AGRONOMY & LEAF INTEGRITY and
PHYTOPATHOLOGY & GENETICS

Virtual Conference

4 – 14 October 2021

PROGRAMME & ABSTRACTS

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CONTENTS

WELCOME MESSAGE

Lea SCOTT
Vice-President of the CORESTA Scientific Commission

Dear Colleagues

It is my pleasure to welcome you to the 2021 Agronomy & Leaf Integrity and Phytopathology & Genetics Study Groups virtual conference. COVID-19 continues to hinder our ability to travel and hold in-person meetings, so CORESTA has decided to offer another virtual conference format to share tobacco scientific knowledge with its members.

The Agronomy & Phytopathology conference is pleased to have 54 oral presentations that will occur across nine live online sessions. We have a diverse group of presentations that covers numerous topics, such as cigar tobaccos, genetic tools and technologies, crop production and sustainability, leaf nicotine, and conventional and biocontrol of pests and diseases. Each presentation is pre-recorded, with a live question and answer session where participants can interact with the presenter. It is our hope that this hybrid approach is a more fulfilling experience for CORESTA members.

Once again, welcome to the Agronomy and Phytopathology virtual conference and we hope you find it to be a worthwhile and enriching experience.

Sincerely and respectfully

G. Lea SCOTT, III
Universal Leaf Tobacco Company
Richmond, VA, U.S.A.

PROGRAMME

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

DAY 1
MONDAY 4 OCTOBER

SESSION - Production impact of nutrition and herbicides

Chair: Lea SCOTT

Co-Chair: Anthony JACKSON

CET Time Zone

13:30-13:45	**AP 01**	**Flue-cured tobacco response to sub-lethal rates of glufosinate** VANN M.C.(1); JORDAN D.L.(1); FISHER L.R.(2) *(1) North Carolina State University, Department of Crop & Soil Sciences, Campus Box 7620, Raleigh, NC 27695, U.S.A.* *(2) North Carolina State University, NC Agriculture Research Service, Campus Box 7643, Raleigh, NC 27695, U.S.A.*
13:45-14:00	**AP 03**	**Diagnosis and amelioration of secondary and micronutrient deficiency on productivity and quality of flue-cured tobacco in Northern light soil region of India** UMA MAHESH M.N.V.A.; REDDY B.S.R.; KALYAN RAMI REDDY L.; G. KRISHNA KUMAR; SRINIVAS P. *Research Department, ITC–ABD, Rajahmundry-533103, East Godavari (Dist.), Andhra Pradesh, India*
14:00-14:15	**AP 04**	**Experiences in evaluation of boron needs in North Carolina flue-cured tobacco production: a summary of plant tissue sufficiency data and impacts on yield and quality** HARDY D.(1); VANN M.C.(2); JERNIGAN C.(3); McGINNIS M.(1); HICKS K.(1) *(1) Agronomic Division, North Carolina Dept. of Agriculture and Consumer Services, NC, U.S.A.* *(2) North Carolina State University, Crop and Soil Science Department, NC, U.S.A.* *(3) Helena Chemical Co., U.S.A.*
14:15-14:30	**AP 05**	**Regulatory effects of topping on K^+ flux and gene expression in tobacco roots** LIANG Taibo(1); GUO Yadi(1); MENG Xiangyu(2); DAI Huaxin(1); ZHAI Zhen(1); WANG Aiguo(1); ZHANG Yanling(1) *(1) Zhengzhou Tobacco Research of CNTC, Zhengzhou 450001, China* *(2) Golden Leaf Manufacturing Center, China Tobacco Henan Industrial Co., Ltd, Zhengzhou 450016, China*
14:30-14:45	**AP 06**	**Auxin herbicide exposure: comparisons of 2,4-D and dicamba drift to flue-cured tobacco** VANN M.C.(1); JORDAN D.L.(1); FISHER L.R.(2) *(1) North Carolina State University, Department of Crop & Soil Sciences, Campus Box 7620, Raleigh, NC 27695, U.S.A.* *(2) North Carolina Agriculture Research Service, North Carolina State University, Campus Box 7643, Raleigh, NC 27695, U.S.A.*

*[**AP 02** cancelled]*

DAY 2
TUESDAY 5 OCTOBER

SESSION - Genetics: tools for tobacco plant breeders

Chair: Dongmei XU

Co-Chair: François DORLHAC de BORNE

CET Time Zone

13:30-13:45	AP 07	**Nematodes: first steps toward the identification of markers related to Rk2 resistance gene against *M. arenaria* and *M. javanica*** JULIO E.(1); COTUCHEAU J.(1); VOLPATTI R.(1); TRIVILIN A.P.(2); DECORPS C.(1); ENDER M.(2); DORLHAC DE BORNE F.(1) *(1) Imperial Brands, Leaf Research, La Tour, 24100 Bergerac, France* *(2) Universal Leaf Tabacos Ltda, BR 471, Km 129,8, P.O. Box 1025, CEP 96.835-642, Santa Cruz do Sul, RS, Brazil*
13:45-14:00	AP 08	**An update to the molecular markers used for rapid screening of pathogen resistant plants** JULIO E.(1); COTUCHEAU J.(1); VOLPATTI R.(1); DECORPS C.(1); VERRON T.(2); CAHOURS X.(2); DORLHAC DE BORNE F.(1) *(1) Imperial Brands, Leaf Research, La Tour, 24100 Bergerac, France* *(2) Imperial Brands, Scientific Research, 200-216 Rue Raymond Losserand, 75014 Paris, France*
14:00-14:15	AP 09	**A novel NAC transcription factor, *NtNAC060* enhances the bacterial wilt resistance and salt stress tolerance in tobacco** LI Xiaoxu(1); PU Wenxuan(1); YI Jianhua(1); GUO Yongfeng(2); GAO Junping(1); WANG Dong(1) *(1) China Tobacco Hunan Industrial Co., Ltd, 386 Laodong Middle Road, Changsha 410007, China* *(2) Tobacco Research Institute, Chinese Academy of Agricultural Sciences, 11 Keyuanjingsi Road, Qingdao 266100, China*
14:15-14:30	AP 10	**Genetic analyses of a major partial disease resistance quantitative trait locus (QTL) in tobacco** SHI Rui(1); JIN Jing(2); SHEW D.(2); LEWIS R.S.(1) *(1) North Carolina State University, Department of Crop and Soil Sciences, Raleigh, NC 27695, U.S.A.* *(2) North Carolina State University, Department of Entomology and Plant Pathology, Raleigh, NC 27695, U.S.A.*
14:30-14:45	AP 11	**Agronomic performance of Polalta-derived breeding lines resistant to tomato spotted wilt virus** KORBECKA-GLINKA G.(1); TROJAK-GOLUCH A.(1); BAKAHER N.(2); GOEPFERT S.(2) *(1) Institute of Soil Science and Plant Cultivation (IUNG) - State Research Institute, Czartoryskich 8, Puławy, Poland* *(2) Philip Morris Products S.A., PMI R&D, Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland*
14:45-15:00	AP 12	**Study on inheritance of the number of leaves per stalk and dimensions of the leaves from the middle belt in tobacco varieties from different types and their F_1 hybrids** KORUBIN-ALEKSOSKA A.(1); DOJCINOV S.(2) *(1) University St. Kliment Ohridski - Bitola, Scientific Tobacco Institute - Prilep, St. Kicevska bb, Prilep, Republic of Macedonia* *(2) Alliance One Macedonia - Kavadarci, Republic of North Macedonia*

DAY 3
WEDNESDAY 6 OCTOBER

SESSION - Biocontrol of tobacco pests and diseases

Chair: Susan DIMBI

Co-Chair: Fabienne LALANDE

CET Time Zone

13:30-13:45	AP 13	**The effect of liquid smoke from tobacco waste on tobacco collar rot (*Sclerotinia sclerotiorum*) in Northern Iran** SAJJADI A.; MORADI ROBATI G.R.; SALAVATI M.R.; NAJAFI M.R.; BARZEGHAR Y.; GHARIB O.; NAJAFI H. *Tirtash Tobacco Research and Education Center, Behshar, Iran*
13:45-14:00	AP 14	**The control effect and mechanism of green manure on soil-borne diseases of tobacco** LIU Yanxia(1); LI Xiang(2); WANG Jinling(3); PENG Yu(2); LI Lei(4); ZHANG Heng(1); ZHU Jingwei(1); GAO Weichang(1); YAO Yunjing(4) *(1) Guizhou Academy of Tobacco Science, Guiyang 550000, China; Guizhou Provincial Academician Workstation of Microbiology and Health, Upland Flue-Cured Tobacco Quality & Ecology Key Laboratory of China Tobacco* *(2) Guizhou Tobacco Company, CNTC, Guiyang 550000, China* *(3) China Tobacco Jiangsu Industrial Co., Ltd, Nanjing 210000, China* *(4) Guizhou University, Guiyang 550000, China*
14:00-14:15	AP 15	**In search of greener nematicides for use on tobacco: current status in Zimbabwe** MAHERE T.S.; CHINHEYA C. *Tobacco Research Board, Kutsaga Research Station, P.O. Box 1909, Airport Ring Road, Harare, Zimbabwe*
14:15-14:30	AP 16	**Molecular characterization of *cry* genes in *Bacillus thuringiensis* native strains isolated from Northern Iran** SHAZDEHAHMADI M.; SAJJADI A.; SHAHADATIMOGHADAM Z. *Tirtash Tobacco Research and Education Center, Behshar, Iran*
14:30-14:45	AP 17	**Evaluation of novel biological control bacteria for management of angular leaf spot in dark tobacco** MARTINEZ-OCHOA N.; SHIELDS C.A.; ARAUJO ALVES M.; JOUBERT A.; MILLER R.D. *University of Kentucky, Department of Plant and Soils, 1401 University Drive, 102 KTRDC, Lexington, KY 40546-0236, U.S.A.*
14:45-15:00	AP 18	**Pathogen identification and biological control of pole rot of flue-cured tobacco caused by *Rhizopus arrhizus* (syn. *R. oryzae*)** LU Canhua(1); GAI Xiaotong(1); SU Jiaen(2); MA Junhong(1); LEI Liping(1); JIANG Ning(1); LIN Zhonglong(3); GAO Chaoyang(4); JIN Yan(1); XIA Zhenyuan(1) *(1) Yunnan Academy of Tobacco Agriculture Sciences, Kunming 650021, China* *(2) Dali Branch of Yunnan Provincial Tobacco Company, Dali 671000, China* *(3) China National Tobacco Corporation Yunnan Company, Kunming 650011, China* *(4) Yanfang Comprehensive Service Center of Agriculture and Rural Affairs of the Zhanyi District, Qujing 655339 , China*

DAY 4
THURSDAY 7 OCTOBER

SESSION - Nicotine impacts of genetics and production practices

Chair: Marcos LUSSO

Co-Chair: Lea SCOTT

CET Time Zone

13:30-13:45	AP 19	**A novel low alkaloid gene** FISHER A.M.(1); PATRA B.(1); WU X.(2); SINGH S.(1); FISHER C.R.(2); JI H.(1) *(1) University of Kentucky, Kentucky Tobacco Research & Development Centre, KTRDC Building, 1401 University Drive, Lexington, KY 40546, U.S.A.* *(2) University of Kentucky, Department of Plant & Soil Sciences, KTRDC Building, 1401 University Drive, Lexington, KY 40546, U.S.A.*
13:45-14:00	AP 20	**Functional characterization of *NIC1*-locus in regulating nicotine biosynthesis in tobacco and its applications in low nicotine variety development** SUI Xueyi; XIE He; WANG Bingwu; TONG Zhijun; SONG Zhongbang; GAO Yulong; ZHANG Yihan; ZHAO Lu; LI Yongping *Yunnan Academy of Tobacco Agricultural Sciences, Kunming, China*
14:00-14:15	AP 21	**Impact of genotype and management on nicotine concentration in Burley tobacco** VANN M.C.; MACHACEK J.L.; CHEEK J.A.; SHORT M.M.; WHITLEY D.S. *North Carolina State University, Department of Crop & Soil Sciences, Campus Box 7620, Raleigh, NC 27695, U.S.A.*
14:15-14:30	AP 22	**The effect of nicotine content on cured leaf quality of Burley** FISHER C.R.(1); JI H.(2); WU X.(2); FISHER A.M.(2) *(1) University of Kentucky, Plant & Soil Science Department, Lexington, KY 40546, U.S.A.* *(2) University of Kentucky, Kentucky Tobacco Research & Development Center, Lexington, KY 40546, U.S.A.*
14:30-14:45	AP 23	**Effects of genotype and cultural practices on flue-cured tobacco growth, development, and chemistry** VANN M.C.; CHEEK J.A.; MACHACEK J.L.; WHITLEY D.S. *North Carolina State University, Department of Crop & Soil Sciences, Campus Box 7620, Raleigh, NC 27695, U.S.A.*

[AP 24 cancelled]

DAY 5
FRIDAY 8 OCTOBER

SESSION - Sustainability through production practices

Chair: Anthony JACKSON

Co-Chair: Marcos LUSSO

CET Time Zone

13:30-13:45	AP 25	**Climate change exacerbates the challenge of pests and diseases on tobacco in Zimbabwe** CHINHEYA C.; DIMBI S.; MAGAMA F. *Tobacco Research Board, Plant Health Services Division, P.O. Box 1909, Harare, Zimbabwe*
13:45-14:00	AP 26	**Effects of wheat straw residue and its biochar on the physical properties and enzyme activities of tobacco-growing soil** WANG Yi(1,2); DU Chuanyin(1); LI Jianlei(3); LIU Zhigang(1); GUAN Ensen(1); WANG Dequan(1); WANG Dahai(2); GAO Kai(1); WANG Shusheng(2) *(1) Weifang Tobacco Co., Ltd, Weifang, Shandong 262200, China* *(2) Tobacco Research Institute, Chinese Academy of Agriculture Sciences, Qingdao, Shandong 266101, China* *(3) Shandong Province Tobacco Monopoly Bureau (Company), Jinan 250098, China*
14:00-14:15	AP 27	**Soil type regulates carbon and nitrogen stoichiometry and mineralization and bacteria following biochar or nitrogen addition** WANG Huanhuan; SHI Hongzhi; ZHAO Yuanyuan; LIU Guoshun *College of Tobacco Science, Henan Agricultural University / Tobacco Harm Reduction Research Center of HAU, Zhengzhou 450002, China*
14:15-14:30	AP 28	**Evaluation of the effect of vermicompost on tobacco plant growth, yield and leaf quality** CHIBUDU C.; MAVUKA R. *Tobacco Research Board, Kutsaga Research Station, P.O. Box 1909, Airport Ring Road, Harare, Zimbabwe*
14:30-14:45	AP 29	**Evaluation of a biological suckercide, pelargonic acid, for the control of suckers in tobacco production in Zimbabwe** ZINYANDU F.; MUKUNGURUTSE C.; KOGA C.; MAVUKA R. *Tobacco Research Board, Kutsaga Research Station, P.O. Box 1909, Airport Ring Road, Harare, Zimbabwe*
14:45-15:00	AP 30	**Investigation of compressed charcoal production from charcoal pyrolysis of Virginia tobacco stalk** MORADI ROBATI G.R.; NAJAFI H.; GHARIB O.; BARZEGHAR E. *Tirtash Tobacco Research and Education Center, Behshar, Iran*

DAY 6
MONDAY 11 OCTOBER

SESSION - Technology applications in genetics and physiology

Chair: Masahiro MIYOSHI

Co-Chair: Dongmei XU

CET Time Zone

13:30-13:45	AP 31	**High quality genome assembly of *Nicotiana tabacum*** GAYSSANT H.(1); DARNIGE E.(1); COTUCHEAU J.(2); DORLHAC DE BORNE F.(2); JULIO E.(2); ZOUINE M.(1) *(1) Laboratoire de Recherche en Sciences Végétales (LRSV), Université de Toulouse, CNRS, UPS, Toulouse INP, 31326 Castanet-Tolosan, France* *(2) Imperial Tobacco Limited and Imperial Brands PLC Companies, Bergerac, France*
13:45-14:00	AP 32	**Understanding the response of tobacco plants to root-knot nematode infection by means of chlorophyll fluorescence imaging** SHAMUDZARIRA G.T.H.; MATANGIRA N.; SHAMUDZARIRA M.C.; MAGAMA F.; CHINHEYA C.; DIMBI S. *Tobacco Research Board, Kutsaga Research Station, P.O. Box 1909, Airport Ring Road, Harare, Zimbabwe*
14:00-14:15	AP 33	**Identification and functional characterization of the pale yellow gene in tobacco** GRUNDMANN L.(1); PRAMOD S.(2); ADAMS A.(2); FREDERICK J.(2); KAENEL A.(1); NOLL G.(1); XU D.(2); PRUEFER D.(1); LUSSO M.(2) *(1) Fraunhofer IME, Schlossplatz 8, 48143 Münster, Germany* *(2) Altria Client Services, 601 E Jackson St., Richmond, VA 23219, U.S.A.*
14:15-14:30	AP 34	**Dynamics of molecular leaf senescence processes occurring during curing of Virginia tobacco cultivar K326** CHEVAL C.; BATTEY J.; SIERRO N.; DULIZE R.; BORNAND D.; SIMICEVIC J.; TITZ B.; GUY P.A.; LASZLO C.; HAIDUC A.; DOSSIN E.; BOVET L.; IVANOV N.; GOEPFERT S. *Philip Morris International R&D, Philip Morris Products SA, CH-2000 Neuchâtel, Switzerland*
14:30-14:45	AP 35	**Two-year evaluation of agronomic practices on standard and low-nicotine tobacco cultivars by non-destructive photonic sensing** TUCCIO L.(1); BARGIACCHI E.(2); MILLI G.(3); MIELE S.(2); FRANCESCHETTI L.(3); AGATI G.(1) *(1) CNR-IFAC, Sesto Fiorentino, I-55019 Firenze, Italy* *(2) Consortium INSTM, I-50121 Firenze, Italy* *(3) Fattoria Autonoma Tabacchi (FAT) & ITT, I-06012 Città di Castello, Perugia, Italy*
14:45-15:00	AP 36	**Classification of Virginia Bright tobacco varieties by non-destructive photonic sensing of leaf flavonoids** TUCCIO L.(1); BARGIACCHI E.(2); MILLI G.(3); MIELE S.(2); FRANCESCHETTI L.(3); AGATI G.(1) *(1) CNR-IFAC, Sesto Fiorentino, I-55019 Firenze, Italy* *(2) Consortium INSTM, I-50121 Firenze, Italy* *(3) Fattoria Autonoma Tabacchi (FAT) & ITT, I-06012 Città di Castello, Perugia, Italy*

DAY 7
TUESDAY 12 OCTOBER

SESSION - Pests and diseases management

Chair: François DORLHAC de BORNE

Co-Chair: Susan DIMBI

CET Time Zone

13:30-13:45	AP 37	**Estimating the effective control of Ditacin 8SL (Ningnanmycin) and Sat 4SL (Cytosinpeptidemycin) with tobacco mosaic virus (TMV), cucumber mosaic virus (CMV), and potato virus Y (PVY) on tobacco plants** NGUYEN VAN Chin(1); DO THI Thuy(2); PHUNG THI Hay(2); NGUYEN VAN Van(1); TAO NGOC Tuan(1); NGUYEN QUOC Tuan(1); NGUYEN VAN Cuong(2) *(1) Vietnam Tobacco Institute, 133 Nguyen Trai Road, Thuong Dinh Street, Thanh Xuan District, Ha Noi City, Vietnam* *(2) Branch of Vietnam Tobacco Institute, Bac Giang Province, Vietnam*
13:45-14:00	AP 38	**Study of the interactions of root-knot nematode (*Meloidogyne incognita*) and tobacco black shank (*Phytophthora nicotianae*) on some tobacco cultivars under natural field pollution conditions** SHAZDEHAHMADI M.; SAJJADI A.; SHAHADATIMOGHADAM Z. *Tirtash Tobacco Research and Education Center, Behshar, Iran*
14:00-14:15	AP 39	**Risk assessment of *Ralstonia solanacearum* to fluazinam and evaluation of combinatory fungicides** MU Wenjun(1); MA Xiaojing(1); HU Liwei(1); ZHANG Zhigao(2); TANG Lina(3); LI Qiuying(3); HUANG Lei(4); FENG Xiaohu(2); SONG Jizhen(1) *(1) Zhengzhou Tobacco Research Institute of CNTC, Key Laboratory of Eco-environment and Leaf Tobacco Quality, No. 2 Fengyang Street, Zhengzhou 450001, China* *(2) Jiangxi Tobacco Company, Fuzhou Branch, No. 1666 Yingbin Street, Fuzhou 344000, Jiangxi, China* *(3) Fujian Institute of Tobacco Science, No. 378 Hualin Road, Fuzhou 350003, China* *(4) China Tobacco Guangdong Industrial Co., Ltd, No. 62 Chiyan Street, Guangzhou 510310, China*
14:15-14:30	AP 40	**Differential susceptibility to angular leaf spot (*Pseudomonas syringae* pv. *tabaci*) in dark tobacco varieties** KEENEY A.B.(1); BAILEY W.A.(1); HANSON Z.R.(2) *(1) University of Kentucky, Department of Plant and Soil Sciences, Research & Education Center, Princeton, KY 42445, U.S.A.* *(2) University of Tennessee, Department of Plant Science, Knoxville, TN 37996, U.S.A.*
14:30-14:45	AP 41	**Evaluation of spray programs for control on angular leaf spot (*Pseudomonas syringae* pv. *tabaci*) in dark tobacco production** KEENEY A.B.; BAILEY W.A. *University of Kentucky, Department of Plant and Soil Sciences, Research & Education Center, Princeton, KY 42445, U.S.A.*
14:45-15:00	AP 42	**Effective light device for trapping tobacco moth (*Ephestia elutella*)** IWAMOTO H.; TAKAHASHI R.; IMAI T. *Japan Tobacco Inc., Leaf Tobacco Research Center, 1900 Idei, Oyama, Tochigi 323-0808, Japan*

DAY 8
WEDNESDAY 13 OCTOBER

SESSION - Cigar tobaccos and alternative uses and crops

Chair: Fabienne LALANDE

Co-Chair: Colin FISHER

CET Time Zone

13:30-13:45	AP 43	**Cigar wrapper response to nitrogen fertilizer rates in western North Carolina** SHORT M.M.; VANN M.C.; CHEEK J.A.; MACHACEK J.L.; WHITLEY D.S. *North Carolina State University, Department of Crop & Soil Sciences, Campus Box 7620, Raleigh, NC 27695, U.S.A.*
13:45-14:00	AP 44	**Development of production recommendations for Connecticut Broadleaf cigar wrapper tobacco in Kentucky and Tennessee** BAILEY W.A.; RODGERS J.C.; KEENEY A.B.; PERKINS C.H.; WITCHER V.F. *University of Kentucky, Department of Plant and Soil Sciences, Research & Education Center, Princeton, KY 42445, U.S.A.*
14:00-14:15	AP 45	**Evaluation of advanced experimental hybrids of cigar wrapper tobacco for yield and quality in Zimbabwe** MUKOYI F.; MAGAMA F.; GARWE D. *Tobacco Research Board, Kutsaga Research Station, P.O. Box 1909, Airport Ring Road, Harare, Zimbabwe*
14:15-14:30	AP 46	**Characterization and functional prediction of microbial community in agricultural processing of cigar leaves from Shifang, Sichuan** ZHANG Qianying(1); CAI W.(1); LIU Y.(2); YE K.Y.(2); ZHANG J.(3); LI D.L.(1) *(1) Technical Research Center, China Tobacco Sichuan Industrial Co., Ltd, Chengdu 610066, China* *(2) Great Wall Cigar Factory, China Tobacco Sichuan Industrial Co., Ltd, Shifang 618400, China* *(3) National Engineering Laboratory for Cereal Fermentation Technology, Jiangnan University, Wuxi 214122, China*
14:30-14:45	AP 47	**Alternative use for tobacco: solvent extraction of oil from Zimbabwe tobacco seed hybrids and parentals** MUSUNA-GARWE C.C.; MUDYAWABIKWA B.; MAGAMA F.; DIMBI S.; GARWE D. *Tobacco Research Board, Kutsaga Research Station, P.O. Box 1909, Airport Ring Road, Harare, Zimbabwe*
14:45-15:00	AP 48	**Industrial hemp production: an evaluation of its adaptability and potential incorporation in tobacco growing systems in Zimbabwe** SHAMUDZARIRA M.C.; MATANGIRA N.; MAGAMA F.; DIMBI S. *Tobacco Research Board, Kutsaga Research Station, P.O. Box 1909, Airport Ring Road, Harare, Zimbabwe*

DAY 9

THURSDAY 14 OCTOBER

SESSION - TSNA impact of genetics and production practices

Chair: Colin FISHER

Co-Chair: Limeng ZHANG

CET Time Zone

13:30-13:45	AP 49	**Consequences of molecular genetic alteration of leaf nitrate levels on TSNAs, alkaloids, flowering time, and biomass in Burley tobaccos** DEWEY R.E.(1); BOVET L.(2); GOEPFERT S.(2); CAMPANONI P.(2); LEWIS R.S.(1) *(1) North Carolina State University, Dept. of Crop and Soil Sciences, Campus Box 8009, Raleigh, NC 27695, U.S.A.* *(2) PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, 2000 Neuchatel, Switzerland*
13:45-14:00	AP 50	**Genetic mapping of a novel low-anatabine gene mutation in tobacco (*Nicotiana tabacum* L.)** KUDITHIPUDI C.; HART F.R. *Altria Client Services LLC, Research Development & Regulatory Affairs, 601 E. Jackson Street, Richmond, VA 23219, U.S.A.*
14:00-14:15	AP 51	**Impact of potassium source on cured leaf moisture and total TSNA in Burley and dark fire-cured tobacco** BAILEY W.A.(1); KEENEY A.B.(1); PEARCE R.C.(1); RICHMOND M.D.(2) *(1) University of Kentucky, Department of Plant and Soil Sciences, Lexington, KY 40546, U.S.A.* *(2) University of Tennessee, Department of Plant Sciences, Knoxville, TN 37996, U.S.A.*
14:15-14:30	AP 52	**Effects of exogenous salicylic acid on photosynthesis and nitrogen metabolism of tobacco under drought stress and transcriptome analysis** FENG Yuqing(1); SHI Hongzhi(1); ZHAO Yuanyuan(1); ZHOU Jun(2); MA Yanjun(2); LI Geng(1) *(1) College of Tobacco Science / Tobacco Harm Reduction Research Center, Henan Agricultural University, Zhengzhou 450002, China* *(2) Beijing Cigarette Factory, Shanghai Tobacco Group Co., Ltd., Beijing 100024, China*
14:30-14:45	AP 53	**Endophytic fungal community of tobacco leaves and their potential role in the formation of Cherry Red tobacco** JIANG Yonglei; ZHAO Gaokun; LIU Jiahong; XIE Yan; LI Yong; ZOU Congming *Yunnan Academy of Tobacco Agricultural Sciences, Kunming, China* *Qujing Branch of Yunnan Tobacco Company, Qujing, China*
14:45-15:00	AP 54	**Effect of fermentation temperature on TSNA contents and aroma quality of cigar filler tobacco and microbial diversity analysis** LI Jingjing(1); SHI Hongzhi(1); QIN Yanqing(2); ZHOU Jun(3); ZHAO Yuanyuan(1); ZHONG Qiu(4); LIU Deshui(3); WANG Jun(4); ZHANG Ruina(4) *(1) College of Tobacco Science of Henan Agricultural University / Tobacco Harm Reduction Research Center of Hau, Zhengzhou 450002, China* *(2) Sichuan Provincial Tobacco Company, Chengdu, China* *(3) Beijing Cigarette Factory of Shanghai Tobacco (Group) Co., Beijing 100024, China* *(4) Deyang Municipal Tobacco Company, Deyang, 618400, China*

ABSTRACTS

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

AP 01

Flue-cured tobacco response to sub-lethal rates of glufosinate

VANN M.C.(1); JORDAN D.L.(1); FISHER L.R.(2)

(1) North Carolina State University, Department of Crop & Soil Sciences, Campus Box 7620, Raleigh, NC 27695, U.S.A.
(2) North Carolina State University, NC Agriculture Research Service, Campus Box 7643, Raleigh, NC 27695, U.S.A.

Glufosinate is a broad-spectrum, contact herbicide that is currently applied to genetically engineered row crops that tolerate exposure to the chemical, such as cotton (*Gossypium hirsutum*), maize (*Zea mays*), and soybean (*Glycine max*). Flue-cured tobacco is susceptible to glufosinate, yet it is commonly grown in close proximity to tolerant crops in North Carolina. The impact of glufosinate drift on flue-cured tobacco is not known. As such, research was conducted to test the effects of sub-lethal rates of glufosinate (19, 37, 75, 146, and 303 g ai ha^{-1}) on flue-cured tobacco yield, visual quality, value, and chemistry. Simulated drift was imposed five weeks after transplanting when tobacco plants were 60 cm tall and had 12-14 expanded leaves. Visual injury increased with exposure rate and ranged from 15-83 % and from 10-83 %, one and two weeks after treatment, respectively. Cured leaf yield was reduced by 45 % at the highest sub-lethal exposure rate and exhibited a linear decline of ≈46 kg ha^{-1} for every 10 g ha^{-1} increase in glufosinate exposure. Visual quality and per acre value were not affected, most likely due to the loss of necrotic tissue and some late-season plant growth compensation as new growth emerged after treatment. Green and cured leaf residues were likewise not detected. In spite of minor impacts to cured leaf quality and value, as well as the inability to detect residues of the herbicide, tobacco exposed to glufosinate is ineligible for commercial sale because it is not approved for application to the crop. The visual injury symptoms documented in this study should be utilized when diagnosing physical drift of glufosinate.

AP 03

Diagnosis and amelioration of secondary and micronutrient deficiency on productivity and quality of flue-cured tobacco in Northern light soil region of India

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Flue-cured tobacco in northern light soils (NLS) in Andhra Pradesh state is grown in 17,000 ha under irrigated conditions. This tobacco is grown on sandy/sandy loam soils and, due to continuous mono cropping of flue-cured tobacco, it started expressing deficiency of certain secondary nutrients and micronutrients. To accentuate the situation, non-availability of calcium ammonium nitrate due to regulatory restrictions, which earlier was used as a major fertilizer source for nitrogen, resulted in deficiency of secondary nutrients such as calcium. Lamina samples were collected in those regions, expressing chlorotic symptoms and analysed for different secondary and micronutrients. Elemental analysis of leaf lamina samples indicated calcium and magnesium in deficient levels. After diagnosis, a two-year study was initiated in 2019-2021, to ameliorate secondary and micronutrient deficiencies and the impact on productivity and leaf quality. Six treatments were studied which included different fertilizer combinations such as ammonium sulphate, urea, mono ammonium phosphate, calcium nitrate, potassium nitrate, micronutrient mixture (B-1.5 %, Cu-0.5 %, Fe-3.4 %, Mg-1.2 %, Mn-3.2 %, Mo-0.05 % and Zn-4.2 %) and a water-soluble fertilizer (19N:11P_2O_5:11K_2O:3Mg). These treatments were studied by following a fertigation protocol. Application of secondary and micronutrients between 40-50 days after planting resulted in a 4 % improvement in productivity and 11 % improvement in grade index without a major shift in lamina chemistry (total nicotinic alkaloids, total sugars and chlorides).

The results of this study indicated a positive influence due to the supplemental application of secondary and micronutrients on productivity and quality of flue-cured tobacco in NLS.

AP 04

Experiences in evaluation of boron needs in North Carolina flue-cured tobacco production: a summary of plant tissue sufficiency data and impacts on yield and quality

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Boron (B), an essential micronutrient for optimum plant growth, is required in trace amounts (<1.1 kg B ha^{-1}) by flue-cured tobacco. In past decades, boron deficiency in North Carolina has not been prevalent. However, recently, its deficiency has been visually and analytically identified to warrant investigation. From 2015-2018, field research was conducted at fourteen locations to evaluate boron plant sufficiency ranges and its application on yield, quality, and leaf chemistry of flue-cured tobacco. Soil-applied rates were 0, 0.6, 1.1, 2.2, 5.6, and 11.2 kg B ha^{-1}. Foliar treatments were evaluated at total rates of 0.6 or 1.1 kg B ha^{-1} applied in combination as singular (at layby) and split treatments (at second cultivation and layby) or as needed based on visual symptoms. Treatments were arranged in a randomized complete block design with four replications. Tissue samples were collected at 3 growth stages: 1) early at 3 weeks after transplanting (WAT) - bud and most recently mature leaf (MRML), 2) midseason at 7 WAT - bud and MRML, and 3) at topping - MRML. Yield, quality, value, and leaf chemistry were evaluated. Deficiency was not visually observed at any site even when tissue concentrations were as low as 11 mg kg^{-1} (sufficiency range, 18 to 75 mg kg^{-1}). Toxicity was observed and analytically verified at rather low rates of application. Boron application affected yield in one of the three years: 2015 - 17 % increase at one location and 27 % to 30 % decrease at one location. The bud or MRML may be used to evaluate boron plant status, but sampling 3 WAT may be too early. If boron concerns exist, soil-applied application up to 1.1 kg ha^{-1} or foliar applications up to 0.6 kg ha^{-1} appear safe. Our findings do not support the need for routine boron application to flue-cured tobacco in North Carolina.

AP 05

Regulatory effects of topping on K^+ flux and gene expression in tobacco roots

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Topping is an important agronomic measure in tobacco field production, but it is a serious artificial injury to tobacco plants and will cause serious potassium loss. However, the variations of K^+ flux in tobacco roots after topping and the relationship between K^+ flux and potassium related gene expression have been rarely studied. In order to analyse the regulatory effect of topping on potassium loss in tobacco, non-invasive micro-test technology was used to study the effects of topping and indole-3-acetic acid (IAA) treatment on plant potassium content, root potassium flux, potassium loss and gene expression in two tobacco cultivars under sand culture conditions. The results showed that topping changed the root potassium flux direction from influx to efflux. Compared with the control, the root potassium efflux rates of EY-1 and Y87 increased by 60.75 $pmol \cdot cm^{-2} \cdot s^{-1}$ and 47.00 $pmol \cdot cm^{-2} \cdot s^{-1}$, respectively, after topping. Topping + IAA treatment decreased the root potassium efflux rates of EY-1 and Y87 by 6.90 $pmol \cdot cm^{-2} \cdot s^{-1}$ and 17.47 $pmol \cdot cm^{-2} \cdot s^{-1}$, respectively, compared with only the topping treatment. Compared with the control, potassium loss of EY-1 and Y87 increased by 60 % and 12 %, respectively, after topping. The application of IAA reduced the potassium loss of the two tobacco cultivars and increased their plant potassium contents slightly, however no significant difference was found. Topping inhibited the synthesis of IAA, promoted the expression of the K^+ outflow channel gene *NTORK1*, and inhibited the expression of the K^+ inner channel gene *NKT1*. The responses of K^+ channel genes to topping were different in different cultivars and different parts of the same cultivar. In conclusion, by inhibiting IAA synthesis, topping promotes the expression of K^+ outflow channel genes, inhibits the expression of K^+ inner channel genes, increases the outflow rate of potassium ions in roots, accelerates potassium loss, and reduces potassium content in tobacco plants. The application of IAA after topping compensates the potassium loss caused by topping.

AP 06

Auxin herbicide exposure: comparisons of 2,4-D and dicamba drift to flue-cured tobacco

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Auxin herbicide application has increased in North Carolina due to the commercialization of 2,4-D and dicamba tolerant crops. Flue-cured tobacco is extremely sensitive to both herbicides, yet is commonly grown in close proximity to genetically modified crops that are resistant to both chemicals. Direct crop response comparisons of each herbicide following a physical drift event have not been reported in flue-cured tobacco. Research was conducted in four North Carolina environments to compare five fractional rates of both herbicides: 1/2, 1/8, 1/32, 1/128, and 1/512 of 280 g acid equivalent (ae) dicamba and 540 g ae 2,4-D ha^{-1}. Pairwise comparisons for each fractional rate were compared. Among each of the fractional rates evaluated, visual injury was greatest following exposure to dicamba. One week after exposure, dicamba injury was generally twice that of 2,4-D. The same trend was also documented two weeks after exposure; however, differences ranged from one-third to two-thirds less, depending upon rate. Cured leaf yield was similar between herbicides at the 1/512 concentration; however, 2,4-D treatments out yielded dicamba at all other rates. Regression analyses were also conducted within each herbicide. 2,4-D injury followed a linear model and was maximized at the 1/2 rate. In contrast, dicamba injury followed a quadratic model and was maximized between the 1/128 and 1/512 exposure rates (95-96 g ae ha^{-1}). The maximum yield reduction within the 2,4-D and dicamba regression models was 1,177 kg ha^{-1} and 1,371 kg ha^{-1}, respectively. Green and cured leaf residues were not detected. Our results demonstrate the damaging potential of the commonly used auxin herbicides in North Carolina and reinforce the caution that should be exercised when applying them in close proximity to flue-cured tobacco.

AP 07

Nematodes: first steps toward the identification of markers related to Rk2 resistance gene against *M. arenaria* and *M. javanica*

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Plant parasitic nematodes belonging to the genus *Meloidogyne* are the most economically harmful nematodes worldwide with the widest host range. In the seventies, commercial tobacco cultivars with the Rk1 gene, which imparts resistance to *Meloidogyne incognita* races 1 and 3 and *Meloidogyne arenaria* race 1, started to be released. This gene has an impact on giant cell formation and was recently characterized on chromosome 17. A second locus, with a different mechanism (referred to as Rk2), was identified in Zimbabwe in plants and determined to be partially resistant to *M. javanica*, and crossed with cultivated tobacco entries to improve agronomic traits, resulting in the breeding line RKT15-1-1. In the nineties, Rk2 was incorporated in commercial varieties together with Rk1 for increased efficiency, against *M. javanica*, and for *M. arenaria*.

Until now, the mechanism of Rk2 remains unclear and several hypotheses have been proposed: dominant, multiple factors or modifying genes related to the background. The difficulty of phenotyping is the main barrier for its characterization. It is not unusual to have galls even with tolerant plants in high pressure conditions. Moreover, the effect of Rk2 alone is less visible than Rk1 alone.

In this study, a population resulting from the cross of two Rk1 tobaccos, and segregating for Rk2, was used in different experimental conditions: F6 recombinant inbred lines were assessed in a nursery in southern Brazil during two crops, in the presence of *M. arenaria* and *M. javanica*. An F_2 population was rated in France, in an open field trial, under *M. arenaria* infestation. Both parents were submitted to re-sequencing experiments and 2,000 single nucleotide polymorphism (SNPs) markers were selected for a linkage mapping and qualitative trait locus (QTL) approach. Both populations were then characterized by SeqSNPs. The first results of this QTLs approach are presented here.

AP 08

An update to the molecular markers used for rapid screening of pathogen resistant plants

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Due to cheaper costs associated with next generation sequencing (NGS) technologies, the availability of transcriptomic and genomic information has enabled the characterization of new molecular markers related to important agronomic traits. Today, most of these markers are based on single nucleotide polymorphism (SNP), which is the most abundant and universal form of polymorphism.

Considering that breeders need fast and reliable techniques, with relative low cost and basic equipment, KASP™ (Kompetitive Allele Specific Polymerase chain reaction (PCR)) has become a preferred technology to select advantageous alleles, as described in recent publications on tobacco breeding. For example, with the *yb1* and *yb2* loci characterizing Burley trait (Edwards et al, 2017) or the broomrape tolerance marker (Julio et al, 2020).

However, most of the markers used for tobacco breeding are associated with pathogen resistance, derived from old fashioned techniques such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) or sequence characterized amplified region (SCAR) (CORESTA Guide No. 16), are still in use today. Therefore, it is important to standardize and simplify the tools, and to convert these markers into KASP™.

SNP-based high density genetic maps, reference cultivar transcriptomes, or genome assembly are very useful resources to develop such markers. Different strategies for marker conversion will be presented, to obtain KASP™ markers associated with black shank (race 0), blue mould, potato virus Y (PVY) and other pathogen resistances.

AP 09

A novel NAC transcription factor, *NtNAC060* enhances the bacterial wilt resistance and salt stress tolerance in tobacco

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Bacterial wilt, as a bacterial disease, seriously impacts the growth of tobacco. The NAC transcription factors comprise one of the largest transcription factor families in plants, which play an important role in stress responses and bacterial disease resistance. Whereas little is known about tobacco NAC members in regulating the resistance to *Ralstonia solanacearum*.

In this study, the *NtNAC060* gene was firstly cloned, then bioinformatics analysis, subcellular localization, transcriptional activation and function analysis were performed.

Results: 1) The *NtNAC060* gene was cloned from tobacco genome and found to encode a typical NAC transcription factor; 2) The phylogenetic analysis revealed that *NtNAC060* was divided into the ATAF1 subgroup; 3) The expression pattern analysis showed that *NtNAC060* gene was expressed in tested tissues and induced by treatments of ABA, salt and pathogenic bacterium *Ralstonia solanacearum*; 4) The subcellular localization and transcriptional activation experiment convinced that the *NtNAC060* serves as a transcriptional activator; 5) The overexpression of *NtNAC060* gene could enhance the tolerance to both *Ralstonia solanacearum* and salt treatments in transgenic lines; 6) The expression levels of bacterial wilt progression related gene *NtPR10* and salt stress responsive genes *NtDREB1A* were significantly induced in the *NtNAC060* gene overexpression lines under pathogenic bacterium *Ralstonia solanacearum* and salt treatment, respectively; 7) The yeast-one hybrid assay indicated that *NtNAC060* may regulate these responsive genes by binding their promoters directly.

Considering these results, the overexpression of the stress inducible *NtNAC060* gene could enhance the bacterial wilt resistance and salt stress tolerance in tobacco by upregulating the stress related genes.

AP 10

Genetic analyses of a major partial disease resistance quantitative trait locus (QTL) in tobacco

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Partial resistance of tobacco to plant pathogens controlled by quantitative trait locus (QTL) is desirable in cultivar development programs because of its increased durability. However, mechanisms underlying this type of resistance are difficult to study. Favorable allelic variability at *Phn7.1*, a major disease resistance QTL, contributes to increased resistance to the soil-borne pathogens *Phytophthora nicotianae* and *Ralstonia solanacearum*, which cause the black shank and bacterial wilt diseases of tobacco, respectively. However, specific DNA sequence information underlying these favorable effects is currently unavailable. In this research, we will describe genetic and RNA/DNA sequencing experiments carried out on nearly isogenic lines that point to the role of a specific gene family on *N. tabacum* linkage group 7 that is likely involved with a high level of partial resistance to these pathogens in tobacco. Results provide insight on systems of polygenic disease resistance in plants and are of value to tobacco breeders working to improve soil-borne pathogen resistance.

AP 11

Agronomic performance of Polalta-derived breeding lines resistant to tomato spotted wilt virus

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Tomato spotted wilt virus (TSWV) is one of the most important pathogens threatening tobacco cultivation. The dark-cured cultivar Polalta is TSWV-resistant, but it cannot be easily incorporated into breeding programs, because its hybridization with other cultivars leads to morphological deformations in the hybrids. Nevertheless, few doubled haploids (DH) have been derived from hybrids of this cultivar at IUNG. These lines carry a *Nicotiana alata* introgression that is associated with TSWV resistance and located in the 0–40 cM region on linkage group 7 (LG7).

This study aimed to assess the phenotype and agronomic performance of advanced breeding lines that are F_4 populations derived from the DH lines and a flue-cured cultivar. The study plant material was derived from segregation of F_2 populations obtained from two DH lines. First, F_2 plants were screened with *N. alata*- and *N. tabacum*-specific markers designed for three loci located in the 0-40 cM region on LG7 to select plants homozygous for the *N. alata* introgression (Ala-Ala-Ala), F_2 plants without the introgression (Tob-Tob-Tob), and a recombinant with a partial introgression (Ala-Ala-Tob). Then, F_4 plants obtained from each of these three F_2 categories were subjected to assessment of morphology, deformation degree, and yield.

The phenotypes of the Ala-Ala-Ala and Tob-Tob-Tob F_4 populations were comparable, as all or nearly all plants had good or very good phenotype and no symptoms of deformation. The yield of the Ala-Ala-Ala F_4 populations was 2290–2522 kg/ha and significantly higher than that of the original DH lines (1686–1719 kg/ha). In contrast, half of the Ala-Ala-Tob F_4 plants had a bad morphology; almost all of them showed thickening and irregularity of the leaf veins, and their yield did not differ significantly from that of the DH lines. Such results suggest a detrimental effect of recombination in the genetic environment of a flue-cured cultivar.

AP 12

Study on inheritance of the number of leaves per stalk and dimensions of the leaves from the middle belt in tobacco varieties from different types and their F_1 hybrids

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Investigations were made with four parent tobacco varieties of different types (Prilep P-23, Prilep P 8-9/80, Floria FL-7 and Samsun S-1) in the role of mother, and MV-1 variety in the role of father, and their four F_1 hybrids for mode of inheritance for morphological properties of the leaves. The crossings were made in 2017, and the experiment with the parent genotypes and their hybrids was set up in 2018, in a field trial at the Scientific Tobacco Institute – Prilep, in a randomized block design with four replications. All appropriate cultural practices were applied during the growing season.

The aim of this work was to study the mode of inheritance of the number of leaves per stalk and the length, width and area of the leaves in the middle belt, in the offspring of the first generation.

The results of the studies indicate the fact that the obtained hybrids did not show a heterotic effect. Intermediate and partial dominance in inheritance is an indicator of good successive selection of individuals in future generations and rapid fixation and stabilization of the studied traits. All crosses (P-23 × MV-1, P 8-9/80 × MV-1, FL-7 × MV-1 and S-1 × MV-1) are rich and interesting selection material.

AP 13

The effect of liquid smoke from tobacco waste on tobacco collar rot (*Sclerotinia sclerotiorum*) in Northern Iran

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The *Sclerotinia sclerotiorum* Bary fungi causes collar rot in the tobacco seedlings in the seedbed. The aim of this study was to investigate the effect of liquid smoke on collar rot disease in the seedbed. This study was carried out based on a randomized complete block design with 13 treatments and 3 replications at the Tirtash Research and Education Center (in Northern Iran) in 2020. Treatments included liquid smoke obtained from tobacco stem, waste from the tobacco company warehouse, wood waste and commercial liquid smoke at three concentrations of 5 %, 10 % and 15 %. The control was a spray with water. The liquid smoke with the above concentrations was used in the seedbed after the two-leaf seedling stage, and applied every two weeks with a total of three foliar applications (spray). In the float system seedbed, every three trays of tobacco seedlings were performed as a replication with respect to the plot spacing. The seedling tray was seeded with totem cultivar. One month to forty days after seeding, inoculation was done on the seedlings' surface by transferring mycelium discs of the *S. sclerotiorum* fungus causing tobacco collar rot near the collar of seedlings. Evaluation of the experimental plot was continued 24 hours after spraying for plant burning, and 72 hours after inoculation by determining the level of infected spots for as long as the fungi was active in the control treatment. Data were analysed by MSTATC software and mean comparison was done by least significant difference (LSD). The results of variance analysis showed that the effect of liquid smoke on the fungus causing tobacco collar rot in the seedbed was significant at 1 % level. Liquid smoke of storage waste and tobacco stem treatments with 10 % concentration showed 73 % and 68 % control of tobacco collar rot disease in the seedbed and were introduced as the most effective treatments.

AP 14

The control effect and mechanism of green manure on soil-borne diseases of tobacco

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Tobacco soil-borne diseases have caused severe damage to tobacco production in Guizhou Province. The investigation of the effect of different varieties of green manure on suppressing the bacterial wilt pathogen (*Ralstonia solanacearum*, Rs) and improving soil microbial activity will provide new ideas and methods for bio-controlling soil-borne diseases. In this research, 15 green manure varieties suitable for planting in southwest tobacco areas were selected. Pot experiments and field trials were conducted to prove the inhibition effect of green manures on Rs and the black shank pathogen (*Phytophthora parasitica* var. *nicotianae*, Pn) in the soil. Ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS) technology was employed to identify and quantify the main substances in root exudates of green manures. Fluorescence quantitative polymerase chain reaction (PCR) was used to study the effect of root exudates on the population of Rs and Pn. High-throughput sequencing of soil microbes was applied to reveal soil microbial community structure and functional gene metabolic expression. (1) The population of Rs in the mung bean planted soil was only 31.26 % of that in the control treatment 80 days after planting, while the number of Pn in February orchid planted soil was only 36.16 % of that in the control treatment. (2) The inhibitory effect of mung bean root exudate on Rs was strongest, with an inhibitory rate of 69.05 %. The root exudates of February orchid had the most obvious inhibitory effect on Pn, with an inhibitory rate of 88.3 %. (3) The root exudates of four green manures were mainly organic acids, amino acids and carbohydrates. The content of amino acids and organic acids in the root exudates of February orchid was higher than any other green manures. The organic acid in the root exudates of February orchid contained 0.72 mg/kg of lactic acid, which was not detected in that of other green manures. The palmitic acid content of legume green manure, including mung bean and hairy vetch was 48.58 % higher on average than that of cruciferous green manure, including rape and February orchid. Besides, only arabinose was detected in the carbohydrates in the root exudates of green manure, with an average content of 2.32 mg/L. (4) After applying green manure in the field, the highest control efficacies of bacterial wilt and black shank were mung bean (55.61 %), and hairy vetch (68.69 %), respectively. (5) The abundance of Rs in the control rhizosphere soil was significantly higher than that in green manure-planted soil, which were 4.35, 4.14, 2.90 and 2.29 times of rape, hairy vetch, mung bean and February orchid, respectively. The abundance of a-Proteobacteria (*Alphaproteobacteria*), Myxobacteria (*Haliangium*) and Klebsiella were higher in mung bean treatment than other treatments; *Phytophthora* was not detected in any treatments, while the abundance of fungi such as *Fusarium* and *Chaetomium* was highest in the control among all the green manure treatments. (6) The soil functions, such as amino acid metabolism, transcription, replication and repair, were enhanced after treatment with green manures. In summary, root exudates of green manure greatly affect the population of soil-borne pathogens. After the application of green manure, the structure and function of soil microbial communities were changed. Mung beans and February orchids are suitable for biological

control of bacterial wilt and black shank. The research results provide a new way for greener prevention and control.

AP 15

In search of greener nematicides for use on tobacco: current status in Zimbabwe

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Increased environmental and animal health concerns from the use of synthetic nematicides have necessitated the development and use of more environmentally-friendly crop protection agents. The use of biological control using nematophagous fungi is one such method. To date several biological control agents including those based on *Trichoderma*, *Mycorrhiza* species have been developed and commercialized for management of plant parasitic nematodes. As the centre for testing and evaluating all crop protection agents targeted for use on tobacco, the Tobacco Research Board has over the years received numerous green products developed worldwide for testing and approval.

The standard protocol for testing is that the highly nematode susceptible K M10 tobacco variety is used. The variety is grown for a season with no nematicides being applied, to build up *M. javanica* nematode populations. In the second year, the K M10 crop to be used for the tests is established on the same land. A randomised complete block design is used. The test nematicide is evaluated against a registered standard. An untreated control plot is included to show the extent of infestation in the season.

Results of the trials have shown that some of these products are efficacious and do give some good control of nematodes while some have failed to work under the Zimbabwean conditions. The selected products have to be used in an integrated pest management (IPM) strategy combined with plant resistance and recommended cultural control methods. As a result of this testing Zimbabwe has several biological nematicides registered for use on tobacco. In this presentation details on the list of all the products tested (active ingredients only) and indications of how well these products performed and their field use status will be shown.

AP 16

Molecular characterization of *cry* genes in *Bacillus thuringiensis* native strains isolated from Northern Iran

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Bacillus thuringiensis (Bt) is a gram positive and spore forming bacterium that has *cry* genes capable of producing the diversified varieties of crystal proteins with insecticidal properties. 60 Bt strains were used in this study. These strains had been previously isolated by the authors from the soils of different tobacco fields in the northern provinces of Iran and characterized by their high toxicity against third instars larvae of tobacco budworm (*Helicoverpa armigera*). In this research, molecular characterization was based on polymerase chain reaction (PCR) analysis using 25 general and specific primers for *cry*1, *cry*2, and *cry*9 genes encoding proteins active against *Lepidoptera*, plasmid profiles, and protein band patterns. Isolates containing *cry*1 were the most abundant (90.1 %), followed by those containing *cry*2 (78.5 %) and *cry*9 (57.6 %). 32 distinct *cry*1-type profiles were identified from only *cry*1-harboring isolates when these were analysed with specific primers that showed high gene diversity of this gene in native strains. The results of *cry*1 gene profiles showed that *cry*1Ab (74.5 %) and *cry*1Ac (65.4 %) had the most frequency, while *cry*1I (18.7 %) and *cry*1F (16.5 %) had the lowest frequency. Several were found to be different from all previously published profiles. Finally, 9.12 % of the native strains did not produce any PCR product. Some native strains were positive by universal primers but negative by specific primers for all known genes of *cry*1, *cry*2 and *cry*9 or gave PCR products of different sizes when assayed with specific primers. These native strains may contain a new gene or genes that seem promising for biological control of insects and management of resistance.

The results of genetic characterization of native Bt strains are important for the selection of strains with high insecticidal toxicity. The results also confirm the presence in the Bt strains of toxic *cry* genes for the biocontrol of pests, including *Helicoverpa armigera*, in the region.

AP 17

Evaluation of novel biological control bacteria for management of angular leaf spot in dark tobacco

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Angular leaf spot (ALS) caused by *Pseudomonas syringae* pv. *tabaci* (Pst) is the most important foliar disease of dark tobacco in Kentucky. In addition to cultural practices such as crop rotation, sanitation, and removal of debris, the antibiotic streptomycin is the only product currently registered for ALS management. However, its widespread use can have severe consequences as resistant strains of the pathogen have already been reported. Due to the nature of bacterial diseases, targeted management practices are extremely limited, and the use of biocontrol agents has become a great option to be considered. The objectives of our experiments were to test 32 known beneficial bacterial strains for their direct effect on the pathogen when tested under laboratory conditions, and to further test those with any antibiosis effect in plant bioassays under greenhouse conditions. Six-week old susceptible KTD8 tobacco seedlings were treated with selected bacterial soil drenches 7-14 days prior to being challenged with foliar sprays of the Pst pathogen. Seedlings were evaluated 14 days later for ALS by using a 0-10 visual scale rating, and by estimating lesion counts in scanned leaves with the Fiji imaging software. *Bacillus cereus*/*proteolyticus* (AP-94) and *Bacillus safensis* (AP-110) were the only bacterial strains that inhibited the Pst pathogen growth when tested *in vitro* in the laboratory. Further greenhouse testing of AP-94, AP-110, as well as *Serratia marcescens* (AP-4) and *Bacillus altitudinus* (AP-281), resulted in some significant ALS disease reductions. One possibility is that these beneficial bacteria are activating the plant defences, in a mechanism called induced systemic resistance (ISR), as documented by many biocontrol researchers around the world since the 1980s. These preliminary findings could serve as the foundation for further testing under field conditions, and hopefully the eventual creation of a biological control product that could help tobacco farmers commonly affected by ALS.

AP 18

Pathogen identification and biological control of pole rot of flue-cured tobacco caused by *Rhizopus arrhizus* (syn. *R. oryzae*)

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The objective of this study was to identify the pathogen and biological control of pole rot on flue-cured tobacco, which causes damage losses during the yellowing stage of leaf curing in several counties of Dali Bai Autonomous Prefecture of Yunnan Province, China.

Infected tissues were used for isolating the causal agent classified by combining results from the morphology and multiple gene sequence analysis. Antagonistic activities of bacteria against the pathogen were also evaluated *in vitro* and *in vivo*.

Two fungi obtained from infected poles and leaves were grouped into the *Rhizopus* genus based on morphological characteristics. Furthermore, all isolates shared high internal transcribed spacer region (ITS) sequence similarity with *R. arrhizus* subspecies *arrhizus* CBS 112.07(T). Similar results were also obtained from multiple sequence alignment and phylogenetic analyses using the ITS, actin (*ACT*), translation elongation factor 1-α (*TEF*), and the largest subunit of RNA polymerase II (RPB1) of all isolates. Above all, the pathogen causing pole rot of flue-cured tobacco in Dali is *R. arrhizus*. Besides, five antagonistic bacteria, including WY11, WYZ, 05-1205, 4-4 and Z002, had robust inhibition activities against the hyphal growth of *R. arrhizus in vitro*. Among these bacteria, Z002 and 05-1205 were selected for biological control efficiency tests in the barn and showed strong evidence to support them as potential biological agents with relative control efficiency of 96 % and 81 %, respectively.

It was concluded that the causal agent of tobacco pole rot during flue-curing in Dali Bai Autonomous Prefecture of Yunnan is *R. arrhizus*. Two antagonists showed robust inhibition activities against *R. arrhizus in vitro* and *in vivo*, which provide promising agents for controlling tobacco pole rot with high efficiency and low toxic measures.

AP 19

A novel low alkaloid gene

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With possible future regulation of nicotine levels, scientists are searching for ways to lower nicotine in ways acceptable to the tobacco industry. The conventionally bred *nic1nic2* low alkaloid (LA) mutants lower nicotine to only about 10 % of the normal high alkaloid (HA) levels. We discovered a spontaneous low alkaloid mutation in a selection of the Burley breeding line L8, which reduced nicotine to 37 % of wild type sister lines. The objective of this preparatory work was to establish the nature of this mutation.

The study included a stable selection of this low alkaloid L8 line (L8-2), a sister line with normal alkaloid levels (L8-1) and the appropriate checks. Leaves of individual plants were sampled for molecular marker analysis, roots and leaves for alkaloid analysis, and roots for gene expression analysis. Molecular marker analysis revealed that both L8 lines were the same high-intermediate (HI) *AAbb* genotype. Nicotine + nornicotine (% DM) for L8-1 and L8-2, respectively, was 3.19 % and 0.87 % in the leaves, and 0.56 % and 0.59 % in the roots. Comparing the two lines, similar levels in the roots but different levels in the leaves suggest that the low alkaloids in L8-2 are a function of nicotine transport rather than synthesis. This is not the case when comparing LA and HA Burley 21 plants, which differ in both root and leaf alkaloid levels. Gene expression analysis also suggested the involvement of a nicotine transport mechanism behind the differential accumulation pattern of nicotine in the two L8 lines.

The mode of action of this novel gene appears to be different from that of the *nic1nic2* mutants, and it is probably not allelic to *nic1*. If so, stacking this gene with *nic1* and *nic2* might lower nicotine beyond the 10 % level of the LA lines.

AP 20

Functional characterization of *NIC1*-locus in regulating nicotine biosynthesis in tobacco and its applications in low nicotine variety development

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The regulation of nicotine biosynthesis in tobacco is mainly controlled by *NIC1* and *NIC2* loci in the tobacco genome. These loci are possibly originated from its ancestral diploids. Previously, a *N. tomentosiformis* originated ethylene response factor (*ERF*) gene cluster was identified as a *NIC2*-locus, which was demonstrated to positively regulate nicotine accumulation. A genomic scan identified novel *NIC2*-locus *ERF*-like homologs that are located at pseudochromosome 7 in the tobacco genome. However, whether this new *ERF* cluster is the *NIC1*-locus, its origination and its function in the regulation of nicotine biosynthesis have not been thoroughly investigated. In this paper, it is reported that the genetic mapping of the *NIC1*-locus proved that this new *ERF* cluster is "*NIC1*-locus", that this locus originated from *N. sylvestris* and that it physically clustered within the tobacco genome. It is specifically expressed in root tissues and co-expressed with *NIC2*-locus *ERF* genes and other nicotine biosynthetic genes and regulators during jasmonic acid (JA) induction. The *RNAi* suppression of *NtMYC2* in transgenic lines caused the down-regulation of most of these *NIC1*-locus *ERF* genes and reduced the expression of the *NtPMT* and *NtQPT*, respectively. Moreover, the expression patterns of both *NIC2*-locus *ERFs* and the *NIC1*-locus *ERFs* are not only highly correlated with each other, but also consistent with nicotine content in wild type tobacco and its low nicotine mutants. Interestingly, *in vitro* binding assays and transient expression assays demonstrate that the *NIC1*-locus *ERF* proteins are able to bind to the promoters of the *NtPMT2* and *NtQPT2*, respectively, and transactivate their expression at various levels. CRISPR-Cas9 based gene editing of *NtERF199* significantly reduced the nicotine contents by 50 % in transgenic tobacco. Our findings not only further our understanding of the transcriptional regulation of nicotine biosynthesis, but also provide gene resources which can be used as candidate targets for low nicotine variety development in tobacco breeding programmes.

AP 21

Impact of genotype and management on nicotine concentration in Burley tobacco

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Proposed standards from the US-FDA suggest that nicotine should be lowered to non-addictive concentrations in cigarettes (0.3 to 0.5 mg g^{-1}). One such method for lowering nicotine in finished products is to source leaf material with low concentrations of the compound. To date, research focused on lowering nicotine in Burley tobacco has not been conducted in North Carolina. The purpose of our study was to pair four Burley tobacco genotypes (HB4488PLC, TN90LC, ITB5101LA, and MSTN90LA) with two production programs (conventional production recommendations and low nicotine production recommendations). Cured leaf yield and quality were highest when the conventional production program was utilized (+476 kg ha^{-1} and +3 indices points, respectively). The main effect of genotype was also significant for yield (HB4488PLC > ITB5101LA = TN90LC = MSTN90LC) and quality (HB4488PLC = ITB5101LA = TN90LC > MSTN90LC). Nicotine concentration in composite cured leaf samples was influenced by the interaction of genotype and management program. Nicotine was highest when TN90LC and HB4488PLC were produced under conventional management programs (49.55 and 35.80 mg g^{-1}, respectively), and declined in low nicotine management programs (TN90LC = 11.76 mg g^{-1} and HB4488PLC = 10.69 mg g^{-1}). The management program did not affect the nicotine concentration measured in MSTN90LC or ITB5101LC (0.51 to 4.14 mg g^{-1}). Our preliminary results indicate that low nicotine management programs can reduce cured leaf nicotine concentration in genotypes with normal nicotine levels; however, these practices may not have an impact when paired with low nicotine genotypes. In addition, these practices are likely to reduce cured leaf yield and quality – which may compromise grower profitability and economic sustainability. Additional research will be conducted to further investigate these findings in future field seasons.

AP 22

The effect of nicotine content on cured leaf quality of Burley

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The low alkaloid (LA) varieties generally have smooth, slick darker green leaves that produce an undesirable cured leaf quality. This can be partially explained by the pleiotropic effect of the recessive nicotine alleles also affecting various stress responses. We did two field tests to determine the extent of the effect of the two nicotine alleles on the leaf morphology and cured leaf quality.

In one test, we grew 400 plants of the F_2 generation of a cross between the standard (HA) TN 90 and LA TN 90 in the field and characterised the nicotine gene combination of each plant with molecular markers. Three weeks after flowering, the leaf type of each of these plants was classified as being the normal "HA" type, or more characteristic of the LA. For the second test, shoots (scion) of 8 to 12 cm long seedlings of LA Burley 21 were grafted onto the roots (stock) of HA Burley 21, and vice versa. Ten replications of up to 10 plants per plot of each combination, as well as plots of ungrafted plants of each variety, and each variety grafted back onto itself, were grown in the field with normal production practices. A few days before harvest, the leaf type of the plants in each plot was classified as either the normal or the "LA" type. These plots were harvested, the grade index of the cured leaf was calculated, and the nicotine content analysed. In the first test, there was a demonstrable gradient of a decreasing proportion of plants with the "LA" leaf type as the proportion of the *Nic1*/*Nic2* alleles increased through the nine possible genotypes. In the second test, the leaf type and grade index were determined exclusively by the genotype of the root stock, regardless of the genotype of the scion.

AP 23

Effects of genotype and cultural practices on flue-cured tobacco growth, development, and chemistry

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The reintroduction of proposed nicotine standards by the US-FDA warrants further investigations of selected tobacco genotypes and management strategies that may reduce concentrations of the metabolite. To quantify these effects, research was conducted in 2020 at two locations in North Carolina. The genotypes NC196, K326, ITB697, and DS2847 were paired with two management programs: current recommendations (14,820 plants ha^{-1}, 84 kg N ha^{-1}, and topped at the early flower stage) and low nicotine recommendations (20,748 plant ha^{-1}, 62 kg N ha^{-1}, and not topped). ITB697 was excluded from this statistical analysis due to losses associated with *P. nicotianae*. Cured leaf yield, quality, price, and value were consistently lowest in plantings of DS2847 (-294 to -484 kg ha^{-1}, -13 to -14 indices points, -0.62 to -0.71 $US kg^{-1}, and -2,202 to -3,265 $US ha^{-1}), when compared to NC196 and K326, respectively. The management program also impacted yield and value, with current recommendations having a higher biomass accumulation (+1,095 kg) and economic value (+$US 3,453) than low nicotine recommendations. The interaction of variety and management practice was significant for cured leaf nicotine, anabasine, and anatabine concentration. Within each of these parameters, alkaloid concentrations were generally greatest in NC196 and K326 grown under current recommendations and lowest in DS2847, regardless of management program. Concentrations of each alkaloid in K326 and NC196 produced under low nicotine management programs were in-between. Nornicotine and reducing sugar concentrations were also highest in K326 and NC196 and lower in DS2847. Ultimately, low nicotine management strategies can be utilized to reduce cured leaf concentrations of alkaloid compounds in conventional genotypes; however, those practices will reduce cured leaf yield and value. In addition, it appears these practices are of limited use with low nicotine genotypes and may not further impact leaf chemistry. More concerning, the lowest nicotine concentrations in the present study were ten times greater than the proposed maximum of 0.3-0.5 mg g^{-1}.

AP 25

Climate change exacerbates the challenge of pests and diseases on tobacco in Zimbabwe

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Climate change characterized by the global increase in temperature and atmospheric CO_2 concentration is widely reported as the biggest threat to mankind. The changes in temperature and CO_2 levels witnessed have an effect on the growth and cultivation of crops as they directly affect the reproduction, spread and severity of plant pests and pathogens, thus posing a threat to food security. Zimbabwe has not been spared as in the last decade, the country has experienced debilitating episodes of drought and floods as a result of the El Nino and La Nina phenomena, characterized by erratic rainfall distribution and extreme weather. The episodes of drought and the occasional excess moisture conditions resulted in significant shifts in pest and disease incidences and prevalence on many agricultural crops including tobacco. For example, there has been a resurgence of some pests and diseases, such as potato virus Y, tobacco etch virus, black shank, fusarium wilt, root-knot nematodes and the tobacco laceworm (*Spodoptera littoralis*) among others, previously considered not economically important on tobacco. Additionally, there has been an emergence of new pests and disease problems on tobacco. In the last two seasons, incidence of pests such as the cotton mealy bug *Phenacoccus solenopsis* Tinsley and stem borers have been on the increase. This is a huge challenge especially coming at a time when many effective crop protection agents that had a broad spectrum of activity, have been phased-out to enhance sustainability in tobacco production. In this presentation an outline of some of the pest and disease challenges that have emerged in the last decade and the management strategies being developed will be given.

AP 26

Effects of wheat straw residue and its biochar on the physical properties and enzyme activities of tobacco-growing soil

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The degradation of Shandong tobacco-planting soil quality, such as soil compaction and microbial activity reduction, has become more and more serious due to the lack of exogenous organic matter input. Therefore, aiming to improve the soil quality and provide the theoretical support of wheat straw reuse, a two-year field experiment (2016-2017) was performed to study the effect of wheat straw and its biochar on the characteristics of soil physics such as bulk density, field water holding capacity and aggregate stability, as well as soil enzyme activities. Four treatments were set up as follows: chemical fertilizer only (CK), 6.75 t hm^{-2} wheat straw plus chemical fertilizer (WS), 2.25 t hm^{-2} (FB1) and 4.50 t hm^{-2} (FB2) wheat straw-derived biochar plus chemical fertilizer. After two years, the results showed that the WS treatment significantly decreased the soil bulk density (by 14 %) and the fractal dimension (D, by 5.5 %) than that of CK at the 0-20 cm soil layer. In contrast, the content of large aggregate ($R_{0.25}$, by 16.53 %), the mean weight diameter (MWD, by 42.53 %) as well as the activities of sucrase, urease and phosphatase (by 112.52 %, 7.81 % and 35.18 %, respectively) were significantly increased ($P < 0.05$). Compared with CK, the FB1 and FB2 treatments significantly increased MWD (by 27.76 % and 37.5 %, respectively, $P < 0.05$), however, the soil enzyme activities were changed non-significantly ($P > 0.05$). Correlation analysis showed that there exists a significant positive correlation between activity of sucrase and MWD and a significant negative correlation between activity of sucrase and D, BD, as well as between activity of phosphatase and D. Overall, compared to biochar, wheat straw is more beneficial to improve soil structure and enzyme activity.

AP 27

Soil type regulates carbon and nitrogen stoichiometry and mineralization and bacteria following biochar or nitrogen addition

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Most studies on the effects of biochar and nitrogen fertilizer on soil carbon (C) and nitrogen (N) mineralization, and microbial C and N content, are restricted to a single tobacco-growing soil type, limiting our understanding of the interactions between these factors and microbial functions. At the same time, the application of biochar in improving tobacco-growing soil and promoting the sustainable development of tobacco requires more extensive soil research. To address this paucity in knowledge, we undertook a three-year field research study using four contrasting tobacco-growing soil types (Ferralsol, Acrisol, Fluvisol, Phaeozem) to assess the role of peanut shell biochar (only biochar) and nitrogen fertilizer (only nitrogen fertilizer) on C and N mineralization, microbial C and N, N stoichiometry and bacteria. Across all four soil types, biochar significantly ($P < 0.05$) increased soil carbon mineralization (Cmin) and nitrogen mineralization (Nmin) over three years compared to nitrogen fertilizer and control. Biochar also increased total C (Csoil) across the four soil types in Year 1, with the Fluvisol type recording the greatest total C in Year 2 and Phaeozem having greatest total C in Year 3. Across all four soil types, biochar resulted in a higher microbial biomass C (Cmic), total N (Nsoil) and microbial biomass N (Nmic); the degree of change was closely related to Csoil and Nsoil. Csoil and Cmic increased following amendment with biochar, which reduced the soil C and N stoichiometric imbalance ($\mathrm{Cimb:\ Nimb} = \frac{\mathrm{Csoil:\ Nsoil}}{\mathrm{Cmic:\ Nmic}}$) and the effect of biochar was most obvious on Fluvisol soil type. However, across all four soil types, nitrogen fertilizer exacerbated the imbalance of soil C and N stoichiometry compared to the control, and this effect was most obvious in Phaeozem. Nitrogen fertilizer also reduced the Csoil:Nsoil and Cmic:Nmic ratio. Biochar reduced the soil bacterial diversity in Ferralsol, Acrisol and Fluvisol, but enhanced bacterial diversity in Phaeozem. It was concluded that biochar reduces the imbalance of soil C and N stoichiometry and had a positive effect on soil carbon and nitrogen metabolism. Biochar improved the tobacco-growing soil by adjusting the soil C, N and microorganisms, which is essential to the sustainable development of tobacco production.

AP 28

Evaluation of the effect of vermicompost on tobacco plant growth, yield and leaf quality

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Vermicompost is a product of compositing organic matter using earthworms and microorganisms to produce a biofertilizer. The resultant compost contains humus, various micro-organisms, macro and micronutrients and has the ability to improve soil properties structurally, nutritionally and biologically. In addition, the compost is reported to increase infiltration and permeability of heavy soils, thus reducing erosion and runoff and improving water holding capacity. Trials to evaluate the efficacy of vermicompost for tobacco production were conducted at Kutsaga Research Station for four seasons from 2017-2020. The main objective of the trials was to evaluate the effect of vermicompost on tobacco growth and yield. A completely randomized design (CRD) with six treatments and replicated three times was used. The treatments used were standard fertilizer (based on soil test), standard fertilizer + 50 % original vermicompost, 25 % standard fertilizer + 75 % original vermicompost, 50 % standard fertilizer + 50 % improved vermicompost, 25 % standard fertilizer + 75 % improved vermicompost, 25 % standard fertilizer + 75 % improved vermicompost + earthworm manure and 100 % vermicompost were used. Results showed that the use of vermicompost on its own gave significantly lower leaf geomean, yields, root and shoot drymass and quality than the standard fertilizer and other vermicompost combinations. The 50 % standard fertiliser in combination with 50 % vermicompost treatment consistently gave good yields that were comparable to the standard. Addition of 50 % vermicompost to standard fertilizer had no extra benefits in yield and quality of tobacco and, therefore, may not be economic in tobacco production. All combinations of the product and standard fertiliser tested gave comparable yields to the recommended fertilizer treatment. Based on leaf geomean, yield and quality results for the four seasons of testing, it is recommended that vermicompost be used in combination with 50 % standard fertiliser as the product has the additional benefits of improving soil organic matter, aeration, nutrient uptake and water holding capacity.

AP 29

Evaluation of a biological suckercide, pelargonic acid, for the control of suckers in tobacco production in Zimbabwe

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Topping and sucker control is an essential exercise in tobacco production as it enables the plant to deploy resources towards maximum leaf development, leading to higher leaf yields. In this experiment, a new organic suckercide, perlagonic acid, was evaluated for efficacy in axillary shoot control in tobacco production. Synthetic environmentally toxic suckercides are no longer acceptable for use in tobacco production and are gradually being phased out. Pelargonic acid is a fatty acid naturally occurring in many plants and animals, and is present in many foods, making it a safer alternative. Field trials were conducted at the Kutsaga Research Station for three seasons from 2017-2020. In all the trials, the following rates of pelargonic acid: 0.25 %, 0.5 %, 0.75 %, 1 %, 1.5 % and 2 % were evaluated in a randomized complete block design. Applications were done at topping, either as a sole application or in combination with a systemic suckercide, flumetralin 150 EC. The parameters measured included sucker counts per plant, sucker dry mass and phytotoxicity assessments per plant. Results from the first season indicated that sole pelargonic acid applications and at high concentrations above 1 %, were effective in controlling axillary shoots but resulted in severe phytotoxicity on tobacco leading to leaf drop as concentration increased. To manage this problem, pelargonic acid was used in combination with a systemic suckercide in the following seasons. The biological suckercide at concentrations ranging from 0.25 %-1.5 % were combined with flumetralin 150 EC at a rate of 1.35 litres/100 litres of water. Results showed that the combination of pelargonic acid at 0.75 % and 1 % with flumetralin 150 EC, significantly controlled tobacco axillary shoots. No phytotoxicity was observed. Pelargonic acid in combination with flumetralin is now recommended for use in tobacco production in Zimbabwe. This presentation will give details of the trial and discuss the implications of these results.

AP 30

Investigation of compressed charcoal production from charcoal pyrolysis of Virginia tobacco stalk

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The aim of this study was the production of compressed charcoal from the pyrolysis of Virginia tobacco stalks. This compressed charcoal has physicochemical properties close to or superior to charcoal from wood. After obtaining charcoal dust from tobacco stalks due to the pyrolysis process, filler and additives were added to increase the burning rate and particle binder. The mixture was then pressed and dried. In this project, wheat starch, corn and tapioca with 5 %, 10 % and 15 % contribution were used to synthesize charcoal dust particles. After completing the compressed charcoal production process, the physicochemical properties of the compressed charcoal were compared with the compressed charcoal available on the market. The results of variance analysis showed that the effect of treatment on moisture content, volatile matter content, ash content, fixed carbon, calorific value, number of hits, ignition duration and burning time factors was significant at the 1 % probability level. Also, the results showed that the calorific value of the compressed charcoal, which is an important property to consider in the study, is almost twice as much as that of the compressed charcoal available on the market.

AP 31

High quality genome assembly of *Nicotiana tabacum*

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Nicotiana tabacum, or cultivated tobacco, is an important plant model whose genome has been explored recently. However, the previous tobacco assemblies based on short reads sequencing showed some flaws. Thanks to new sequencing technologies, the genome assembly quality and its completeness was improved. Using 10X Genomics linked read and Hi-C data, we first produced a 4.2 Gb draft genome assembly (N_{50} = 61Mb, L_{50}=19).

By anchoring the genetic maps, we generated a 3,04 Gb chromosome level genome assembly. In total, 244 scaffolds were anchored into 24 pseudomolecules with single nucleotide polymorphism (SNPs) and simple-sequence repeats (SSRs) genetic markers. The completeness of the genome assembly has been checked by Benchmarking Universal Single-Copy Orthologs genes analysis (BUSCO). The current genome assembly showed the highest BUSCO score of 93.5 % compared to the published tobacco genomes. Previous studies have shown that tobacco descends from two ancestral diploids plants: *Nicotiana sylvestris* and *Nicotiana tomentosiformis*. By proceeding to the mapping of short reads generated from the genomes of these two presumed ancestors on the tobacco pseudomolecules, we were able to pinpoint which tobacco genome region corresponds to which ancestor allowing to gain insight into the evolutionary history of the current tobacco genome. The release of this chromosome-scale genome will provide an important resource for further studies at the fundamental and applied research level.

AP 32

Understanding the response of tobacco plants to root-knot nematode infection by means of chlorophyll fluorescence imaging

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The use of chlorophyll fluorescence imaging provides a non-invasive, non-destructive method with which to measure changes in photosynthetic metabolism in plants infected by pathogens. This technique can be enhanced to better understand the host physiology by manipulating the atmosphere around the leaf to enable synchronized measurements of gas exchange. The main objective of this experiment was to use fluorescence and gaseous exchange to track the physiological response of tobacco plants infected by the root-knot nematode (RKN). Three tobacco varieties which included an RKN resistant variety, K RK64, tolerant K RK66 and susceptible KM10 were used in this experiment. Plants were grown in 2 litre pots under greenhouse conditions and then inoculated with RKN populations of 0, 10 000 and 20 000 eggs per pot. Gaseous exchange (stomatal conductance, ETR, assimilation, transpiration rates, CO_2 exchange) and chlorophyll fluorescence (NPQ, Fv/Fm) were measured using the LI6800 portable photosynthesis machine at 7, 14, 21, 35 and 49 days after inoculation. Results showed that when under attack, resistant varieties significantly increased their photosynthetic rates. Furthermore, the assimilation rates (A), transpiration rates (E), intercellular CO_2(C_i) rates, stomatal conductance to water and CO_2 also showed significantly different responses to susceptible treatments ($P < 0.05$). Results obtained further show that nematode attack affects chlorophyll fluorescence, particularly the effect on Electron Transport rates, Net Photosynthetic Quenching (NPQ) and the quantum efficiency of Photo System 2. This presentation will detail how the photosynthetic apparatus of the three varieties responded to *Meloidogyne javanica* infection and how future plant screening for root knot nematode resistance can be achieved more efficiently through gaseous exchange and chlorophyll fluorescence measurements.

AP 33

Identification and functional characterization of the pale yellow gene in tobacco

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The pale yellow (PY) trait in tobacco (*Nicotiana tabacum* L.) was first described in 1969 and was found to be controlled by a single dominant gene. Plants containing a PY gene show accelerated chlorophyll breakdown compared to wild type plants. In commercial tobacco varieties, the accelerated chlorophyll breakdown trait mediated by the PY locus could help growers to better manage the crop and minimize losses due to unfavorable weather conditions and diseases. However, the PY locus and its associated PY gene are still uncharacterized, so that the inclusion of the PY locus in a breeding program results in a time-consuming and subjective selection process. In a previous study, we mapped the PY locus on the tobacco genome and identified single nucleotide polymorphism (SNP) markers to enable accelerated breeding of the trait. We generated and analyzed a mapping population (F_2 generation resulting from a cross between dark tobacco variety Narrow Leaf Madole LC [non-PY-variety] and TI1372 [source of the PY locus]) and found a quantitative trait locus (QTL) explaining 75 % of variance in the PY trait. Within this QTL region, putative candidate genes were identified by RNASeq differential gene expression analysis and confirmed with quantitative real time polymerase chain reaction (PCR). In the present study, we analyzed two candidate genes in more detail by knockdown (RNAi) and overexpression studies and could validate one gene as responsible for the phenotype. While overexpression lines showed delayed chlorophyll breakdown, RNAi lines had an accelerated chlorophyll breakdown phenotype with a lower leaf chlorophyll content and increased expression levels of leaf senescence-related genes. Our study demonstrates the utility of a high-density SNP array for mapping of traits and, most importantly, provides a key target gene to enable more precise and faster breeding of the PY trait in commercial tobacco cultivars.

AP 34

Dynamics of molecular leaf senescence processes occurring during curing of Virginia tobacco cultivar K326

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Plant senescence is the process of aging. It is influenced primarily by genetics and developmental processes as well as environmental cues. However, because of their significant complexity, the molecular networks by which genetics and the environment control senescence remain elusive.

It is fundamental to understand the molecular mechanisms that define leaf senescence during leaf ripening and curing, which are important for the quality of tobacco leaves.

Here, we examine the senescence process by using a combination of transcriptomics, proteomics, and metabolomics approaches. Samples were collected over time and at different leaf positions during curing of K326 Virginia tobacco.

The integration of different techniques allowed us to resolve the spatiotemporal dynamics of the molecular networks responsible for the modifications that occur during curing. The fundamental knowledge resulting from this work will help create a framework from which new strategies can be implemented for improving the quality traits of future tobacco products.

AP 35

Two-year evaluation of agronomic practices on standard and low-nicotine tobacco cultivars by non-destructive photonic sensing

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In the last few years, the interest in low nicotine cultivars and agronomic practices that could reduce nicotine accumulation in tobacco leaf has increased with the aim to reduce nicotine content in cigarettes and consequently overall smoking addiction. This study aimed to apply a non-destructive photonic sensing method to the proximal detection of the plant nitrogen (N) status at the stage of maximal plant N assimilation, to find any correlation with leaf nicotine content (%) at harvest.

For two consecutive years a varietal test was carried out at the Fattoria Autonoma Tabacchi (FAT - Città di Castello [PG], Italy), comparing standard and new low-nicotine cultivars of Virginia Bright tobacco managed with local best practices (standard N fertilization and topping) and with low-nicotine agronomic practices (reduced N and no topping). The fluorescence sensor provided indices of leaf chlorophyll (CHL), flavonols (FLAV) and nitrogen (Nitrogen Balance Index, NBI = CHL/FLAV), allowing non-destructive comparisons among agronomic practices. The upper sun-exposed side of a single leaf for each plant was measured in the field by a portable fluorescence sensor at the stage of the maximal plant N assimilation, and before harvest.

Significant differences were found among the differently managed varieties for leaf CHL, FLAV and NBI. The in-field NBI index at maximal plant N assimilation was found to be correlated to the leaf nicotine content (%) destructively measured at harvest.

The study indicates the usefulness of integrating a photonic sensing technology in tobacco cultivation to provide additional information in the identification of new appreciable low-nicotine varieties and in the evaluation of the impact of agronomical practices on the resulting leaf nicotine content.

AP 36

Classification of Virginia Bright tobacco varieties by non-destructive photonic sensing of leaf flavonoids

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Tobacco leaf is rich in polyphenols, which affect its colour and quality. Among the polyphenols, the flavonoid compounds play an important role in the stress resistance of the plant against UV radiation, fungal pathogens, herbivores, oxidative cell injury and they have potential for tobacco mosaic virus inhibition (anti-TMV activity). Flavonoids can also be considered as potential biopharmaceuticals for their high medicinal value.

This study aimed to compare the epidermal flavonoids content of different Virginia Bright varieties by a non-destructive photonic sensing method.

A comparative test was carried out at the Fattoria Autonoma Tabacchi (FAT - Città di Castello [PG], Italy), for three consecutive years monitoring different varieties of Virginia Bright tobacco, from the companies ProfiGen and Bergerac Seed and Breeding (BSB), grown under the same conditions each year. A portable fluorescence sensor provided an index of flavonols (FLAV), allowing rapid, representative and non-destructive comparison among the varieties. The upper sun-exposed side of a sub-apical single leaf for each plant was measured in the field two months after transplant.

Every year, significant differences were found among the varieties compared. FLAV index of most ProfiGen cultivars showed results higher than BSB cultivars. Cluster analysis reported the main and relevant differences between the examined varieties.

The study showed the usefulness of integrating a photonic sensing technology that provides additional information for the selection and characterization of tobacco varieties.

AP 37

Estimating the effective control of Ditacin 8SL (Ningnanmycin) and Sat 4SL (Cytosinpeptidemycin) with tobacco mosaic virus (TMV), cucumber mosaic virus (CMV), and potato virus Y (PVY) on tobacco plants

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In recent years, viral diseases have been developing strongly and causing significant damage to the yield and quality of tobacco in Vietnam. TMV, CMV, and PVY are the most common diseases in the tobacco field. The determination of biological antivirus agents plays an important role in the management of viral diseases in tobacco plants.

To control some viral diseases, the Vietnam Tobacco Institute applied two biological antivirus agents, Ditacin 8SL (Ningnanmycin) and Sat 4SL (Cytosinpeptidemycin) that are isolated from *Streptomyces noursei* var. *xichangenis* and *Streptomyces achygroscopicus* var. *liaoningensis*. The experiment was performed in the net-house and under field conditions in Bac Giang Province, Vietnam, in 2020. The field experiment was arranged in a randomized complete block design with three replications. The results showed that Ditacin 8SL and Sat 4SL were effective in controlling some viral diseases of TMV, CMV, and PVY on tobacco. In the net-house, treating with Ditacin 8SL three to six times before plant infection resulted in effective control of PVY, TMV, and CMV in the range of 24-60 %; treatment with Sat 4SL showed 20,0-35,6 % effectiveness after 21 days of inoculation. Under field conditions, the effective control with Ditacin 8SL was 100 % for TMV; PVY: 93,5 %; CMV: 60,5 % and tomato necrotic ringspot virus (TNRV): 52,1 %. The effective control with Sat 4SL reached 70,9 % for TMV; CMV: 58,3 %; PVY: 96,4 % and TNRV: 36,9 %. Spraying Ditacin 8SL and Sat 4SL at the stage from transplanting (3-4 leaves/plant) up to the 16-18 leaves/plant stage showed the best control of viral diseases, increased the yield and grade index, and had less impact on the smoke properties.

Ditacin 8SL and Sat 4SL were effective in controlling TMV, CMV, PVY, and TNRV on tobacco plants. To manage the virus diseases CMV, PVY, and TNRV, a mixture of virucide, insecticide, and foliar fertilizer should be sprayed. For TMV, only the virucide and foliar fertilizer should be used. All diseased plants in the field need to be destroyed.

AP 38

Study of the interactions of root-knot nematode (*Meloidogyne incognita*) and tobacco black shank (*Phytophthora nicotianae*) on some tobacco cultivars under natural field pollution conditions

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The most important soilborne pathogens of tobacco include soilborne pathogenic fungi and root-knot nematodes that are scattered all over the world and cause great economic damage to tobacco products.

The most effective way to manage them is to use resistant cultivars. The purpose of this research was to study the interaction of some air-cured tobacco cultivars with *Phytophthora nicotianae* and tobacco root-knot nematode (*Meloidogyne incognita*) in order to introduce a superior cultivar.

The experiments were performed in a randomized complete block design (RCBD) with paired plots with five treatments and three replications, including tobacco cultivars Burley Geel3, K17, BCE, BB16A and Burley 21 as a check (control) under natural field pollution conditions. Field evaluation of tobacco black shank (*P. nicotianae*) infestation was performed on a weekly basis according to the method of Van-Jarsold et al, using indexes 1 to 5. Evaluation of the resistance of cultivars to root-knot nematodes was performed based on gall index, number of egg masses and average eggs per mass at the end of the growing season based on scoring 0 to 10. Important morphological, agronomic, functional and qualitative traits were also measured in the field. The results showed that the interactions of *M. incognita* and *P. nicotianae* were synergistic, so that the simultaneous presence of nematode and fungus had a significant increase in the degree of symptoms and root necrosis.

The results showed that in terms of all agronomic traits, yield and disease evaluation indicators, the BCE cultivar was resistant to black shank and root-knot nematode. The cultivars K17 and BB16A were in the semi-resistant group. Also, Burley Geel3 and Burley 21 cultivars were very sensitive to tobacco black shank and root-knot nematode.

AP 39

Risk assessment of *Ralstonia solanacearum* to fluazinam and evaluation of combinatory fungicides

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Tobacco bacterial wilt is a destructive soil-borne vascular disease in tobacco production, but the varieties of effective control bactericides are limited at present. Therefore, it is particularly important to explore fungicides with a unique mechanism and excellent control effect. In this study, the sensitivity of tobacco bacterial wilt to five fungicides was determined, the resistance risk of tobacco bacterial wilt to fluazinam was evaluated, and the synergistic toxicities of fluazinam and SYP-14288 with five fungicides (nano-sulfur, nano-copper, nano-silver, benziothiazolinone and kasugamycin) were evaluated by the Wadley method. The results showed that the average EC_{50} values of fluazinam, SYP-14288, streptomycin, benziothiazolinone and kasugamycin were 1.0840, 0.0378, 0.5463, 2.4299, 9.9273 µg/mL, respectively. The sensitivity baselines of the five fungicides to 40 strains of tobacco bacterial wilt were of normal distribution and presented continuous single peak curves, which could be used for monitoring the resistant strains in fields. The 15 mutants with different resistance levels had strong temperature adaptability and weak pathogenicity. The results of comprehensive evaluation showed that the resistance risk of tobacco bacterial wilt to fluazinam was moderate. The mixtures of fluazinam with benziothiazolinone, kasugamycin, nano-sulfur at the ratio of 1:80, 1:80, 1:40, respectively, had synergistic effects. The synergistic effect of SYP-14288 and nano-sulfur mixture at the ratio of 1:40 was the most obvious. This study provides more alternative fungicides for tobacco bacterial wilt control and a reference for a resistance management strategy.

AP 40

Differential susceptibility to angular leaf spot (*Pseudomonas syringae* pv. *tabaci*) in dark tobacco varieties

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Angular leaf spot (ALS) is a bacterial disease caused by *Pseudomonas syringae* pv. *tabaci*. Since 2015, ALS has become the most prevalent and damaging foliar disease of dark tobacco. The objective of this study was to evaluate common dark tobacco varieties grown in western Kentucky and Tennessee for susceptibility to ALS. Field trials were conducted in 2019 and 2020 at the Highland Rim Research and Education Center in Springfield, TN, and at the University of Kentucky Research and Education Center in Princeton, Kentucky, in 2020. All varieties in all trials were inoculated with a streptomycin-sensitive isolate of *Pseudomonas syringae* pv. *tabaci* at approximately five weeks after transplanting and then evaluated every 7 to 14 days after inoculation for symptoms of ALS. Dark tobacco varieties evaluated in the trials included NL Madole, PD 7309, DT 538, KY 171, Little Crittenden, TR Madole, PD 7312, Shirey, DT 558, PD 7305, KT D8, KT D6, KT D14, KT D17, VA 309, TN D950, and the experimental variety DFH 1404. Across the three trials, plot evaluation data consistently showed less susceptibility to ALS infection in PD 7309 and DT 538, while the varieties KT D8 and KT D17 appeared to be most susceptible. Lower susceptibility to ALS appears to be related to varieties that are later maturing and have better holdability before harvest, while varieties that are more susceptible tended to be earlier maturing with lesser holdability before harvest.

AP 41

Evaluation of spray programs for control on angular leaf spot (*Pseudomonas syringae* pv. *tabaci*) in dark tobacco production

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Angular leaf spot (*Pseudomonas syringae* pv. *tabaci*) has become the most prevalent and problematic foliar disease in dark tobacco since 2015. Field trials focusing on angular leaf spot control began in 2015 and have been ongoing each year at the University of Kentucky Research and Education Center in Princeton, Kentucky and at Murray State University in Murray, Kentucky. These field trials have been established to evaluate direct and plant-mediated inhibitory effects of fifteen different antibiotic, biocontrol and/or synthetic bactericide products. Products tested are either registered or have the potential to be registered for dark tobacco. Streptomycin is an antibiotic bactericide and the standard control used by dark tobacco producers to combat this bacterial disease. Since 2016, resistance assays have shown that 24 out of 101 plant tissue samples from angular leaf spot outbreaks on private farms have shown resistance to streptomycin. A monitoring project was established in 2020 to observe how different environments and management practices used by dark tobacco growers may influence angular leaf spot incidence and severity. Seventeen variables are being investigated in this monitoring study from greenhouse management to harvest. Out of 30 fields monitored in 2020, four fields presented with angular leaf spot symptoms. Tissues samples were taken from these four fields and screened to confirm the presence of *P. syringae* pv. *tabaci*. Bacterial isolates from these samples were then screened for resistance to streptomycin and copper oxide. One of the four samples screened presented with resistance to streptomycin.

AP 42

Effective light device for trapping tobacco moth (*Ephestia elutella*)

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Many nocturnal insects are attracted by light. Light traps, generally equipped with black light fluorescent tubes (BL), have been used in manufacturing facilities and warehouses to catch such insects. The tobacco moth (*Ephestia elutella*) is one such species that exhibits a phototactic behaviour. Earlier laboratory studies have demonstrated that adults of this species are attracted by ultraviolet-light-emitting diodes (UV-LED). However, tobacco moths are seldom caught in commercial light traps equipped with BL. This study was conducted to investigate reasons why commercial light traps cannot catch tobacco moths and to provide bases for trap device improvement. The predominant BL and UV-LED emitted wavelengths are almost identical, but the directivities of light emitted from the respective devices differ greatly. First, commercial light traps were used for release-capture tests conducted in a chamber to confirm UV-LED and BL effects as trap lures. To evaluate the LED attractiveness, existing light sources were replaced by LED devices. The irradiant LEDs were then adjusted to five levels of intensity. Moth behaviours in scotophase under light exposure were observed using video recordings. Adults in a petri-dish were irradiated with UV light emitted from an LED device for 10 seconds. Results showed that tobacco moths were captured at 0.01 W/m^2 intensity, but the number of moths caught decreased at higher irradiance levels. When UV light at intensity of 0.1 W/m^2 was irradiated to active moths for 10 seconds under dark conditions, the locomotive or flight activities ceased within 15 seconds. These results suggest that UV light induces attraction and interferes with moth locomotion, depending on the irradiance. When irradiance on the prospective flight paths to the sticky surface of a trap is kept at approximately 0.01 W/m^2, tobacco moths were likely to be caught by a light trap.

AP 43

Cigar wrapper response to nitrogen fertilizer rates in western North Carolina

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Cigar wrapper tobacco is a new and emerging cash crop in western North Carolina. To develop nitrogen (N) fertilizer recommendations, field trials were initiated in 2020 to quantify the impact of N application rate to cigar wrapper tobacco yield, cured leaf grade distribution, and cured leaf chemistry. Connecticut Broadleaf and Pennsylvania Seedleaf (PA41) tobacco types were evaluated in our study. Cured leaf yield for Connecticut Broadleaf (2,037 kg ha^{-1}) and PA41 (2,340 kg ha^{-1}) was maximized at 159 and 191 kg N ha^{-1}, respectively. Wrapper (7 %) and binder (31 %) grades as well as total alkaloid concentration in cured leaves (3.91 %) increased linearly with N application in PA41 varieties and were maximized at 224 kg N ha^{-1}. In contrast, wrapper (5 %) and binder (23 %) grades were maximized between 173 and 181 kg N ha^{-1} in Connecticut Broadleaf varieties. Straight strip and filler grades likewise declined as N rate increased in both cigar wrapper types. Total alkaloid concentration was highest (3.78 %) when 148 kg N ha^{-1} was applied to Connecticut Broadleaf varieties. Our results indicate that PA41 has a higher yield potential than Connecticut Broadleaf and should be fertilized with a greater quantity of N for these yields to be achieved. Our results also suggest that N application may need to be higher than what is required for maximized yield in order to increase wrapper and binder grades. Additional site years and N application rates should be further tested in these environments in order to further refine these recommendations.

AP 44

Development of production recommendations for Connecticut Broadleaf cigar wrapper tobacco in Kentucky and Tennessee

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Leaf dealers have recently expressed interest in expanding production of Connecticut Broadleaf cigar wrapper tobacco into Kentucky and Tennessee. In an effort to develop production recommendations for Connecticut Broadleaf grown in Kentucky and Tennessee, basic agronomic field trials were conducted at the University of Kentucky Research and Education Center in Princeton, Kentucky, in 2019, 2020, and 2021. Trials included a nitrogen rate trial, a variety trial, and a fungicide trial in 2019-2020, and a larger fungicide trial and lower leaf removal/topping height trial in 2021. Nitrogen rates of 84, 112, 140, 168, 196, 224, and 252 kg N Ha^{-1} were evaluated in 2019 and 2020. Nitrogen source was ammonium nitrate with 84 kg N Ha^{-1} applied broadcast prior to transplanting and the remainder applied as sidedress applications at 2 weeks after transplanting. Highest percent wrapper grades were generally found from nitrogen rates of 168 to 196 kg N Ha^{-1}. In the variety trial, varieties evaluated included 'A1', 'B1', 'B2', 'D1', 'D2' from the breeding efforts of the Connecticut Valley Experiment Station, compared to two selections of the commercial standard 'C33'. Highest total yield and percent wrapper was seen from 'B2', 'D2', and the selections of 'C33'. In a fungicide trial, treatment comparisons included no foliar fungicides applied, azoxystrobin (144 g ai Ha^{-1}) applied at layby (4 weeks after transplanting), or azoxystrobin at layby followed by mancozeb (1.68 kg ai Ha^{-1}) 10 days later followed by azoxystrobin at topping. Total yield and percent wrapper increased as the number of fungicide applications increased. Treatments that included the most frequent fungicide applications reduced, but did not totally eliminate, the occurrence of the "greenspot" phase of frogeye leafspot (*Cercosporea nicotianae*) in cured leaf of Connecticut Broadleaf.

AP 45

Evaluation of advanced experimental hybrids of cigar wrapper tobacco for yield and quality in Zimbabwe

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Cigar wrapper production is suited in niche environments in Zimbabwe where it may provide a viable and lucrative alternative to other crops grown. Zimbabwe has an impeccable record of producing flavour styles of tobacco and the potential to produce quality wrappers exists if fundamental gaps in varietal selection, production, cultural and agronomic practices are addressed. The overall objective of the study was to assess the field performance of test cigar wrapper varieties by measuring several agronomic traits that include days to topping, leaf measurements, saleable yield, grading, pricing and smoke quality assessments from an organoleptic panel. Eighteen experimental test hybrids (ETH), nine each from exotic collections and advanced hybrids recently bred at the Tobacco Research Board were evaluated in full agronomic trials using a randomized complete block design (RCBD) at two sites (Burma Valley and Banket Research Station) for two seasons (2018-2020). Multivariate analysis of variance was done using GenStat 17th edition. Preliminary results show significant differences ($p < 0.05$) among ETH for days to topping and yield but no significant differences in largest leaf and penultimate leaf across test sites. Smoke quality assessments of fermented leaf by an organoleptic panel showed that three test hybrids had excellent smoking acceptability scores especially on satisfaction and no after-smoking irritation parameters. The availing of adaptable cigar wrapper varieties along with the cultural practices needed will be a boon to the envisaged widespread production of export quality cigar wrappers in Zimbabwe. This presentation will provide results of the agronomic traits measured and avail critical information for investments into the budding cigar manufacturing industry in Zimbabwe.

AP 46

Characterization and functional prediction of microbial community in agricultural processing of cigar leaves from Shifang, Sichuan

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Shifang, located in Sichuan Province, is known as the hometown of the Chinese cigar. In Shifang, cigar leaf has been cultivated for nearly 400 years. However, microbial community diversity is poorly understood in the agricultural processing of cigar leaves from Shifang. In this study, based on 16S rRNA and internal transcribed spacer region (ITS) gene Illumina Miseq high-through sequencing, characterization and functional prediction of the microbial community in freshly harvested, air-cured and agricultural fermented cigar leaves were revealed. Results showed that the differences of microbes in periods during agricultural processing were greater than those among varieties. The microbial richness of freshly harvested cigar leaves was the lowest, and microbial diversity of air-cured tobacco leaves was the highest. The richness and diversities of bacterial communities, and numbers of bacterial functional genes in all samples were higher than those of fungal. The dominant microbes belonged to 16 genera, including unclassified_*Enterobacteriaceae*, *Pseudomonas*, *Chloroplast*, *Acinetobacter*, *Pantoea*, *Sphingomonas*, *Staphylococcus*, *Aquabacterium*, unclassified_*Burkholderiaceae*, *Methylobacterium*, *Caulobacter*, *Brevundimonas*, *Aspergillus*, *Alternaria*, *Sampaiozyma*, and *Plectosphaerella*, of the *Proteobacteria*, *Cyanobacteria*, *Firmicutes*, *Basidiomycota*, and *Ascomycota*. Unclassified_*Enterobacteriaceae*, *Alternaria*, and *Mycosphaerella* were significantly positively correlated with freshly harvested cigar leaf group, while unclassified_*Burkholderiaceae*, *Novosphingobium*, *Pectobacterium*, and *Aspergillus* were significantly negatively correlated with it. *Pectobacterium* and *Aspergillus* were significantly positively correlated with agricultural fermented cigar leaf group, while *Alternaria*, *Penicillium*, *Mycosphaerella*, *Moesziomyces*, *Filobasidium*, and *Nigrospora* were significantly negatively correlated with it. The functions of bacteria and fungi were associated with fatty acid/lipid, amino acid, and carbohydrate biosynthesis, and fermentation. Compared to freshly harvested cigar leaves, air-cured and agricultural fermented cigar leaves were more functionally active regarding fatty acid/lipid biosynthesis and aromatic compound degradation from bacteria, and amino acid degradation from fungi. Results in this study advances our understanding of cigar leaf microbiota and their functions, and is of great importance for the production and quality improvement of Chinese cigar leaf.

AP 47

Alternative use for tobacco: solvent extraction of oil from Zimbabwe tobacco seed hybrids and parentals

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The perceived health hazards associated with the traditional form of tobacco consumption has led to research and development efforts the world over towards developing and commercializing a wide range of Next Generation Products (NGPs). This will, no doubt, lead to a massive reduction in the demand for tobacco leaf destined for traditional combustible cigarettes. This is a potentially devastating scenario for countries such as Zimbabwe where tobacco contributes significantly to the nation's foreign currency earnings. The objective of this trial was, therefore, to explore alternative uses of tobacco, namely as a source of tobacco seed oil (TSO). Laboratory trials were conducted to develop methods for TSO extraction, quantify the oil content of test hybrid seed varieties and breeding lines (tobacco seed) and establish the quality of the extracted oil. Tobacco seed from the Kutsaga tobacco germplasm collection were used. Oil was extracted using the solvent extraction method. Results showed that the tested tobacco seed had 30-40 % oil content and is of good quality. This is comparable to seed oil yield and quality of oil from other crops. This presentation will provide detail on the yield of the individual test varieties, elaborate on the quality of the extracted oil and give recommendations on the next steps that need to be taken to take the project forward.

AP 48

Industrial hemp production: an evaluation of its adaptability and potential incorporation in tobacco growing systems in Zimbabwe

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Zimbabwe is highly dependent on tobacco as a cash crop with it accounting for 30 % of total exports and 10 % of gross domestic product. However, in the face of the World Health Organization's Framework Convention on Tobacco Control (WHO FCTC) and global market volatilities, the need for crop diversification is strategic. The recent relaxation of legislation around the growing of hemp in the country has raised considerable interest from tobacco farmers considering its potential economic importance. In Zimbabwe, an industrial hemp value chain and business friendly policies are under development, but before these can be fully promulgated, insightful science-based research, technology development and transfer are needed. The main objectives of the study were to screen and select for hemp grain and fibre germplasm adaptable to the Zimbabwean growing environments and also to establish the pest and disease spectrum to enable the development of management options for hemp production. Trials with twelve varieties that included five from Canada, three from China and four from France were set up at Kutsaga Research Station as a factorial design and three plant densities (p0 = 70 seeds/m^2, p1 = 110 seeds/m^2 and p2 = 150 seeds/m^2) were evaluated. Seed was sown in the field in January, just as reaping of the irrigated tobacco crop would have been nearing completion. Results show that there was variation in the performance of the test varieties. While some varieties succumbed to photoperiod sensitivity that resulted in early-flowering and consequent yield and quality losses of harvestable organs, other varieties were identified as adaptable. The adaptable varieties had fast growth cycles and good yields of seed and fibre and can thus be potentially used as a sequential crop and also for rotations. This presentation will provide results of agronomic traits measured, pest, disease and weed problems observed and give recommendations for the management of an integrated hemp and tobacco farming enterprise in Zimbabwe.

AP 49

Consequences of molecular genetic alteration of leaf nitrate levels on TSNAs, alkaloids, flowering time, and biomass in Burley tobaccos

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We have previously demonstrated that tobacco-specific nitrosamine (TSNA) levels in Burley tobaccos can be substantially lowered using two distinct molecular genetic techniques designed to impede the plant's ability to store high levels of free nitrate in the cured leaf: (1) overexpression of a mutant nitrate reductase (NR) enzyme that is in a continually activated state and (2) downregulation of two closely related nitrate transporters, designated *CLCNt2-S* and *CLCNt2-T*. We have conducted multiple-year field trials to assess the effects of these genetic modifications on both the chemical (nitrate, alkaloids, and TSNAs) and agronomic (flowering time and plant biomass at harvest) properties of the plant. Plants expressing a constitutively activated NR enzyme flower prematurely and accumulate less biomass than wildtype control Burley tobaccos. However, by making F_1 hybrids with Burley genotypes known to flower atypically late, the biomass and/or flowering time were largely restored to normal without compromising the ability of the NR transgene to lower nitrate and TSNA levels. Reducing leaf nitrate levels via the genome editing-mediated knockout of *CLCNt2-S* and *CLCNt2-T* can also influence flowering time and aerial biomass. Disruption of *CLCNt2-S*, but not *CLCNt2-T*, confers a very late-flowering phenotype, suggesting that the two closely related gene isoforms are not functionally redundant. The non-equivalence of *CLCNt2-S* and *CLCNt2-T* is also supported by the results of transcript profiling assays of the two genes across different tissue types and developmental stages. Nevertheless, in order to significantly lower cured leaf nitrate and TSNA levels, both *CLCNt2-S* and *CLCNt2-T* must be rendered non-functional. The results of these studies provide key insights into how promising TSNA-reduction techniques can best be deployed in a manner that minimally impacts key agronomic properties.

AP 50

Genetic mapping of a novel low-anatabine gene mutation in tobacco (*Nicotiana tabacum* L.)

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Anatabine is a minor alkaloid found in tobacco and constitutes about 3 % of the total alkaloids. It is a precursor for *N*-nitrosoanatabine (NAT), one of four tobacco-specific nitrosamines (TSNAs) found in cured tobacco leaf. Depending on the tobacco type, agronomic practices, and environmental conditions, NAT content in the lamina ranges from 35-50 % of total TSNAs. Therefore, reduction or elimination of anatabine content in tobacco could significantly reduce total TSNA content in tobacco products. Since regulation of anatabine synthesis is not completely understood, identifying molecular markers associated with the anatabine trait will be useful in identifying and/or developing tobacco varieties that are low in anatabine and NAT.

A novel group of mutant lines has been developed and characterized that exhibit 80-95 % reduction of anatabine while maintaining normal levels of other tobacco alkaloids. Microsatellite markers closely linked to low anatabine were identified and used to genotype F_2 populations, revealing that the mutation is recessive. The low-anatabine trait was mapped to linkage group six, 0.3 centimorgan (cM) away from PT60878 on the high-density tobacco map. The results from this study will be useful in isolating the locus that regulates anatabine synthesis through map-based cloning, as well as identifying candidate gene assays and will help in elucidating the late stages of the alkaloid biosynthetic pathway.

AP 51

Impact of potassium source on cured leaf moisture and total TSNA in Burley and dark fire-cured tobacco

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Evaluations on the effect of potassium source on the formation of tobacco-specific nitrosamines (TSNA) in dark and Burley types of tobacco have been of recent interest. Previous field research at the University of Kentucky has observed reductions in total TSNA associated with applications of potassium chloride (KCl) compared to potassium sulfate (K_2SO_4). It is now recommended that Burley and dark tobacco producers incorporate potassium chloride with a maximum rate of 113 kg/ha in the potassium fertility program. The objective of this study was to further investigate how potassium source and a combination of potassium sources to supply the total potassium requirement impact the moisture content and total TSNA in cured leaf. Applied field research experiments were conducted in dark fire-cured tobacco at Princeton, Kentucky, and Burley tobacco at Lexington, Kentucky, imposing four treatments (untreated control; 100 % KCl; 100 % K_2SO_4; 70 % K_2SO_4 + 30 % KCl). Preliminary data suggests that potassium applications that utilized 100 % KCl had statistically higher moisture in the cured leaf for both dark fire-cured and Burley tobacco types. The impact of these treatments on total TSNA content will be presented. Results from this study will be used to supply updated information for potassium fertility recommendations in Burley and dark tobacco types.

AP 52

Effects of exogenous salicylic acid on photosynthesis and nitrogen metabolism of tobacco under drought stress and transcriptome analysis

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Water is a major ecological factor for the growth, physiological metabolism and quality formation of tobacco. Drought causes not only the decrease of tobacco yield and quality, but also the lowering of nitrogen utilization rate, leading to nitrate accumulation and increased levels of tobacco specific nitrosamine in cured and stored leaves. Salicylic acid, a phenolic compound and signal substance, is involved in regulating many plant physiological processes. The flue-cured tobacco variety K326 and Burley tobacco variety TN90 were used in the pot experiments to investigate the physiological responses of tobacco to salicylic acid under PEG drought stress and natural drought stress conditions. The pigment contents, photosynthetic characteristics, key nitrogen metabolism enzyme activities, antioxidant enzyme activities, nitrate contents etc., were measured after 0.3 $mmol \cdot L^{-1}$ SA treatment. Transcriptome sequencing and GO/KEGG analysis were also performed. The results showed that under drought stress conditions, the photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr) decreased significantly, while the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and the contents of malondialdehyde (MDA), proline and nitrate were all increased. Compared with PEG drought stress (D), SA spray significantly improved all the physiological parameters, with the Pn, Gs and Tr increasing by 45.74 %, 26.82 %, 52.22 %, and the activities of SOD, POD, CAT, protein and proline content increasing by 44.27 %, 50.18 %, 25 %, 34.69 % and 24.88 %, respectively; and nitrate content was reduced significantly. Compared with natural drought stress, SA treated leaves had higher Pn, Gs, Tr and Ci of 23.41 %, 46.88 %, 50.93 % and 52.27 %, respectively; POD activity and proline contents also increased, while MDA content decreased; nitrate reductase (NR) and glutamine synthetase (GS) activities increased by 47.08 % and 23.63 %, and nitrate content decreased by 12.98 % over the drought stress control. GO and KEGG analysis showed that SA treatment was able to up-regulate the genes involved in photosynthesis (RCA1, PSBO, PSBR, PSAO), photosynthesis-antenna proteins (lhcA-P4, LHC4.2, CAB50), carbon metabolism (FBP, FBA1, RBCS), porphyrin and chlorophyll metabolism (POR1, hemC, HEMA1, CHLM) and starch and sucrose metabolism (SS1, bglX, AGPS1). The conclusion is that SA application would effectively improve the photosynthesis of tobacco under drought conditions, thus increase nitrogen use efficiency and reduce the accumulation of nitrate which is the precursor of TSNA.

AP 53

Endophytic fungal community of tobacco leaves and their potential role in the formation of Cherry Red tobacco

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Cherry Red tobacco is the superior variant showing red dapples in cured leaves due to the demethylation of nicotine to nornicotine during maturation and curing. Fungi is known for its capacity of converting nicotine to nornicotine. However, to the best of our knowledge, the endophytic community of Cherry Red tobacco is never reported. Here we sampled mature leaves from Cherry Red and ordinary tobacco at lower, centre and upper plant sections, and analysed the internal transcribed spacer region (ITS) diversity using high-throughput sequencing. Results revealed a significantly different fungal community of foliar endophyte in Cherry Red and ordinary tobacco. In comparison to the ordinary control, a higher diversity and co-occurrence network complex was found in Cherry Red samples, especially in the centre and upper leaves, where the red dapples had mainly emerged. More taxa were enriched in the Cherry Red than ordinary tobacco leaves at all plant sections. Particularly, *Aspergillus*, a fungus that has been demonstrated to efficiently convert nicotine to nornicotine, was specifically enriched in upper Cherry Red leaves, which presented the most red dapples after curing. A less robust network structure was detected in the Cherry Red tobacco compared to the ordinary tobacco, corresponding to the vulnerability of red dapple production to agronomic and environmental disturbances. The nearest taxon index and β nearest taxon index indicated that the local community structuration of tobacco endophytic fungi was mainly driven by deterministic process, while the community turnover among plant sections was stochastic. In conclusion, our study provides the earliest information of the endophytic fungal community in Cherry Red tobacco leaf; the community diversity, composition and network features synchronously varied with the appearance of red dapples, suggesting their potential role in the formation of Cherry Red tobacco.

AP 54

Effect of fermentation temperature on TSNA contents and aroma quality of cigar filler tobacco and microbial diversity analysis

LI Jingjing(1); SHI Hongzhi(1); QIN Yanqing(2); ZHOU Jun(3); ZHAO Yuanyuan(1); ZHONG Qiu(4); LIU Deshui(3); WANG Jun(4); ZHANG Ruina(4)

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Fermentation is indispensable for quality cigar tobacco production and fermentation temperature plays a pivotal role. Meanwhile, the fermentation process is also an important stage in the formation of tobacco specific nitrosamine (TSNA), and temperature may also have a role to play. Therefore, understanding the impacts of temperature on both quality improvement and TSNA formation, as well as the relationship between temperature and microbial changes, is necessary for proper temperature control to balance quality improvement and harm reduction. In this experiment, the upper leaves of cigar variety Shiyan 1 were used and subjected to fermentation at 25 °C, 35 °C, 45 °C and 55 °C in the fermentation chamber for six weeks. Samples were collected at a one-week intervals during the process and measured for TSNA content and their precursors, neutral aroma components and mildew. 16s rRNA sequencing was also performed to investigate microbial diversity changes during fermentation. The results showed that with the increase of fermentation temperature from 25 °C to 45 °C, the total amount of neutral aroma components increased significantly, but the further increase of temperature did not result in increased aroma component contents. After six weeks of fermentation at 45 °C, the total amount of neutral aroma components increased by 31.51 % over the unfermented control; among them, solanone and megastigmatrienone were 1.52 times and 1.29 times higher than the control. TSNA content increased continuously and significantly with the temperature and with the fermenting time, and six-week fermentation at 55 °C resulted in the highest total TSNA accumulation, an increase of 103.22 %. The contents of alkaloid and nitrate decreased with the fermentation time. For unfermented tobacco, the dominant bacterial groups were *Pseudomonas*, *Sphingomonas* and *Methylobacterium*; the bacteria varied with temperatures, with low-temperature fermented tobacco dominated by *Aureimonas*, *Sphingomonas* and *Pseudomonas*, and high temperature fermented tobacco dominated by *Pseudomonas*, *Ralstonia* and *Mesoplasma*. The results suggested that fermentation at 45 °C for six weeks may be beneficial to balance quality improvement and TSNA reduction.

AUTHOR INDEX

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PROGRAMME SUMMARY

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	Chair: SCOTT Co-Chair: JACKSON	**Chair: XU Co-Chair: DORLHAC**	**Chair: DIMBI Co-Chair: LALANDE**	**Chair: LUSSO Co-Chair: SCOTT**	**Chair: JACKSON Co-Chair: LUSSO**
CET	**PRODUCTION**	**GENETICS**	**BIOCONTROL**	**NICOTINE**	**SUSTAINABILITY**
13:30 - 13:45	**AP01** VANN	**AP07** JULIO	**AP13** SAJJADI	**AP19** FISHER A.	**AP25** CHINHEYA
13:45 - 14:00	**AP03** MNVA Uma Mahesh	**AP08** JULIO	**AP14** LIU Yanxia	**AP20** SUI Xueyi	**AP26** WANG Yi
14:00 - 14:15	**AP04** HARDY	**AP09** LI Xiaoxu	**AP15** MAHERE	**AP21** VANN	**AP27** WANG Huanhuan
14:15 - 14:30	**AP05** LIANG Taibo	**AP10** LEWIS	**AP16** SHAZDEHAHMADI	**AP22** FISHER C.	**AP28** CHIBUDU
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CET	**TECHNOLOGY**	**PESTS & DISEASES**	**CIGARS / ALTERNATIVES**	**TSNA**
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14:00 - 14:15	**AP33** GRUNDMANN	**AP39** MU Wenjun	**AP45** MUKOYI	**AP51** RICHMOND
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Full Session Titles

Day 1 PRODUCTION impact of nutrition and herbicides
Day 2 GENETICS: tools for tobacco plant breeders
Day 3 BIOCONTROL of tobacco pests and diseases
Day 4 NICOTINE impacts of genetics and production practices
Day 5 SUSTAINABILITY through production practices
Day 6 TECHNOLOGY applications in genetics and physiology
Day 7 PESTS & DISEASE management
Day 8 CIGARS tobaccos and ALTERNATIVE uses and crops
Day 9 TSNA impact of genetics and production practices

Appendix: TIME ZONES

Time zone equivalents to CET 13:30 Conference start time

Time Zones	City	Time	Hour difference with Paris
CET	**Paris**	**13:30**	**0**
PDT	San Francisco	04:30	**-9**
CST	Managua	05:30	**-8**
EDT	New York	07:30	**-6**
BRT	São Paulo	08:30	**-5**
BST	London	12:30	**-1**
CAT	Harare	13:30	**0**
EET	Bucharest	14:30	**+1**
GST	Dubai	15:30	**+2**
IST	New Delhi	17:00	**+3:30**
CST	Beijing	19:30	**+6**
JST	Tokyo	20:30	**+7**
AEST	Sydney	21:30	**+8**

CET Central European Time
PDT Pacific Daylight Time
CST Central Standard Time
EDT Eastern Daylight Time
BRT Brasilia Time
BST British Summer Time
CAT Central Africa Time
EET Eastern European Time
GST Gulf Standard Time
IST Indian Standard Time
CST China Standard Time
JST Japan Standard Time
AEST Australian Eastern Standard Time