. *nicotianae*, with decreased morbidity and decreased disease index; the rhizosphere bacterial community structure and community richness of different preceding crops were revealed by the T-RFLP method.

Our present study indicated that barley, which showed the highest control efficiency on restraining and reducing tobacco black shank pathogens, was the best preceding crop for tobacco cultivation. We also demonstrated that the rhizosphere bacterial community structure, which is critical for the health of land plants, was dramatically affected by different preceding crops.

AP 43

Tobacco crop protection against blue mould: what strategy with organic and biocontrol products?

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Blue mould, *Peronospora tabacina*, is one of the most dangerous diseases for tobacco crops in Europe and particularly in France. Protection against this disease is systematic during the period of high risk as all registered crop protection agents are rather preventive.

Currently, the control of blue mould revolves around six active ingredients: Mancozeb + Dimethomorph, Acibenzolar-S-Methyl + Metalaxyl-M, Cyazofamid and Orange peel oil. The latter is the only active ingredient allowed in organic farming and is also a compound of biocontrol products. Almost all the conventional active substances are facing hurdles in the European procedure for the renewal of their reapproval due to their unfavourable toxicological and/or ecotoxicological profile, hence a need to look for new products to manage this disease.

In France, between 2009 and 2019, experiments against blue mould have been set up each year with several organic or biocontrol products in order to screen the most effective which are selected to go through the approval stage.

In 2009 and 2010, the products tested alone presented low efficacies. From 2011 onwards, an increase in efficacy was noticed as the rates of the treatments were increased and also because product associations were investigated. From 2016 onwards, trials have consistently shown good efficacy with biocontrol and organic products.

Today, despite a higher cost than current conventional registered solutions, it can be confirmed that new effective organic and biocontrol solutions exist to control blue mould and that these products have their place in an Integrated Pest Management.

AP 45

The role of laboratory Quality Management Systems in ensuring compliance in the tobacco industry: the case of the Tobacco Research Board's Analytical Chemistry Services (TRB ACS)

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The role of analytical chemistry services in agricultural production cannot be over-emphasised. Fundamental questions emanating from soil quality right up to agro-produce quality can never be fully addressed without an accurate and precise analytical chemistry answer. It is, therefore, crucial that the analytical chemistry services be of the highest quality so that ensuing decisions are made from an informed position. Agricultural laboratories should, thus, implement a credible quality management system robust enough to support the agricultural industry. The Tobacco Research Board (TRB) laboratory offers a wide range of analytical services, primarily to the tobacco industry, which include soil analysis, pesticide residue analysis, water analysis, plant chemistry analysis and agro-chemical analysis. Central to these services is the issue of competence and credible results. This review highlights how the TRB laboratory has managed to develop and implement a quality management system that has promoted confidence in the operation of the laboratory and has also enabled the laboratory to demonstrate operational competence. Key pillars to the establishment of the quality management system will be highlighted as well as the current and potential benefits of implementing such a system.

AP 47

In field photonic sensing of tobacco leaves for the non-destructive assessment of nitrogen content and quality prediction

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Nitrogen (N) fertilisation is one of the most important aspects of tobacco cultivation. Right N rates increase leaf yield and quality, and influence vegetative cycle, disease resistance, leaf nicotine and sugar content. Good N availability to the plants must be guaranteed until plant topping, without making intensive use of fertiliser or inducing soil depletion. This study was aimed at defining a new optical method for the proximal detection of N status and pigments of tobacco leaves.

Four different N levels (0, 50, 100 and 150 kg ha^{-1}) were applied to a total of 264 Virginia Bright tobacco plants cultivated at Fattoria Autonoma Tabacchi (FAT - Città di Castello [PG], Italy). The upper sun-exposed side of a single leaf for each plant was measured in the field by a portable fluorescence sensor in middle June, middle-end July and early August 2018. The fluorescence sensor provided indices of leaf chlorophyll (CHL), flavonols (FLAV) and nitrogen (the Nitrogen Balance Index, NBI=CHL/FLAV). It was found that the NBI could discriminate the four plots treated at the four different N levels. The NBI was significantly (R^2 = 0.69) linearly correlated to the leaf N content, assessed by destructive Kjeldahl method. A good (R2=0.85) linear correlation was found between the CHL index and the actual leaf chlorophyll content. The NBI measured in the field at the stage of the maximal plant N assimilation (middle-end of July) was strongly (R^2 = 0.98) linearly correlated with leaf nicotine content (%) destructively measured at harvest (beginning of September).

The results of this study indicate the usefulness of the developed photonic sensing method for determining the nitrogen status of plants and applying precision fertilisation. It was also as an interesting tool for early prediction of final leaf nicotine content.

AP 48

A comparison of methods for handling data that is Missing Completely at Random (MCAR) in crop experiments at Kutsaga

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Missing values represent a potential source of bias in research trials and thus every effort should be made to ensure that all the relevant data are collected and availed for analysis. However, in reality, there are times when some data are missing and in agricultural experiments missing data occur for example when test tubes accidentally break, plants die due to an extraneous factor that is not the treatment under study and or part of the crop is lost during handling or processing. This results in the loss of statistical power and introduces bias which leads to misinterpretation of the actual treatment effect and ultimately the wrong conclusions may be drawn. However, there are methods of handling missing data to enable an analysis that yields a result as close to the true value as is possible. These include the Complete Case Analysis, the Mean Substitution, Regression Imputation and Multiple Regression Imputation using the Fully Conditional Specification (FCS) method. To establish the best method to use, a study was done in which a complete data set of plant height measurement from a Kutsaga nematological trial was obtained and analysed. Thereafter, some readings were randomly removed from the data set to obtain subsets with data that was Missing Completely At Random (MCAR). Two such data sets, one with 5 % and another with 20 % missingness were obtained. These two data sets were subjected to the four different methods of handling missing data. Analysis Of Variance (ANOVA) of the data obtained from each of the methods was computed for comparison with that obtained from the complete data set. The results showed that Multiple Regression Imputation using the Fully Conditional Specification (FCS) method was the most ideal and had the least bias. In this paper, a detailed demonstration of the application of the four different methods of handling missing data will be done.

AP 49

Nicotine variability of low nicotine cultivars versus normal nicotine cultivars

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Nicotine is the most abundant alkaloid in cultivated tobacco (*Nicotiana tabacum*), typically constituting more than 90 % of total alkaloids. Recently, the U.S. Food and Drug Administration (FDA) issued an Advance Notice of Proposed Rulemaking (ANPRM) to obtain information for consideration in developing a tobacco product standard to set the maximum nicotine level in cigarette filler to "minimally addictive or non-addictive" levels. Nicotine levels are highly variable across different years and locations as data collected over decades in the Minimum Standards Programs show. Analysis of the Burley check varieties in the Minimum Standards Program showed relative standard deviations ranging from 20 % to 28 % for individual varieties across locations from 2012 to 2018. Though there are decades of nicotine analysis from the Minimum Standards Program, there is not much data collected and publicly available on nicotine variability in low nicotine cultivars. The purpose of this study is to determine variability in nicotine content across multiple years and locations from low nicotine cultivars and compare it to normal nicotine cultivars. To initiate this long term study we analyzed low nicotine cultivars alongside normal nicotine cultivars at the same locations from 2012 to 2018. Assuming the variability in nicotine content for low alkaloid cultivars is similar to or greater than the variability in normal nicotine cultivars, it will have major impacts on meeting any regulatory standard. This could suggest that to meet a standard of 0.3-0.5 mg/g nicotine content the cultivars themselves may need to have much lower nicotine levels to consistently meet the proposed standard year after year. This will have major implications on the technical achievability of such a standard.

AP 50

Reducing nicotine in Burley tobacco by combining low alkaloid varieties and agronomic practices

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The United States Food and Drug Administration (FDA) has requested comments on a possible nicotine standard of 0.3-0.5 mg/g in tobacco filler. There are currently no publicly available varieties that approach this level. The objective of this study was to test whether this level could be achieved if the currently available low alkaloid (LA) varieties were grown using all agronomic practices that are known to lower nicotine. One standard variety, TN 90LC (check), and three LA varieties, LA TN 90, ITB 1501 and ITB 259, were grown with standard recommended practices and also with practices designed to lower nicotine (no topping, close spacing, low nitrogen, early harvest, irrigation). None of the varieties grown with standard practices had nicotine levels below 0.5 mg/g, in any stalk position. When grown with the nicotine-reducing practices, all three LA varieties had nicotine < 0.5 mg/g in the primings (lowest stalk position) only. In the mid-stalk lugs and leaf, and the overall weighted concentration, only ITB 1501 had nicotine 0.4-0.5 mg/g, and in the tips, all varieties were > 0.5 mg/g. The abnormally wet 2018 growing season resulted in atypically low nicotine levels. The check nicotine was only 36 mg/g: historically, this is at the lower limit of the normal nicotine range. In the standard agronomic practices, the two ITB varieties had the highest yield (2,351 kg/ha and 2,287 kg/ha) and LA TN 90 the lowest (1,653 kg/ha). In the nicotine-reducing practices, LA TN 90 again had the lowest yield (1,338 kg/ha) and the other three varieties had similar yields (1,553-1,568 kg/ha). LA TN 90 also had the lowest grade index in both the standard and nicotine-reducing agronomic practices (36 and 29, respectively). The two ITB varieties had much better quality, not very different from the check. All LA lines were heavily infested with aphids.

AP 51

Genetic and biochemical analysis of very low nicotine tobacco leaf

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Recently, the U.S. Food and Drug Administration issued an advance notice of proposed rulemaking to obtain information for consideration in developing a tobacco product standard to set the maximum nicotine level in cigarette filler. Tobacco industry and public institutions have been working on further understanding nicotine biosynthesis and on developing tobacco varieties with reduced nicotine levels. Genetic alteration via traditional breeding, mutation breeding, and genetic engineering has been effective to reduce nicotine to various levels. However, historically, low alkaloid traits are associated with poor leaf quality with characteristics such as delayed maturation, thin body and rigid cured leaf etc. To understand the effect of lowering nicotine on leaf quality, experiments were conducted to obtain transcriptomics and metabolomics expression data using seven Burley and four flue-cured lines at different plant growth stages. An integrative analysis of the "omics" data has provided insightful information towards understanding the complex and tightly regulated tobacco leaf developmental and maturation process. Computational analysis has led to the identification of more than 200 potential candidate genes that include transcription factors, secondary metabolism, cell-wall and senescence-related elements that could be associated with leaf quality. Seventy-four of these genes were validated using real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR). Functional analysis of some of these genes is under way.

AP 52

Changes in TSNA contents and sensory quality of ultra-low nicotine tobacco leaves produced by grafting with eggplant

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Ultra-low nicotine tobacco production has been given broad attention due to the possible regulation of nicotine content in cigarette tobacco. Based on grafting with eggplant (eggplant as rootstock, flue-cured variety Yunyan 87 as scion), the feeding selection behaviour of budworms (*Heliothis assulta*) was compared between ultra-low nicotine tobacco and regular tobacco leaves, and the content of TSNAs and neutral aroma components, smoke parameters and sensory quality of cigarettes were also determined and evaluated. Tobacco samples were from the following three treatments: “tobacco /tobacco”, “tobacco /eggplant without earthing up”, “tobacco /eggplant with earthing up”. The results showed that in the enclosed environment, budworms preferred ultra-low nicotine tobacco leaves to regular tobacco leaves, and the budworms began to feed on the regular tobacco leaves only after the ultra-low nicotine leaves had been eaten up. The contents of NNN, NNK, NAT and total TSNAs in ultra-low nicotine tobaccos were reduced dramatically, with NNN, NNK and TSNAs contents in upper leaves from tobacco /eggplant without earthing up being 82.7 %, 79.5 % and 68.8 % lower than that of regular tobacco, respectively, and being 79.7 %, 79.8 % and 68.6 % lower for middle leaves. The differences in content of neutral aroma components were not significant. Nicotine content in mainstream cigarette smoke made from ultra-low nicotine tobacco was markedly lower than that in control cigarettes, while there were no significant differences in total particle matter, moisture, CO, tar level and puff number. There were no significant differences in aroma quality, smoke density, irritation and combustibility, while the aroma quantity, odour score and after taste were decreased to some extent. Significant reductions were perceived for strength, consumer satisfaction and acceptance. The results showed that the selective reduction of nicotine by grafting had different impacts on leaf and smoke chemistry and sensory parameters.

SUSTAINABILITY FORUM

In 2015, the United Nations published 17 sustainable development goals and their corresponding targets to be achieved by 2030. Like other sectors, the tobacco and alternative product sectors are likely to impact most of the goals. Consequently, it is important to understand and measure these impacts for organisations aiming to enhance their positive impacts, and reduce the others. In 2018, CORESTA decided to support a project with the objective to identify available scientific tools for performance assessment from which a framework could be elaborated. 16 sectorial gaps were identified and each translated into proposals for guideline and method developments. In 2019, the CORESTA Scientific Commission has decided to arrange a panel discussion during the AP Conference in Victoria Falls to exchange on topics associated with these gaps.

Four experts were invited to present on topics related to tobacco supply chains, and on projects conducted to improve social and environmental performance along this chain.

Luke Wilde, Chief Executive of twentyfifty Limited, has worked in leadership and organisational change for over two decades, with a specific focus on corporate responsibility, human rights and sustainability. Luke has led twentyfifty's engagement on the implementation of its agricultural labour principles in its third-party supply chain since 2015, and currently leads tobacco supply chain impact assessments. During his presentation, Luke explains that all major tobacco companies have made commitments to respecting human rights and conducting 'human rights due diligence'. He highlights the limits to audit approaches and the insights (and challenges) of new approaches to understanding the impact on farmers, workers and their communities.

Warwick Evans, Senior Manager responsible for Agronomy and Sustainability in Leaf at imperial Brands, explains in detail the scope, objective and implementation of a sustainable tobacco programme built with stakeholders on a risk-based approach.

Eduardo Royo, currently Manager Sustainable Agriculture for PMI, being responsible of Crop and Environmental Pillars in African origins where PMI sources tobacco, presents the WASH (Water Sanitation and Hygiene) initiatives he is currently leading in the region, with the aim to generate a positive impact on people's lives.

Finally, Enrica Bargiacchi, a Research Agronomist at the Interuniversity Consortium for Science and Technology of Materials in Firenze, Italy, presents multiple initiatives to support more sustainable tobacco crop production.

2019 CORESTA JOINT STUDY GROUPS CONFERENCE

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

The effect of *Mycorrhiza* enriched biofertiliser on tobacco growth and yield

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Due to the inherent poor fertility of soils on which tobacco is grown in Zimbabwe, production relies on inorganic fertilisers to provide the crop with macro and micronutrients required for optimum plant growth and maintenance of soil fertility. Globally, however, total reliance on the use of mineral fertilisers has been shown to pose environmental risks and increase tobacco production costs. Thus, the trend towards organic production practices in agriculture has increased in the recent past. Arbuscular mycorrhizal fungi have been reported to improve the uptake of many nutrients including phosphorus (P), nitrogen (N), copper (Cu) and zinc (Zn) and thus, improve nutrient acquisition from fertilisers. In addition, colonisation of plant roots by the mycorrhizal fungi enhances plant water relations and increases resistance to cultural and environmental stresses, and consequently improves plant growth. In an endeavour to reduce Zimbabwe's tobacco production environmental footprint, a biofertiliser that contains arbuscular mycorrhizal fungi was tested for its efficacy on tobacco growth and yield. The trial was conducted at Kutsaga Research Station for two seasons from 2017-19. Three rates 30, 40 and 50 kg/ha of the product were tested against the standard mineral fertiliser treatment in a complete randomised block design with five replications. Results showed that leaf geomean was significantly higher in standard fertiliser treatment and that this was not significantly different from that in plots treated with 40 kg/ha of the biofertiliser. The mean tobacco yield in the standard mineral fertiliser treatment was, however, significantly higher at 2880 kg/ha than in the biofertiliser treated plots where yields ranged from 1909-2162 kg/ha. A cost benefit analysis of using either of these nutrient sources will be discussed in this paper.

APPOST 02

Effects of spraying exogenous glycerol and molybdate on nitrate accumulation in pot-grown Burley tobacco leaves

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Burley tobacco is well known as yellow-green leaf tobacco with reduced chlorophyll content and lower carbohydrate accumulation and lower nitrogen assimilation ability, resulting in the accumulation of nitrate, one of the precursors of TSNAs. Enhancing the ability of carbon and nitrogen assimilation is assumed to be an efficient approach to reduce nitrate accumulation. Glycerol has been proved to be able to promote photosynthesis carbon fixation and carbohydrate formation and molybdate has been known to have the ability of promoting nitrate reductase activity. Based on this knowledge, pot experiments were carried out to investigate the effect of glycerol and molybdate on reducing nitrate content in Burley tobacco leaves. The Burley tobacco variety TN90 at root elongation stage was sprayed with A (0.1 % glycerol only), B (the combination of 0.1 % glycerol + 10 mg/kg auxin), and C (1.0 % sodium molybdate), with water as control. The results showed that after spraying 0.1 % glycerol for seven days, the NO_3-N content of tobacco leaves decreased by 53.22 %, and photosynthetic rate and nitrate reductase activity increased by 34.11 % and 22.53 %, respectively, carbohydrate contents also increasing significantly. The expression of key genes (GLPK, gpmA, PGK, SPS, SUS2-2) in tobacco leaf carbon fixation and sucrose synthesis pathway and key genes (NLP7, NIA1, NPF3.1, NPF7.3) involved in nitrogen reduction and assimilation pathway increased significantly. After spraying 0.1 % glycerol + 10 mg/kg auxin, the NO_3-N content of tobacco leaves decreased by 76.72 %, and the dry matter accumulation of leaves increased by 36.35 %, the photosynthetic rate and nitrate reductase activity increasing by 63.55 % and 43.45 %, respectively. In addition, after spraying 1.0 % sodium molybdate, the photosynthetic rate and nitrate reductase activity of tobacco leaves increased by 12.76 % and 30.71 %, respectively, and the content of NO_3-N in tobacco leaves decreased significantly, indicating that molybdate directly increased nitrate reductase activity and nitrogen utilisation, leading to the reduction of nitrate content in tobacco leaves. This study provided a new technical approach for effectively reducing nitrate accumulation and TSNA content in tobacco leaves.

APPOST 03

Effect of delaying harvest on tobacco quality of upper six leaves and climatic requirement in central Henan of China

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Upper tobacco leaves have higher quality potential and play an important role in low tar cigarette production. Upper leaves being rich in organic matter and tight in structure, high maturity is the key to produce high quality cured leaves. According to the current harvesting standards, the maturity of upper leaves is generally low in reality and the degradation is not complete, so delaying harvest of upper leaves to increase maturity is an important approach to improve availability of upper leaves. A series of experiments were carried out in central Henan Province from 2017 to 2018 to investigate the effect of delaying harvest time on leaf quality, and to establish climatic indexes required for high quality upper tobacco production. Harvesting date experiment included five treatments (normal date, delayed 4, 8, 12, 16 days). Another two-factor field experiment included transplanting date and harvesting date, and the relationship between climatic parameters during upper six leaf growth and cured leaf quality was investigated. The results showed that with the delay of harvesting date, the soil plant analysis development (SPAD) value, weight per leaf and yield of the upper six leaves showed a continuous downward trend. The output value and sensory evaluation quality increased to a point before declining, reaching maximum values at delayed 10 to 12 days with 19.03 % and 7.36 % higher than that of normal harvest control, respectively, characteristic of richer aroma, better taste and more satisfaction. SPAD value of 5.0-6.1 was proved to be a proper index to decide the optimum maturity. The analysis on the relationship between quality of upper six leaves of flue-cured tobacco and temperature and other climatic indexes revealed that the full flavour style was more significant when the average daily temperature during the mature stage of the upper six leaves was between 25.3~27.1 °C. The quality was the best when the accumulated > 20 °C temperature was between 263.0~313.3 °C for the mature period of upper six leaves and between 497.9~544.5 °C for the whole growth stage of upper six leaves, respectively. This study provided technical standards and climatic indicators for high maturity and high availability of upper tobacco leaf production.

APPOST 04

Optimising carbon footprint of international meetings

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It is broadly acknowledged that global warming is intimately influenced by the extracted carbon from the earth, and transferred to the atmosphere mainly as carbon dioxide (in addition to other greenhouse gases converted into CO_2eq). Considering the anticipated economic, environmental and social impacts of a global temperature increase, a number of political initiatives have been taken since the Rio de Janeiro summit in 1992. Many regulations invite organisations to report their carbon emissions. Some countries even apply tax payment per ton of CO_2 emitted. Such policies aim at increasing transparency and encouraging behaviour changes to reduce carbon emissions, and ultimately mitigate the impact of global warming on populations.

People travelling to international meetings is one of many emission sources. Such events are important in many cases, but emissions can be reduced by making an informed choice of the meeting locations. To support this goal, a web interface tool has been developed to estimate carbon emissions. Participant locations are the input parameters; flying distances are calculated and converted into CO_2eq considering each individual location as a potential meeting location. The process consists in converting locations into GPS coordinates using geolocation application programming interface, then estimating distances and converting them into CO_2eq by using an acknowledged approach developed by the French Environment and Energy Management Agency. The output is a graphical representation helping meeting organizers make an informed decision.

This poster will present the principles of calculations, the functionality of the tool and examples illustrating its potential benefits.

Note: Not travelling individually will not prevent the plane from flying. But fewer travellers will eventually mean fewer planes. Behaviour changes of a small group of individuals are usually not enough, however, population behaviour is a sum of individual behaviours, and global changes will come if individuals change first.

APPOST 05

Influence of disease resistance on the yield and quality in Burley tobacco

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Tobacco breeders recognise empirically that introduction of certain disease resistance is accompanied by negative effects on agronomic traits such as decrease in yield and quality. Only a few studies have quantitatively clarified the negative effects of disease resistance. For this study, after preparing disease-resistant and susceptible lines with genetic background of Burley 21, the influence of six disease resistances on yield and quality considering the genotype were evaluated. Experiments were conducted at the JT Leaf Tobacco Research Center in Japan. Regarding PVY resistance, results showed that the resistance derived from Bursana and Virgin A Mutant decreased the yield, although PVY resistance derived from Kerti No. 1 did not. Results also suggest that introduction of dominant resistance genes as a heterozygous genotype might be desirable for black root rot, root-knot nematode or tobacco mosaic virus (TMV) resistance because of their less negative effects on yield or quality than those of the homozygous genotype. In the case of wildfire resistance, neither homozygous nor heterozygous genotype showed negative effect on yield or quality. Powdery mildew resistance derived from Kokubu (Japanese domestic) was controlled genetically by two recessive genes. The recessive homozygous line showed lower yield and quality than those of Burley 21. Those of heterozygous lines were intermediate between those of the mating parents. These results agreed well with what was recognised in the tobacco breeding process.

APPOST 06

Physiological, transcriptomic and secondary metabolic responses of tobacco seedlings to well-cellar style transplanting

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The well-cellar style transplanting (WCST) spread in China was an original transplanting method of flue-cured tobacco, and had obvious comparative advantages when compared to the conventional transplanting (CT). In order to demonstrate the mechanism in WCST, this study analysed the physiological, transcriptomic and secondary metabolic responses of tobacco seedlings compared with conventional transplanting. The results showed that the average air temperature and the average relative humidity of atmosphere were 22.29 °C and 97.10 % in WCST, enhanced by 4.76 °C and 58.97 %; however, the average photosynthetically active radiation (PAR) was 221.57 $\mu mol\, m^{-2} s^{-1}$ in WCST, reduced by 20.53 % compared to CT. After transplanting, the plant height and maximal leaf area in WCST were significantly improved, the leaf water potential was enhanced between 29.92 % and 64.46 %, furthermore SOD, POD and MDA were reduced by 14.48 %, 8.86 % and 9.83 % compared with CT, respectively. There were 4845 differentially expressed genes identified, including 2693 down-regulated genes according to transcriptome sequencing. Some down-regulated genes associated with “oxidation-reduction process” and “antioxidant activity” were significantly enriched according to Gene Ontology annotation. Some down-regulated genes involved in “flavonoid biosynthesis” was significantly enriched according to KEGG pathway annotation. WCST decreased the gene expression of *CHI*, *F3H*, *FLS*, *F3'H* and *A3RT* related with the flavonoid biosynthesis, and significantly reduced the contents of the flavones taxifolin, rutin, kaempferol-3-glucoside, hyperoside, luteolin, isorhamnetin-3-rutinoside and kaempferol-3-rutinoside. There was no obvious abiotic stress, furthermore the higher air temperature, higher air humidity and lower PAR environment was beneficial to the growth and development of tobacco seedlings in WCST.

APPOST 07

Effects of wheat straw and biochar addition on physiochemical properties and organic carbon fractions in flue-cured tobacco planting soil

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Soil organic carbon (SOC) is the core component in maintaining soil fertility. However, soil total organic carbon (TOC) gains and losses are difficult to distinguish over a short time period. Previous studies showed that labile organic carbon fractions, compared with TOC, could be sensitive indicators of soil quality changes due to management practices. Based on a 2-year field experiment, effects of wheat straw and its biochar addition on soil physiochemical properties and organic carbon fractions were determined to provide the theoretical basis for the quality promotion of flue-cured tobacco planting soil. Four treatments were designed, including chemical fertiliser alone (conventional treatment, CK), chemical fertiliser plus wheat straw (FS), chemical fertiliser plus biochar of 2.25 $t \cdot hm^{-2}$ (FB) and 4.5 $t \cdot hm^{-2}$ (FB2). Each treatment was repeated three times. In comparison with CK, FS treatment significantly increased the concentrations of microbial biomass carbon (MBC), hot-water extractable C (HWC) and labile organic carbon by 252.39 %, 107.01 % and 68.95 %, respectively, while there was no significant change in total organic carbon (TOC) and light organic carbon (LFOC). The concentrations of TOC, MBC and LFOC under FB and FB2 treatments were significantly increased whereas no significant change in LOC and HWC were found among FB, FB2 and CK treatments. Compared to CK, FS and FB treatments improved soil physiochemical properties, and FS treatments had the best effect. FS treatment had the highest value of carbon pool management index (CPMI), which was significantly higher than CK by 73.51 %. However, biochar treatments (FB and FB2) decreased CPMI in comparison with CK. Overall, the activity and quality of soil organic carbon improved more by the continuous incorporation of wheat straw, whereas the stability of soil organic carbon was improved more by the addition of biochar, which is beneficial for the long-term stable fixation of soil organic carbon.

APPOST 08

Characterisation and genetic mapping of a novel premature leaf senescence mutant yellow leaf 1 in common tobacco (*Nicotiana tabacum* L.)

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Ripe (yellowing) leaves are harvested and cured in tobacco farming. Leaf senescence therefore has a great impact on yield and quality of tobacco. However, little is known about the molecular mechanism of tobacco leaf senescence regulation. The objective of this study was to explore the functional genes related to tobacco leaf senescence regulation through ethyl methanesulfonate (EMS) mutagenesis followed by genetic mapping and functional analysis of the mutated genes. In this paper, the characterisation and genetic mapping of an EMS induced tobacco mutant yellow leaf 1 (*yl1*) that displays premature leaf senescence phenotypes is described. The phenotypes of leaf senescence were studied using physiological parameters and senescence marker genes. Map-based cloning was conducted to locate the mutant gene on the chromosomes. Exogenous phytohormone treatments of leaves were performed to determine whether the mutant gene is involved in hormone signaling pathways. The results showed that compared with wild type, *yl1* mutant plants exhibited an obvious phenotype of early leaf yellowing. Analyses of physiological parameters and senescence marker genes further indicated that the mutant phenotype is closely related to premature leaf senescence. Genetic analyses indicated that the *yl1* mutant phenotype is controlled by a single recessive gene. Using simple sequence repeat markers, the mutant gene was mapped to a specific interval of tobacco linkage group 11. Treatments with exogenous phytohormones *in vitro* showed that the gene might participate in the regulation of leaf senescence through the salicylic acid signaling pathway. Mapped-based cloning and functional analysis of this gene would lead to a better understanding of the molecular regulation of leaf senescence in tobacco.

APPOST 09

Estimating leaf chlorophyll content in tobacco based on various canopy hyperspectral parameters

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Leaf chlorophyll content (LCC) is an important physiological parameter in the process of plant growth. Thus monitoring LCC can be used to detect and study plant mutations, pressure, and nutritional status. Wu et al. found that crop LCC had a linear relationship with canopy hyperspectral parameters by considering the effect of 670 nm fast saturation and relatively low LCC. According to their study on the tobacco, they found a good regression relationship between the linear combination of LCC and the spectral reflectance at 620 nm and 740 nm; as well as the linear combination of the spectral reflectance at 350 nm and 800 nm, both of which were independent of the tobacco cultivars.

The objective of this study was to make a comprehensive analysis of the relationships between tobacco LCC and various leaf hyperspectral parameters, to identify the sensitive hyperspectral parameters indicating LCC in tobacco, and to determine quantitative relationships applicable for estimating LCC in tobacco. In this paper, the quantitative relations between LCC and three kinds of leaf hyperspectral parameters on five potassium treatments in tobacco were investigated. The results indicated that a linear relationship of LCC with the raw spectral reflectance value at 732 nm and an exponential relationship of LCC with first order differential spectral reflectance value at 837 nm were performed to estimate LCC, giving R^2 of 0.854 and 0.881, RMSE of 0.366 mg g^{-1} and 0.301 mg g^{-1}, and RE of 18.34 % and 15.62 % respectively. Both could serve as optimal techniques to estimate tobacco LCC. Nevertheless, the better one was $(SD_r-SD_y)/(SD_r+SD_y)$ with $y = 88.033 + 111.514 \times (SDr-SDy)/(SDr+SDy)$, $R^2 = 0.948$, RMSE = 0.127 mg g^{-1}, and RE = 9.31 %, respectively, indicating that $(SD_r-SD_y)/(SD_r+SD_y)$ was suitable to estimate LCC. These results suggest that the new normalised variable $(SD_r-SD_y)/(SD_r+SD_y)$ to estimate LCC is more effective than raw spectral reflectance, first order differential spectra and red edge spectral parameters.

APPOST 10

The influence of sodium carbonate buffer on TSNAs accumulation and leaf quality during tobacco storage

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Storage of tobacco leaves is an important stage in tobacco processing. Tobacco Specific *N*-nitrosamines (TSNAs) in tobacco leaves gradually increase during storage. The purpose of this study was to investigate the influence of sodium carbonate on the accumulation of TSNAs and the quality of tobacco leaves during long-term storage. Sodium carbonate buffer was used to adjust the pH value of Maryland and Burley tobacco leaves during the threshing and redrying process. The treated tobacco leaves were naturally stored for 18 months. The tobacco samples were periodically collected to measure the pH value, the content of TSNAs, the appearance of tobacco leaves, the moisture content, chemical composition and sensory quality. The results showed that the pH value of the treatment group was higher than that of the control group and remained stable with the storage time. The TSNA levels in the treatment group were significantly lower than that in the control group. There was no difference for the appearance of tobacco leaves between the treatment group and the control group. The moisture content of the treatment group was higher than that of the control group. The total alkaloids and total sugar content in the treatment group were slightly lower than that in the control group. The ammonia and total volatile alkali in the treatment group were lower than that in the control group. The sensory quality of the treatment group is better than that of the control group. It can be seen that sodium carbonate buffer can reduce the accumulation of TSNAs during storage of tobacco leaves, and there is no negative effect on the quality of tobacco leaves.

APPOST 11

Effect and mechanism of controlling tobacco weather fleck by *Bacillus velezensis* GJ11

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Tobacco weather fleck is a kind of non-infective disease caused by O_3 injury at low temperature. Plant growth-promoting rhizobacteria (PGPRs) such as *Bacillus velezensis* confer benefits to crops by producing volatile organic compounds to trigger induced systemic tolerance (IST). Here, *B. velezensis* GJ11 was isolated and screened within the tobacco root, which can produce 2,3-butanediol and acetoin to trigger IST against O_3 injury in tobacco plants. Further analysis found acetoin played a more important role in triggering IST against O_3 injury than 2,3-butanediol. For a higher acetoin production, the *bdh* gene was deleted to block the metabolic pathway from acetoin to 2,3-butanediol in GJ11. The knockout strain GJ11Δ*bdh* produced acetoin about four times more than GJ11, showing a much stronger activity against O_3 injury than the wild-type strain. Both acetoin and GJ11Δ*bdh* could effectively enhance the antioxidant enzymes activity (e.g. SOD and CAT) in tobacco that was favourable for scavenging reactive oxygen species such as H_2O_2 in leaves after exposure to O_3. Thereby, less H_2O_2 was observed in the tobacco leaves pre-treated with acetoin or GJ11Δ*bdh*. The result of field experiments also paralleled the result of the laboratory experiment. Both acetoin and GJ11Δ*bdh* could well protect tobacco plants from O_3 injury in the high mountain areas after application by root-drench. The results presented in this study, from both the laboratory and fields, can broaden our knowledge on the application of PGPRs and their volatiles such as acetoin to protect crops from O_3 injury.

APPOST 12

Influences of plant growth promoting rhizobacteria (PGPR) inoculum combined with the reduction application of chemical fertilizer on the community structure of *nosZ* denitrifying bacteria in flue-cured tobacco soils

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Variation of the denitrifying bacterial communities in the flue-cured tobacco cultivation soils treated with plant growth promoting rhizobacteria (PGPR) plus reduction of chemical fertiliser (RCF) was investigated so as to reveal the microbial mediate mechanism of the denitrifying process in tobacco cultivation soils, establish reasonable fertiliser regimes and provide theoretical foundations for maintaining soil quality. A traditional flue-cured tobacco cultivation field experiment was established in Miyi county, Sichuan Province. Chemical analysis and terminal restriction fragment length polymorphism (T-RFLP) were used to determine the variation of soil physicochemical parameters and *nosZ*-type denitrifying bacterial community composition under the impact of PGPR+RCF fertiliser, respectively. The results showed that the soil physicochemical properties varied under the treatment of PGPR+RCF fertiliser. Compared with the conventional fertilisation (CK), the soil available potassium content reached the peak in the soil amended with 10 % reduction of the total amount of chemical fertilisers (10 % RCF), while the soil organic carbon, total nitrogen, alkaline nitrogen and available phosphorus were highest in the soil treated with 30 % reduction of the total amount of chemical fertilisers (30 % RCF). The T-RFLP analysis showed that diversity of the *nosZ*-type denitrifying bacterial community structure increased in the soil treated with PGPR and other fertilisers. *Polymorphumgilvum*, *Rhodobacter* and *Bacterium* were the predominant denitrifying bacteria genus in all the five soils, while *Bradyrhizobium* and *Azospirillum* were only detected in the PGPR inoculated soil. The most complex and diverse *nosZ*-type denitrifying bacteria communities were detected in the soil with 30 % RCF. Redundancy Analysis (RDA) showed that soil pH, soil organic matter and available phosphorus were the major factors in shaping *nosZ*-type denitrifying bacterial community. The treatment of PGPR+RCF changed the soil physicochemical properties thus modifying the composition of the *nosZ*-type denitrifying bacterial community composition and increasing the diversity of the *nosZ*-type denitrifying bacterial community.

APPOST 13

Agronomic and qualitative performance of some experimental Burley hybrids in Malawi

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Malawi mainly depends on tobacco for forex generation, with Burley as a major contributor. However, the country has a limited choice of high yielding and disease resistant varieties against a plethora of disease constraints, particularly root-knot nematodes, fusarium wilt and angular leaf spot. Six improved experimental hybrids and four checks were simultaneously screened in randomised complete blocks (RCBD) with three replications in disease hotspots, and evaluated for yield and quality in rotated lands at four sites in central Malawi in the 2017-2018 and 2018-2019 seasons. Screening focussed on reaction of genotypes to root-knot nematodes (assessed on a 0-10 scale) and proportion of plants infected by fusarium wilt diseases. BRK4 and ABH12 were used as nematode resistant checks while ABH31 and ABH43 served as nematodes and fusarium wilt resistant checks. Two-row plots were used in each case with an inter-row spacing of 1.2 m. Each row had 12 plants spaced at 0.6 m apart. Yield evaluation followed recommended production practices for Burley while no topping and desuckering were done in screening plots. Data was collected on disease ratings for root-knot nematodes and fusarium wilt and other traits of agronomic relevance. Results revealed significant differences with regard to reaction of test hybrids and checks to root-knot nematodes at 6 WAT and 9 WAT ($P < 0.05$). Except HIP-15-20, all other hybrids were as resistant as the nematode resistant check, BRK4. All test hybrids except HIP-15-18, recorded significantly ($P < 0.05$) lower mean fusarium wilt infected plants compared to the checks. Yield, colour spectrum and grade outturn of cured leaf did not show significant differences between the hybrids and checks implying that agronomic performance was similar.

APPOST 14

Forestry sustainability in the tobacco sector in Malawi: a community concern requiring community approaches

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Tobacco farmers depend heavily on tree resources for poles and firewood used in curing. Malawi tobacco farmers predominantly grow Burley tobacco which requires the least amount of tree resources per unit volume in comparison to dark-fired and flue-cured tobacco. Nonetheless, the sheer volume of Burley tobacco produced annually demands considerable amount of poles for barn construction. This has exerted immense pressure on existing tree resources in Burley tobacco growing areas. Associated with small land holding sizes, smallholder farmers' tree planting and management has not equalled the amount of wood used. To address this, several tree planting and management approaches have been tried. These include: the provision of tree nursery inputs to individual farmers; issuing of free tree seedlings; and lately the inclusion of tree planting and management in contract growing conditions. The success of such approaches has not been satisfactory. Stakeholders, particularly the buyers under the integrated production system, resolved to directly get involved in tree planting and management. More often such plantations are established away from where the farmers are. As from last year ARET initiated an approach that aims at ensuring that the trees are planted by the farmers within their own areas. The approach recognises that the tobacco farmers are part of a community and so the community becomes the entry point. People are mobilised to identify their forestry needs. ARET provides technical and necessary material support. Where tree seedlings are required, they raise their own. The programme has run for one year in five pilot sites across Malawi with encouraging results: 25,300 trees planted in community woodlots, 17 live barns and 14 agro-forestry fields established. Woodlots so established are well cared for and survival of planted trees is close to 100 %. Depending on the success, the programme will be rolled out to other areas.

APPOST 15

Analysis of mainstream cigarette smoke constituents prioritized by the World Health Organization in a core collection of tobacco accessions: variability and correlations

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Among the different strategies proposed for tobacco regulation, the WHO study group on Tobacco Product Regulation (TobReg) has proposed mandating ceilings on smoke constituents. These constituents were selected according to their potential toxicity, their variability among brands and the potential for the constituents to be lowered with up-to-date technologies.

To assess the potential use of the natural diversity of tobacco plants to reduce the proposed constituents, the variability of different leaf and smoke constituents was evaluated in a core collection of tobacco accessions, representative of genetic diversity available before intensive modern breeding. A panel of 145 tobacco varieties from the Imperial Tobacco collection was grown in the field with three repeats in a split-splot design. After curing, handmade cigarettes were prepared to be analysed for smoke constituents. A statistical approach, taking into account multiple factors linked to the growing environment and weight of tobacco actively burnt during puffing, was designed to identify potential differences between varieties.

Significant differences were found for some constituents between tested varieties, but for others, low variability and inverse correlation are major bottlenecks. The WHO strategy on ceilings cannot be implemented in the state of the art, even if some elements can be treated individually.

APPOST 16

Effects of biochar and seaweed fertiliser on tobacco growth, soil properties and bacterial wilt occurrence

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Bacterial wilt of tobacco incited by *Ralstonia solanacearum* is prevalent worldwide and causes severe economic losses every year. Up until now there is no promising control method available against the disease. This study aimed to elucidate the effect of soil amendments by application of different quantities of biochar and seaweed extract fertilisers on the growth of the tobacco plant and development of resistance against wilt-causing bacteria. The experiment was a split plot in randomised complete block design (RCBD). NC102 tobacco cultivar was planted in disease endemic areas. On the basis of conventional fertilisation, six treatments were set as follows: CK (control), BC1 (9000 $kgha^{-1}$ biochar), BC2 (15000 $kgha^{-1}$ biochar), BC3 (22500 $kgha^{-1}$ biochar), SW (2250 $kgha^{-1}$ seaweed fertilizer) and BC2 + SW (15000 $kgha^{-1}$ biochar + 2250 $kgha^{-1}$ seaweed fertiliser). Each treatment was replicated three times. The results revealed that the tobacco plant is capable of growing under bacterial wilt attack. Soil amendments with seaweed fertilizer and biochar either applied individually or together restrict the growth of *Ralstonia solanacearum*. Although excessive use of biochar inhibits the growth of the tobacco at rosette stage, it is promoted at vigorous growing stage. Moreover, the application of biochar and seaweed fertilisers significantly increases soil pH and nutrient contents thereby reducing the disease incidence and severity. A significantly positive effect was observed on the yield of flue-cured tobacco. The chemical composition and proportion of middle and crown leaves of tobacco were also improved. The Pearson correlation coefficient was used to reflect the relationship between the physical and chemical properties of soil and their relative effect on disease progression in the different treatments. Therefore, it can be concluded that proper management of soil pH plays a key role in bacterial wilt disease suppression and improves the quality of flue-cured tobacco leaves.