2020 Online
CORESTA Congress

12 October – 12 November 2020

PROGRAMME & ABSTRACTS

"Integrated Science:
Opportunities and Challenges"

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PROGRAMME SESSIONS

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

[CORESTA wishes to thank Japan Tobacco Inc., the original hosts of the planned physical Congress in Vienna, Austria, for their permission to adapt the original logo, colours and design to the online Congress]

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

SESSION 1: Biotechnology

Chairman: Dongmei XU

Abstract No.	Oral Presentation
AP 01	**Identification and verification of lncRNA against herbivores in *Nicotiana attenuata*** JIN Jingjing(1,2); RAN Li(3); BALDWIN I.T.(2) *(1) Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, P.R. China* *(2) Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, D-07745 Jena, Germany* *(3) Institute of Insect Sciences, Zhejiang University, 310058 Hangzhou, P.R. China*
AP 02	**The AP2 transcription factor *NtERF172* confers drought resistance by modifying *NtCAT*** HU Risheng(1); LI Yangyang(1); LIU Dan(2); ZHAO Qiang(3) *(1) Hunan Tobacco Research Institute of CNTC, No. 628, Section 1, Furong South Road, Tianxin District, Changsha 410000, Hunan Province, P.R. China* *(2) Tobacco Research Institute, Chinese Academy of Agricultural Sciences, Qingdao 266101, P.R. China* *(3) College of Horticulture, Qingdao Agricultural University, Qingdao 266109, P.R. China*
AP 03	**Tobacco yield improvement by photosynthetic antenna engineering** SHEN Y.; DAVIS G.; XU D. *Altria Client Services LLC, Product Design and Maintenance, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*
AP 04	**Reduction of cadmium in tobacco leaves** KUDITHIPUDI C.; MORRIS J.W. *Altria Client Services LLC, Product Design and Maintenance, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

SESSION 2: Diseases

Chairman: François DORLHAC de BORNE

Abstract No.	Oral Presentation
AP 05	**Biological control of bacterial wilt by constructing tobacco rhizosphere microbial community at the seedling stage** LIU Yanxia(1); LI Xiang(2); SUN Guangjun(2); LI Guanglei(2); JIANG Chaoying(2); JIAO Jian(2); XIA Zhilin(2); GUO Liang(2); SHEN Hong(2) *(1) Guizhou Academy of Tobacco Science of CNTC, Guiyang 550000, P.R. China* *(2) Guizhou Tobacco Company of CNTC, China National Tobacco Corporation, Guiyang 550000, P.R. China*
AP 06	**Coping with spotted wilt in tobacco** BERTRAND P.; MOORE J.M.; LUO Xuelin; La HUE S. *University of Georgia, Crop and Soil Sciences, 2360 Rainwater Road, Tifton, GA 31793-5766, U.S.A.*
AP 07	**The *eIF(iso)4E-T* deficient *va* tobacco is superior to Virgin A Mutant in resistance to Potato Virus Y** TAKAKURA Y.; UDAGAWA H.; SHINJO A.; KOGA K. *Japan Tobacco Inc., Leaf Tobacco Research Center, 1900 Idei, Oyama, Tochigi 323-0808, Japan*
AP 08	**Potential fungicides and biocontrol agents to manage target spot (*Thanatephorus cucumeris*) on flue-cured tobacco in Virginia** JOHNSON C.S. *Virginia Tech, Southern Piedmont Agricultural Research and Extension Center, 2375 Darvills Road, Blackstone, VA 23824, U.S.A.*

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

SESSION 3: Low Nicotine

Chairman: Marcos LUSSO

Abstract No.	Oral Presentation
AP 09	**Multi-omics data revealed nov-miR1170 regulates nicotine biosynthesis of *Nicotiana tabacum*** XU Yalong; JIN Jingjing; CHEN Qiansi; XIE Xidong; LIU Pingping; ZHAI Niu; LU Peng; LI Zefeng; CAO Peijian *China Tobacco Gene Research Center, Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, P.R. China*
AP 10	**Evaluation of low-nicotine tobacco cultivars and agronomic production practices by non-destructive photonic sensing** TUCCIO L.(1); BARGIACCHI E.(2); MILLI G.(3); MIELE S.(2); MICHELETTI F.(1); AGATI G.(1) *(1) CNR-IFAC, I-55019 Sesto Fiorentino, FI, Italy* *(2) Consortium INSTM, I-50121 Firenze, Italy* *(3) Fattoria Autonoma Tabacchi (FAT) & ITT, I-06012 Città di Castello, PG, Italy*
AP 11	**Analysis of the surface chemistry of high- and low-alkaloid Burley varieties/lines grown using standard agronomic practices and under low-nicotine field management** MIHAYLOVA-KROUMOVA A.(1); FISHER A.M.(1); FISHER C.R.(2); WAGNER J.G.(1) *(1) University of Kentucky, Kentucky Tobacco Research and Development Center, 1401 University Dr., Lexington, KY 40546, U.S.A.* *(2) University of Kentucky, Plant Science Building, 1405 Veterans Drive, Lexington, KY 40546, U.S.A.*
AP 12	**Reducing nicotine in Burley tobacco by combining low alkaloid varieties and agronomic practices – a very different second year** FISHER A.M.; FISHER C.R.; JI Huihua *University of Kentucky, 1401 University Drive, 200L KTRDC Bldg, Lexington, KY 40546, U.S.A.*

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

SESSION 4: Nutrition

Chairman: Susan DIMBI

Abstract No.	Oral Presentation
AP 13	**Effect of tilling depth on bacterial community structure and enzyme activities of flue-cured tobacco rhizosphere soil in mountainous areas of Yunnan** DENG Xiaopeng(1); TONG Wenjie(1); LIU Qi(2); LI Junying(1); YANG Min(3); XU Zhaoli(1); MA Erdeng(1); YU Lei(3) *(1) Yunnan Academy of Tobacco Agricultural Sciences of CNTC, 33 Yuantong Street, Kunming City, Yunnan Province, P.R. China* *(2) Tobacco College of Yunnan Agricultural University, 95 Jinhei Road, Kunming City, Yunnan Province, P.R. China* *(3) Agricultural College of Kunming University, 2 Puxin Road, Kunming City, Yunnan Province, P.R. China*
AP 14	**A comparison of traditional and alternative fertilizer programs for flue-cured tobacco production** SHORT M.M.; VANN M.C.; CHEEK J.A.; WHITLEY D.S. *North Carolina State University, Department of Crop and Soil Sciences, 101 Derieux Street, Raleigh, NC 27695, U.S.A.*
AP 15	**Nitrogen fertilizer source and the impact to flue-cured tobacco nutrient assimilation, yield, quality, value, and chemistry** VANN M.C.; WOODLEY A.L.; SUCHOFF D.H.; FISHER L.R. *North Carolina State University, Department of Crop & Soil Sciences, NCSU Campus Box 7620, Raleigh, NC 27695, U.S.A.*
AP 16	**Nitrogen application programs for fine-textured soils of the North Carolina Piedmont** VANN M.C.(1); WHITLEY D.S.(1); MASON J.H.(1); HAMBRICK T.(2); STRADER W.(3); DABBS D.C.(4) *(1) North Carolina State University, Department of Crop & Soil Sciences, NCSU Campus Box 7620, Raleigh, NC 27695, U.S.A.* *(2) Forsyth County Cooperative Extension, 1450 Fairchild Road, Winston-Salem, NC 27105, U.S.A.* *(3) Rockingham County Cooperative Extension, 525 NC Hwy 65 – Suite 200, Reidsville, NC 27320, U.S.A.* *(4) Alamance County Cooperative Extension, 209-C North Graham-Hopedale Road, Burlington, NC 27217, U.S.A.*

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

SESSION 5: Organic / Sustainable Production

Chairman: Fabienne LALANDE

Abstract No.	Oral Presentation
AP 17	**Establishing nitrogen fertility recommendations for the production of organic Burley tobacco** SUCHOFF D.H.; VANN M.C.; FISHER L.R. *North Carolina State University, Department of Crop and Soil Sciences, 101 Deriuex Place, Raleigh, NC 27695, U.S.A.*
AP 18	**Effects of polyethylene mulches on pest management and yield in organic flue-cured tobacco** MACHANOFF C.A.; SUCHOFF D.H.; VANN M.C.; WOODLEY A.L. *North Carolina State University, Department of Crop and Soil Sciences, 101 Derieux Street, Raleigh, NC 27695, U.S.A.*
AP 19	**Impacts of conservation tillage and cover crop mulch on weed emergence and leaf yield and quality in organic flue-cured tobacco** MACHANOFF C.A.; SUCHOFF D.H.; VANN M.C.; WOODLEY A.L. *North Carolina State University, Department of Crop and Soil Sciences, 101 Derieux Street, Raleigh, NC 27695, U.S.A.*
AP 20	**Winter cover crop management in the production of organic flue-cured tobacco** WOODLEY A.L.; HAHN S.L.; VANN M.C.; OSMOND D.L. *North Carolina State University, Department of Crop & Soil Sciences, NCSU Campus Box 7620, Raleigh, NC 27695, U.S.A.*

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

SESSION 6: Production

Chairman: Anthony JACKSON

Abstract No.	Oral Presentation
AP 21	**Impacts of lower-leaf removal timing, number, and nitrogen application to flue-cured tobacco** VANN M.C.(1); FINCH C.E.(1); FISHER L.R.(1); WELLS R.(1); BROWN A.B.(2) *(1) North Carolina State University, Department of Crop & Soil Sciences, NCSU Campus Box 7620, Raleigh, NC 27695, U.S.A.* *(2) North Carolina State University, Department of Agricultural & Economic Resources, NCSU Campus Box 8109, Raleigh, NC 27695, U.S.A.*
AP 22	**Cigar wrapper tobacco production in western North Carolina** VANN M.C.; MACHACEK J.L.; CHEEK J.A.; WHITLEY D.S. *North Carolina State University, Department of Crop & Soil Sciences, NCSU Campus Box 7620, Raleigh, NC 27695, U.S.A.*
AP 23	**Industrial hemp: the benefits, concerns, and unknowns for North Carolina tobacco farmers** SHORT M.M.(1); McGINNIS M.(2); VANN M.C.(1); SUCHOFF D.H.(1); EDMISTEN K.L.(1) *(1) North Carolina State University, Department of Crop and Soil Sciences, 101 Derieux Street, Raleigh, NC 27695, U.S.A.* *(2) North Carolina Department of Agriculture & Consumer Services, Agronomic Division, 4300 Reedy Creek Road, Raleigh, NC 27607, U.S.A.*
AP 24	**Economic factors influencing lower leaf removal decisions** BLALOCK C.(1); VANN M.C.(1); FISHER L.R.(1); BROWN A.B.(2) *(1) North Carolina State University, Department of Crop & Soil Sciences, NCSU Campus Box 7620, Raleigh, NC 27695, U.S.A.* *(2) North Carolina State University, Department of Agricultural & Economic Resources, NCSU Campus Box 8109, Raleigh, NC 27695, U.S.A.*

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SESSION 7: TSNAs

Chairman: Colin FISHER

Abstract No.	Oral Presentation
AP 25	**Analysis of TSNAs and their relationship with alkaloids in cigar wrapper and filler tobaccos from different regions and varieties** SHI Hongzhi(1); ZHOU Di(1); SUN Yuqi(1); WANG Jun(2); ZHOU Jun(3); ZHAO Yuanyuan(1); ZENG Dailong(4); QIN Yanqing(2); BAI Ruoshi(3); YANG Xingyou(2); LI Jingjing(1) *(1) College of Tobacco Science of Henan Agricultural University / Tobacco Cultivation Key Laboratory of China Tobacco / Tobacco Harm Reduction Research Center of HAU, Zhengzhou 450002, P.R. China* *(2) Sichuan Tobacco Company, Chengdu 618400, P.R. China* *(3) Beijing Cigarette Factory of Shanghai Tobacco (Group) Co., Beijing 100024, P.R. China* *(4) China Tobacco Sichuan Industrial Co., Ltd, Great Wall Cigar Factory, Shifang, P.R. China*
AP 26	**Rapid screening of nicotine converters from tobacco seedling population** LI Yong; PANG Tao; CHEN Xuejun; SHI Junli; SUI Xueyi; GU Huaguo *Yunnan Academy of Tobacco Agricultural Sciences of CNTC, Yunnan, P.R. China*
AP 27	**Three-year N-nitrosonornicotine data for stable reduced converter (SRC) dark tobacco crop** LION K.; LUSSO M.; ADAMS A.; MORRIS W.; DAVIS G. *Altria Client Services LLC, Research, Development & Regulatory Affairs, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*
AP 28	**Correlation between post-curing TSNA increase and alkaloid and nitrite contents in cured leaves** KAWANA M.; MASUDA S.; SATO N. *Japan Tobacco Inc., Leaf Tobacco Research Center (LTRC), 1900 Idei, Oyama, Tochigi 323-0808, Japan*

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

POSTER SESSION 1: Pest & Diseases

Abstract No.	Poster Presentation
APPOST 01	**Study of fungicides effect of Vista and Nativo on tobacco collar rot (*Sclerotinia sclerotiorum*) in Northern Iran** SAJJADI A.; NAJAFI M.R.; HOSSEINI A.; MASOUDI A.; SAFFAR F. *Tirtash Tobacco Research and Education Center, Behshar, Iran*
APPOST 02	**Production of wettable powder biopesticide formulation from two superior *Bacillus thuringiensis* strains native to Northern Iran** SHAZDEH AHMADI M.; SAJJADI A.; SALEHI JOUZANI Gh.R.; ASSEMI H.; SHAHADATI MOGHADAM Z. *Tirtash Tobacco Research and Education Center, Behshar, Iran*
APPOST 03	**Field evaluation of the efficacy of formulations produced from *Bacillus thuringiensis* (*Bt*) and nuclear polyhedrosis virus (NPV) isolates native to Northern Iran against *Helicoverpa armigera*** SHAZDEH AHMADI M.; SAJJADI A.; SALEHI JOUZANI Gh.R.; ASSEMI H.; SHAHADATI MOGHADAM Z. *Tirtash Tobacco Research and Education Center, Behshar, Iran*
APPOST 04	**Identification of a PVY isolate breaking down the resistance of *va*-genotye tobacco in China** LI Ruo; WAN Xiuqing; QIAO Chan; GUO Zhaokui *Heilongjiang Institute of Tobacco Science of CNTC, Hayao Road 17, Harbin, P.R. China*
APPOST 05	**Phosphorous acid effects on Pythium root rot in tobacco float bed** HARADA H. *Japan Tobacco Inc., Leaf Tobacco Research Center, 1900 Idei, Oyama, Tochigi 323-0808, Japan*
APPOST 06	**Diagnosis of leaf curl and crooked tip diseases on tobacco plants in Tay Ninh Province, Vietnam** NGUYEN VAN C.(1); VIET HA C.(2) *(1) Tobacco Institute, 133 Nguyen Trai, Thanh Xuan, Ha Noi, Vietnam* *(2) Vietnam National University of Agriculture, Vietnam*

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

POSTER SESSION 2: Production & Nutrition

Abstract No.	Poster Presentation
APPOST 07	**Evaluation of the effects of organic and chemical fertilizer on quality and yield of flue-cured tobacco under irrigated and rainfed conditions** AHMADI M.(1); MOHSENZADEH R.(1); DAVANLO A.(1); YAGHOBI Y.(1); SHAHADATI MOGHADAM Z.(1); GHOLIZADEH A.Gh.(1); ALINEJAD R.(1); HOSSEINI A.(1); SALAVATI M.R.(1); NOROZI A.(2); LATIFI N.(3); DASTA N.(2) *(1) Tirtash Tobacco Research and Education Center, Behshar, Iran* *(2) Azad University, Iran* *(3) Gorgan University of Agricultural Science and Natural Resources, Iran*
APPOST 08	**Growth, nitrogen uptake and soil N_2O emissions in flue-cured tobacco as influenced by drip fertigation strategy** MA Erdeng(1); GAO Tian(2); ZHANG Guangbin(3); XU Zhaoli(1); LI Junying(1); TONG Wenjie(1); Deng Xiaopeng(1) *(1) Yunnan Academy of Tobacco Agricultural Sciences of CNTC, No. 33 Yuantong Rd, Kunming 650031, P.R. China* *(2) College of Tobacco Science, Yunnan Agricultural University, Jinhei Road, Kunming 650201, P.R. China* *(3) State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, No. 71 East Beijing Road, Nanjing 210008, P.R. China*
APPOST 09	**Dynamic change of soil pH, physi-chemical characters and enzymatic activity under restructuring arable layer** CHEN Jin(1,2); DENG Xiaohua(2); PENG Shuguang(3); LIU Yongjun(3); DENG Yongsheng(3); WANG Zhenhua(3); PENG Deyuan(3); LI Yuanhuan(2); SU Gexuan(2) *(1) Ningxiang Branch, Changsha Tobacco Company, Hunan, Ningxiang 410600, P.R. China* *(2) College of Agronomy, Hunan Agricultural University, Changsha 410128, P.R. China* *(3) Hunan Tobacco Company of CNTC, Changsha 410004, P.R. China*
APPOST 10	**Improved soil fertility and microbial diversity by microbial organic fertilizers in continuous tobacco cropping areas** GUO Hui(1); YANG Huijuan(1); SUN Junwei(2); WEI Yuehui(1); SHI Hongzhi(1) *(1) College of Tobacco Science, Henan Agricultural University, Zhengzhou 540002, P.R. China* *(2) Dali State Company of CNTC, Dali 671000, P.R. China*
APPOST 11	**Ensuring nicotine uniformity by measuring tobacco leaf using NIR spectrometer** UEYAMA S.(1); ISHIHARA K.(1); ISSHIKI H.(2); SEINO Y.(2); IGA H.(2); SUMITOMO T.(2) *(1) Japan Tobacco Inc., Leaf Tobacco Research Center, 1900 Idei, Oyama, Tochigi 323-0808, Japan* *(2) Japan Tobacco Inc., Leaf Services Division, 2-2-1 Toranomon, Minato-ku, Tokyo 105-8422, Japan*

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

Abstract No.	Poster Presentation
APPOST 12	**A study for inheritance of chemical components in dried leaves of tobacco varieties of different types and their F1 progenies** KORUBIN-ALEKSOSKA A.(1); DOJCINOV S.(2) *(1) "St. Kliment Ohridski" University - Bitola, Scientific Tobacco Institute - Prilep, Kicevska bb, Prilep, Republic of North Macedonia* *(2) Alliance One Macedonia - Kavadarci, Zapaden Bulevar 105, 1430 Kavadarci, Republic of North Macedonia*
APPOST 13	**Tobacco production programming in the Suwannee River Valley of North Florida** VANN C.D.(1); WYNN K.(2); BROUGHTON D.(3); MOORE J.M.(4); VANN M.C.(5) *(1) University of Florida - Institute of Food and Agricultural Sciences, Lafayette County Extension, Mayo, FL 32066, U.S.A.* *(2) University of Florida - Institute of Food and Agricultural Sciences, Hamilton County Extension, Jasper, FL 32052, U.S.A.* *(3) University of Florida - Institute of Food and Agricultural Sciences, North Florida Research & Education Center - Suwannee Valley, Live Oak, FL 32060, U.S.A.* *(4) University of Georgia, Department of Crop & Soil Sciences, Tifton, GA 31793, U.S.A.* *(5) North Carolina State University, Department of Crop & Soil Sciences, Raleigh, NC 27695, U.S.A.*
APPOST 14	**The effect of silicate solubilizing bacteria and mycorrhizal symbiosis on potassium fertilizer requirements of tobacco (*Nicotiana tabacum* L.)** RANJBAR R.(1); SEPEHR E.(2); SAMADI A.(2); SADAGHIANI M.H.(2); DOVLATI B.(2); BARIN M.(2) *(1) Urmia Tobacco Research Center, Urmia, Iran* *(2) Soil Science Department, Faculty of Agriculture, Urmia University, Urmia, Iran*

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

CORESTA SUB-GROUP & TASK FORCE REPORTS

Agronomy & Leaf Integrity

Group	
AA	Sub-Group Agrochemical Analysis
GMO	Sub-Group Proficiency Testing for Detection of Transgenic Tobacco
LNTP	Task Force Collaborative Study of Low Nicotine Tobacco Agronomic Production Practices
PSMST	Sub-Group Pest and Sanitation Management in Stored Tobacco
RFT	Sub-Group Agrochemical Residue Field Trials
TSNA	Sub-Group TSNA in Air-Cured and Fire-Cured Tobacco

Phytopathology & Genetics

Group	
BIO	Sub-Group Efficacy of Biological and Eco-Friendly CPAs
IPM	Sub-Group Integrated Pest Management
TAG	Task Force Tobacco Alkaloid Genetics
TBO	Task Force Tobacco Biotechnology and Omics
XDES	Sub-Group Extended Diagnostic Expert System

SMOKE SCIENCE and PRODUCT TECHNOLOGY

SESSION 1: Cigarette Design

Chairman: Bernhard EITZINGER

Abstract No.	Oral Presentation
ST 01	**Numerical simulation of cigarette smoking process: Effects of multifactor variations on cigarette burning behaviour and releases of tar and CO** LI Qiaoling(1); ZHONG Hongxiang(1); LIN Kai(1); CAI Guohua(1); WANG Daoquan(1); CHEN Xin(1); ZHENG Quanxing(1); LIU Xiucai(1); MA Pengfei(1); DENG Xiaohua(1); XU Hanchun(1); CHEN Xiaodong(2); LI Bin(3); LI Yuefeng(1) *(1) Technology Center, China Tobacco Fujian Industrial Co., Ltd., of CNTC, No. 298, Binshui Road, Jimei District, Xiamen 361021, Fujian, P.R. China* *(2) College of Chemistry and Chemical Engineering, Xiamen University, No. 422, South Siming Road, Xiamen 361005, Fujian, P.R. China* *(3) Key Laboratory of Tobacco Processing Technology of CNTC, Zhengzhou Tobacco Research Institute of CNTC, No. 2, Fengyang Street, Hi-Tech District, Zhengzhou 450001, P.R. China*
ST 02	**Paper filter – a market changer?** MOSTOVOJUS V.; TUCINSKAS G. *Nemuno Banga LLC, Kestucio str. 1, Lentvaris, Lithuania* CANCELLED

SESSION 2: E-Vapour

Chairman: Rob STEVENS

Abstract No.	Oral Presentation
ST 03	**Method development for the analysis of mono-carbonyl compounds in e-vapor products by LC-MS** ZHU J.; HEREDIA A.; TWEEDY J.; TAYYARAH R. *ITG Brands and Fontem USA, P.O. Box 21688, Greensboro, NC 27420, U.S.A.*
ST 04	**Method optimization on analysis of TSNA in electronic cigarette liquids and N-nitrososarcosine (NSAR) in smokeless tobacco by UHPLC-MS/MS** WU Jingcun; QIN Feng *PerkinElmer Health Sciences Canada, Inc., 501 Rowntree Dairy Road, Unit 6, Woodbridge, Ontario L4L 8H1, Canada*
ST 05	**Determination of glycidol in e-liquids and emissions from e-cigarettes** WANG Jiaming; RODRIGUEZ-LAFUENTE A.; JOZA P. *Labstat International Inc., 262 Manitou Drive, Kitchener, Ontario N2C 1L3, Canada*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

SESSION 3: HTP Aerosol Analysis

Chairman: Jutta PANI

Abstract No.	Oral Presentation
ST 06	**eHTP aerosol generation: mass balance method to evaluate the contribution of stick elements and device design** DUROT N.; ROUILLARD S.; RAVERDY-LAMBERT D. *SWM INTL c/o LTR Industries, Usine Le Mans, 72702 Allonnes Cedex, France*
ST 07	**Investigation on HTP aerosol release dynamics with a new puff by puff HTP vaping machine** DEJOIE S.(1); DUROT N.(1); BINARD F.(2); ROUILLARD S.(1); RAVERDY-LAMBERT D.(1) *(1) SWM Intl c/o LTR Industries, Usine Le Mans, 72702 Allonnes Cedex, France* *(2) SWM Intl c/o PDM Industries, Kerisole, 29300 Quimperlé, France*
ST 08	**Development and validation of a routine method for the determination of carbonyl compounds in heated tobacco products (HTPs) by UPLC-MS** JABLONSKI J.J.; MARTIN A.M.; GILLMAN I.G. *Enthalpy Analytical, LLC, 1470 E Parham Road, Richmond, VA 23228, U.S.A.*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

SESSION 4: HTP and E-Vapour Design

Chairman: Jutta PANI

Abstract No.	Oral Presentation
ST 09	**Influences of tobacco powder particle size on the reconstituted tobacco slurry process for electrically heated tobacco products** TIAN Yongfeng; DONG Gaofeng; MIAO Mingming; TANG Jianguo; ZHU Donglai; SHANG Shanzhai; ZHANG Xia; HE Pei; TANG Shiyun; YANG Chen; LIU Zhihua *Technical Center, China Tobacco Yunnan Industrial Co., Ltd., of CNTC, Yunnan Key Laboratory of Tobacco Chemistry, No. 367, Hongjin Road, Kunming 650231, P.R. China*
ST 10	**Modular new product development in highly dynamic markets** KÜCHENHOF J.(1); NIEBUHR G.(2); SCHMIDT R.(2); KESSLER M.(2); KRAUSE D.(1) *(1) Hamburg University of Technology, TUHH, Institute for Product Development and Mechanical Engineering Design (PKT), Denickestraße 17, 21073 Hamburg, Germany* *(2) Hauni Maschinenbau GmbH, Dev. Vaping Technologies, Kurt-A.-Körber-Chaussee 8-32, 21033 Hamburg, Germany*
ST 11	**Extractable and leachable testing of electronic nicotine delivery systems** MORLEY N.; McGUIGAN S.; THOMAS J.; FEILDEN A. *Hall Analytical Laboratories Ltd, Millbrook Business Centre, Floats Road, Manchester M23 9YJ, U.K.*
ST 12	**Thermal behaviour of tobacco pads for aerosol generation** JAMALI A.(1); BÄUMKER E.(1); SABERI M.(1); PELZ U.(1); SHEER C.(2); KESSLER M.(2); SCHMIDT R.(2); GOLDSCHMIDTBÖING F.(1) *(1) University of Freiburg, IMTEK, Design of Microsystems, Georges-Koehler-Allee 102, 79110 Freiburg, Germany* *(2) Hauni Maschinenbau GmbH, Vaping Technologies, Barnerstraße 14, Aufgang D, 22765 Hamburg, Germany*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

SESSION 5: *In vitro* Toxicology

Chairman: Kei YOSHINO

Abstract No.	Oral Presentation
ST 13	***In vitro* biological assessment of nicotine administration efficiency of a prototypic ENDS with a novel volumetric heater** MIRDOGAN A. *Hauni Maschinenbau GmbH, Kurt-A.-Körber-Chaussee, 8-32, 21033 Hamburg, Germany*
ST 14	***In vitro* assessment of acute respiratory toxicity** SHARMA M.(1); STUCKI A.(1); VERSTRAELEN S.(2); FRIJNS E.(2); MAES F.(2); CLIPPINGER A.J.(1) *(1) PETA International Science Consortium Ltd., Society Building, 8 All Saints Street, London N1 9RL, U.K.* *(2) VITO, Flemish Institute for Technological Research, Boeretang 200, 2400 Mol, Belgium*

SESSION 6: Nicotine Pouches

Chairman: Bin HU

Abstract No.	Oral Presentation
ST 15	**Characterization of on!® nicotine pouches – Part 2: Nicotine dissolution release profiles** ALDEEK F.; WAGNER K.A.; SMITH C.R.; McCUTCHEON N; GRISEVICH H; SUAREZ C.J.; McFARLANE C.B.; DANIELSON T.L. *Altria Client Services LLC, Research, Development & Regulatory Affairs, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*
ST 16	**Harm reduction opportunities with a portfolio of oral tobacco-derived nicotine containing pouches** SARKAR M. *Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

SESSION 7: Non-Targeted Analysis of Smoke Free Product Aerosols

Chairman: Guy JACCARD

Abstract No.	Oral Presentation
ST 17	**The dos and don'ts of non-targeted screening by LC–HRAM-MS for chemical characterization of smoke-free products** WACHSMUTH C.; ARNDT D.; BUCHHOLZ C.; BENTLEY M.; GOUJON-GINGLINGER C. *Philip Morris Products S.A., PMI R&D, Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland*
ST 18	**Computer-assisted structure identification (CASI) for high-throughput identification of small molecules by GC×GC–HRAM-TOFMS** KNORR A.; ALMSTETTER M.; MARTIN E.; CASTELLON A.; POSPISIL P.; BENTLEY M.; GOUJON-GINGLINGER C. *Philip Morris Products S.A., PMI R&D, Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland*
ST 19	**Non-targeted chemical characterization of complex matrices by nominal- and high-resolution accurate-mass GC×GC–TOFMS** ALMSTETTER M.; KNORR A.; RHOUMA M.; MARTIN E.; CASTELLON A.; POSPISIL P.; BENTLEY M.; GOUJON-GINGLINGER C. *Philip Morris Products S.A., PMI R&D, Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland*
ST 20	**Non-targeted analysis using gas chromatography mass spectrometry for evaluation of chemical composition of e-vapor products** MILLER IV J.H.; SHAH N.H.; NOE M.R.; AGNEW-HEARD K.A.; GARDNER W.P.; PITHAWALLA Y.P. *Altria Client Services LLC, 600 East Leigh Street, Richmond, VA 23219, U.S.A.*
ST 21	**Untargeted chemical characterization of the aerosol generated by a heated tobacco product** BENTLEY M.; ALMSTETTER M.; ARNDT D.; KNORR A.; MARTIN E.; POSPISIL P.; MAEDER S. *Philip Morris Products S.A., PMI R&D, Quai Jeanrenaud 5, 2000 Neuchâtel, Switzerland*
ST 22	**Can liquid chromatography scan techniques be as useful a tool for e-liquids as gas chromatography scan techniques have been for cigarette tobaccos?** LAUTERBACH J.H. *Lauterbach & Associates, LLC, 211 Old Club Court, Macon, GA 31210, U.S.A.*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

SESSION 8: Product Use Behaviour

Chairman: Xavier CAHOURS

Abstract No.	Oral Presentation
ST 23	**New measures for assessing the abuse liability of connected ENDS** CAPONE M.J. *Hauni Vaping Technologies, Weidenbaumsweg 103, 21033 Hamburg, Germany*
ST 24	**Dos and don'ts in the design of indoor air quality studies on smoke-free products** MITOVA M.I.; GOUJON-GINGLINGER C.; GOMEZ LUESO M.; ROTACH M.; MAEDER S. *Philip Morris Products S.A., PMI R&D, Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland*
ST 25	**Human abuse liability assessment of tobacco and nicotine products: considerations to meet current regulatory recommendations** BAXTER S.(1); VANSICKEL A.(2); SHERWOOD N.(3); CAMPBELL L.(1); KONG M.(4) *(1) RAI Services Company, Winston Salem, NC, U.S.A.* *(2) Altria Client Services, Richmond, VA, U.S.A.* *(3) Neil Sherwood Consulting, Nyon, Switzerland* *(4) Altasciences Clinical Research, Laval, QC, Canada*

SESSION 9: Risk Assessment

Chairman: Paul HARP

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ST 26	**Comparative risk assessment of heated tobacco product and electronic cigarette aerosols with cigarette smoke based on cancer potency and margin of exposure** RODRIGO G.; JACCARD G.; TAFIN DJOKO D.; KORNELIOU A.; ESPOSITO M.; BELUSHKIN M. *Philip Morris Products S.A., PMI R&D, Rue des Usines 56, CH-2000 Neuchâtel, Switzerland*
ST 27	**Cardiovascular, carcinogenic and reproductive effects of nicotine exposure: a narrative review of the scientific literature** MARTINEZ J.; PRICE L. *JT International SA, 8 Rue Kazem Radjavi, 1202 Geneva, Switzerland*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

SESSION 10: Smoke and Tobacco Analysis

Chairman: Karl WAGNER

Abstract No.	Oral Presentation
ST 28	**Transfer of aroma components in slim cigarettes flavoured by different methods** WU Bingyu; FEI Ting; LUO Chen; BI Yanjiu; MA Lichao; TAO Liqi; WU Da *Shanghai Tobacco Group Co., Ltd., of CNTC, No. 3733, Xiupu Road, Shanghai 201315, P.R. China*
ST 29	**Comparison of reference cigarette variability, smoke yields, and filler HPHC content of 1R6F and 3R4F** MORTON M.J.; BLAKE T.L.; WAGNER K.A. *Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*
ST 30	**Dokha tobaccos – does their chemistry follow their growth in popularity?** LAUTERBACH J.H. *Lauterbach & Associates, LLC, 211 Old Club Court, Macon, GA 31210, U.S.A.*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

POSTER SESSION 1: Cigarettes and Cigars

Abstract No.	Poster Presentation
STPOST 01	**An algorithm for inspecting breakable capsules with "Off-Centre" defects based on machine vision technology** SONG Xuyan; PAN Xi; LI Ran; WEI Min; HE Yunlu *China Tobacco Hubei Industrial Co., Ltd. of CNTC, Yellow Crane Tower Science Park, No. 1355, Jinshan Avenue, Dongxihu District, Wuhan 430040, Hubei, P.R. China*
STPOST 02	**Analysis of highly volatile flavouring compounds in cigarettes smoke** PINTO M.I.; PORTER R.; GHELLI J.; GOSS C.; THOMPSON N.; DALTON D.; WRIGHT C. *British American Tobacco, R&D Centre, Regents Park Road, Millbrook, Southampton, SO15 8TL, U.K.*
STPOST 03	**Conversion of glycerin in cigar smoke to formaldehyde in DNPH trapping solution** JABLONSKI J.J.; GILLMAN I.G. *Enthalpy Analytical, LLC, 1470 E Parham Road, Richmond, VA 23228, U.S.A.*
STPOST 04	**Assessing the sources of smoke variability in machine-made cigars** HILLDRUP J.P.; GILLMAN I.G. *Enthalpy Analytical, LLC, 1470 E Parham Road, Richmond, VA 23228, U.S.A.*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

POSTER SESSION 2: HTP and E-Vapour Analysis

Abstract No.	Poster Presentation
STPOST 05	**A screening method by gas chromatography–mass spectrometry for the quantitation of 33 aerosol constituents from a heat-not-burn tobacco product** HOFER I.; GAUTIER L.; CORTES SAUTEUR E.; DOBLER M.; PYTHON A.; O'REILLY C.; GISI D.; TINGUELY E.; WEHREN L.; GARCÍA FIDALGO E.; CUKURCAM L.; HENNEMANN M.; MATERA R.; ROTA D.; SANTOS CH.; SEQUEIRA C.; EPARS T. *Philip Morris Products S.A., PMI R&D, Quai Jeanrenaud 5, CH-2000 Neuchatel, Switzerland*
STPOST 06	**Assessment of filter pre-treatment for metal analysis in e-vapour aerosol** IMAI R.; NAGAE H.; FUKAI Y.; SHIMAZU A.; TAKAYAMA H. *Japan Tobacco Inc., Scientific Product Assessment Centre, 6-2, Umegaoka, Aoba-ku, Yokohama, Kanagawa 227-8512, Japan*
STPOST 07	**New developments in vacuum photoionisation TOF-MS technique to analyse smoking products on-line and in real time** EHLERT S.(1,2); HEIDE J.(2); WALTE A.(1); ZIMMERMANN R.(2) *(1) Photonion GmbH, Hagenower Str. 73, 19061 Schwerin, Germany* *(2) University of Rostock, Dept. of Analytical Chemistry, Dr.-Lorenz-Weg 2; 18059 Rostock, Germany*
STPOST 08	**Determination of α-tocopherol acetate (vitamin E acetate) in e-liquids and cannabis liquids samples - a comparison between HPLC-DAD and LC-MS/MS methods** RODRIGUEZ-LAFUENTE A.; JOZA P. *Labstat International Inc., 262 Manitou Drive, Kitchener, Ontario N2C 1L3, Canada*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

POSTER SESSION 3: Tobacco and Smokeless Tobacco

Abstract No.	Poster Presentation
STPOST 09	**Study of thermal decomposition of Iranian Virginia tobacco components** MORADI ROBATI G.R.; SAJJADI A.; SALAVATI M.R. *Tirtash Tobacco Research and Education Center, Behshar, Iran*
STPOST 10	**Characterization of on!® nicotine pouches – Part 1: HPHCs** WAGNER K.A.; BALLENTINE R.M.; BROWN A.P.; JIN X.C.; LOPEZ V.F.; SHARIFI M.; McFARLANE C.B.; MELVIN M. S.; MORTON M.J.; DANIELSON T.L. *Altria Client Services LLC, Research, Development & Regulatory Affairs, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*
STPOST 11	**Factors influencing pyrolysis and smoke release characteristics of tobacco particles at low temperature** CAO Yun(1); ZHOU Shun(1,2); WANG Xiaofeng(1); ZHANG Yaping(1,2); ZHANG Xiaoyu(1); WANG Chenghu(1); LI Yanyan(1); GUAN Mingjing(1); CHEN Gang(2); HUANG Lan(2) *(1) Key Laboratory of Combustion & Pyrolysis Study of CNTC, Anhui Tobacco Industrial Co., Ltd., of CNTC, No. 9, Tianda Road, Hefei 230088, P.R. China* *(2) Key Laboratory for Tobacco Chemistry of Anhui Province, Anhui Tobacco Industrial Co., Ltd., of CNTC, No. 9, Tianda Road, Hefei 230088, P.R. China*
STPOST 12	**A rapid quantitative optimization method for technological parameters of threshing based on uniform experimental design** YIN Fan; CHEN Zhuangyu; LUO Xianhua *Chenzhou Redrying Factory of Hunan Tobacco Redrying Co., Ltd., of CNTC, No. 1728, Chenzhou Avenue, Huatang Town, Beihu District, Chenzhou, 423000, Hunan, P.R. China*
STPOST 13	**Analysis of menthol optical isomers in tobacco products** SI Xiaoxi; ZHU Ruizhi; TANG Jianguo; MIAO Mingming; LIU Zhihua *Yunnan Key Laboratory of Tobacco Chemistry, R&D Center of China Tobacco Yunnan Industrial Co., Ltd. of CNTC, No. 367, Hongjin Road, Kunming 650231, Yunnan, P.R. China*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

POSTER SESSION 4: Toxicology

Abstract No.	Poster Presentation
STPOST 14	**Mode-of-action analysis of the induction of micronuclei by a flavouring compound *in vitro*** WATANABE T.; MUNAKATA S.; ISHII T.; SAITO J.; ERAMI K.; HASHIZUME T. *Japan Tobacco Inc., R&D Group, Scientific Product Assessment Center, 6-2, Umegaoka, Aoba-ku, Yokohama, Kanagawa 227-8512, Japan*
STPOST 15	**The comparative analysis of cytokine production by a human 3D airway tissue model following exposure to traditional cigarette smoke, tobacco-heated product and e-cigarette aerosol** BEDFORD R.(1); ROTHWELL E.(1); MARTIN S.(1); O'HANLON C.(1); McCUNE A.(2); HOLLINGS M.(1) *(1) Genetic Toxicology, Covance Laboratories Ltd, Harrogate, North Yorkshire, U.K.* *(2) Immunology and Immunotoxicology, Covance Laboratories Ltd, Harrogate, North Yorkshire, U.K.*
STPOST 16	**Comparison of *in vitro* cytotoxicity and genotoxicity of condensates derived from electronic-cigarettes and reference combustible cigarette** DOSHI U.; GARDNER W.; LEE K.M. *Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*
STPOST 17	**Comparison of smoking machines for sample preparation for *in vitro* assays** SEKIGUCHI H.; ITO H. *Japan Tobacco Inc., Scientific Product Assessment Center, 6-2, Umegaoka, Aoba-ku, Yokohama, Kanagawa 227-8512, Japan*

SMOKE SCIENCE and PRODUCT TECHNOLOGY

CORESTA SUB-GROUP & TASK FORCE REPORTS

Smoke Science

Group	
BMK	**Sub-Group Biomarkers**
CROM	**Task Force Consumer Reported Outcome Measures Consortium**
IVT	**Sub-Group *In Vitro* Toxicity Testing**
NGTX	**Task Force 21st Century Toxicology for Next Generation Tobacco and Nicotine Products (NGTX)**
PUB	**Sub-Group Product Use Behaviour**
SMA	**Sub-Group Smoke Analytes**

Product Technology

Group	
CSM	**Sub-Group Cigar Smoking Methods**
EVAP	**Sub-Group E-Vapour**
HTP	**Task Force Heated Tobacco Products**
PTM	**Sub-Group Physical Test Methods**
RAC	**Sub-Group Routine Analytical Chemistry**
TTPA	**Sub-Group Tobacco and Tobacco Products Analytes**

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ABSTRACTS

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

2020 CORESTA CONGRESS ONLINE

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

ORAL PRESENTATIONS

AP 01

Identification and verification of lncRNA against herbivores in *Nicotiana attenuata*

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Long noncoding RNAs (lncRNAs) have been shown to play important roles in various biological processes in plants, including defense against pathogens. However, it is still unknown whether lncRNAs mediate defense against herbivore attack. In order to explore lncRNA's function in herbivore response, lncRNA sequencing for wild tobacco, *Nicotiana attenuata*, was performed. In total, 1290 significant differentially expressed lncRNA responses to herbivore elicitation were identified, and long intergenic non-coding RNAs (lincRNAs) were the most abundant. Further, these up-regulated lincRNAs were classified into early (< 1 h) and late (> 3 h) responders based on their expression pattern. Silencing two early responders significantly reduced the accumulation of jasmonates (JAs), JA-regulated defensives and resistance to *Manduca sexta* attack, which revealed lncRNA's roles in regulating JA-mediated plant defense. By lncRNA sequencing of JA-deficient mutants, most late responder lincRNAs were predicted to be transcriptionally regulated by JA signaling. Our study uncovers a new role for lncRNAs in mediating JA-mediated herbivore response.

AP 02

The AP2 transcription factor *NtERF172* confers drought resistance by modifying *NtCAT*

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Drought stress often limits plant growth and global crop yields. Catalase (CAT)-mediated hydrogen peroxide (H_2O_2) scavenging plays an important role in the adaptation of plant stress responses, but the transcriptional regulation of the *CAT* gene in response to drought stress is not well understood. In this study, an APETALA2/ETHYLENE RESPONSIVE FACTOR (AP2/ERF) domain-containing transcription factor (TF), *NtERF172*, which was strongly induced by drought, abscisic acid (ABA), and H_2O_2, was isolated from tobacco (*Nicotiana tabacum*) by yeast one-hybrid screening. *NtERF172* localized to the nucleus and acted as a transcriptional activator. Chromatin immunoprecipitation, yeast one-hybrid assays, electrophoretic mobility shift assays, and transient expression analysis assays showed that *NtERF172* directly bound to the promoter region of the *NtCAT* gene and positively regulated its expression. Transgenic plants overexpressing *NtERF172* displayed enhanced tolerance to drought stress, whereas suppression of *NtERF172* decreased drought tolerance. Under drought stress conditions, the *NtERF172*-overexpressed lines showed higher catalase activity and lower accumulation of H_2O_2 compared with wild-type (WT) plants, while the *NtERF172*-silenced plants showed the inverse correlation. Exogenous application of amino-1,2,4-triazole (3-AT), an irreversible *CAT* inhibitor, to the *NtERF172*-overexpression lines showed decreased catalase activity and drought tolerance, and increased levels of cellular H_2O_2. Knock down *NtCAT* in the *NtERF172*-overexpression lines displayed a more drought stress-sensitive phenotype than *NtERF172*-overexpression lines. We propose that *NtERF172* acts as a positive factor in drought stress tolerance, at least in part through the regulation of *CAT*-mediated H_2O_2 homeostasis.

AP 03

Tobacco yield improvement by photosynthetic antenna engineering

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Increasing tobacco yield and productivity is important for tobacco farmers and the tobacco industry. To improve tobacco quantity and maintain sustainability of tobacco production, different efforts have been involved, such as variety evaluation, agronomy practice management, soil and fertilization management. The objective of this research on photosynthetic antenna engineering is to increase the tobacco's efficiency in turning atmospheric carbon dioxide into energy and biomass through photosynthesis.

During photosynthesis, at low sunlight intensities, absorbed photons (solar energy) are utilized efficiently. As the level of irradiance increases further (high sunlight intensity), photosynthesis becomes saturated and reaches a plateau since the carbon reactions (photosynthesis) cannot keep up with the linear increase in light absorption. Under bright sunlight conditions (higher light intensity), wild type plant lines with their fully developed light-harvesting antenna utilize photons inefficiently; only about 20 % of the incoming sunlight energy is converted into useful photosynthesis, while excess absorbed energy is dissipated by the non-photochemical quenching (NPQ) process, which also needs extra energy from the plant.

Downregulation in the expression of the sunlight harvest particle *SRP43* gene in tobacco conferred a Truncated photosynthetic Light-harvesting Antenna (TLA) property mutant tobacco line, resulting in a greater leaf-to-stem ratio and improved photosynthetic productivity and canopy biomass accumulation under high-density cultivation conditions. The performance of TLA transgenic plants will be discussed in detail.

AP 04

Reduction of cadmium in tobacco leaves

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Cadmium is a heavy metal classified as a Class 1 "known human carcinogen" by the International Agency for Research on Cancer (IARC) and is present in both combustible and smokeless tobacco products. Tobacco plants are particularly efficient in accumulating cadmium with most of it being translocated to the leaves. Specialized membrane transport proteins in the form of channels, carriers or pumps mediate the movement of heavy metals through membranes. Heavy metal P-type ATPases (HMAs) are a subgroup of the P-type ATPases super family that contributes to long distance translocation of heavy metals. To reduce tobacco leaf cadmium, a

tobacco HMA RNAi construct was introduced into three commercial varieties of different tobacco types, flue-cured, Burley and dark. Control and transgenic lines were produced under controlled greenhouse conditions with and without the addition of cadmium to the medium. These plants were tested for accumulation of cadmium in roots and aerial portions of the plant. Results demonstrated that leaf cadmium reduction varied among selected transgenic plants and ranged from 88 (TN90 06T44) to 91 % (VA359 06T498). Cadmium content in other aerial parts such as bark and pith were also reduced in the range of 72-91 % and 79-92 % respectively. In contrast, root cadmium levels increased up to 20-fold in transgenic plants compared to controls. Results confirmed that the tobacco HMA is involved in root to shoot cadmium transport and disruption of its function reduces cadmium levels in aerial parts of the tobacco plant under experimental conditions.

AP 05

Biological control of bacterial wilt by constructing tobacco rhizosphere microbial community at the seedling stage

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Tobacco bacterial wilt caused great damage to tobacco production in China, especially in the southwest. It is a new approach to explore the use of the suppressive-soil to construct tobacco healthy rhizosphere soil for biological control of bacterial wilt, and it will provide a theoretical basis for novel biological control of tobacco. The disease-suppressive (hardly affected with disease) and -conducive soils (easily affected with disease) in Guizhou Province were used in seedling and potted simulation experiments. The treatments were designed as follows: Cn+Cp-conducive-soil in both seedling and pot experiments; Cn+Sp-conducive-soil in seedling and suppressive-soil in pot experiment; Sn+Cp-suppressive-soil in seedling and conducive-soil in pot experiment; Sn+Sp-suppressive-soil in both seedling and pot experiment. The high-throughput sequencing and Biolog technology were used to study the changes in the structure, function, and metabolic genes of soil microbial communities. The results obtained show that the control effect of Sn+Cp treatment was significantly higher than that of the Cn+Sp treatment. There was no significant difference of the functional diversity of soil microbial community between Sn+Cp and Cn+Sp treatments at 90 d. However, *Gemmatimonadetes* and *Nitrospirae* of the Sn+Cp treatment were significantly higher than those of Cn+Sp treatment, while Actinobacteria and Acidobacteria showed a reverse trend in Sn+Cp and Cn+Sp. The metabolic genes of general function prediction only, protein turnover and signal transduction mechanisms of the Sn+Cp treatment were up-regulated compared with those of Cn+Sp treatment, while transcription and carbohydrate transport and metabolism of Sn+Cp treated soil were down-regulated. Furthermore, there were more significant correlations between soil microorganisms and soil properties in the Sn+Cp treatment, compared with that in Cn+Sp

treatment. In summary, the use of suppressive soil microorganisms in the seedling stage can construct healthy microbial communities in tobacco rhizosphere, thus reducing the occurrence of bacterial wilt. This result will provide new approaches for the biological control of tobacco bacterial wilt.

AP 06

Coping with spotted wilt in tobacco

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Spotted wilt, caused by tomato spotted wilt virus, is a major cause of disease loss in Georgia tobacco. The disease begins in spring weeds where juvenile tobacco thrips (*Frankliniella fusca*) acquire the virus. When these juveniles develop into adults they are able to transfer the virus to tobacco. Spotted wilt was first noted in Georgia tobacco in 1985. A minor epidemic developed causing no significant loss from 1989 to 1994. Beginning in 1995 the situation changed and a major epidemic causing high state wide loss occurred for the next 15 years. Since 2010 losses have been less but still significant in places. In 1997 tray drench treatments with imidacloprid for soil insect control were found to reduce spotted wilt.

In 1999 a greenhouse seedling treatment with acibenzolar-s-methyl (Actigard) showed promise for reducing spotted wilt. A management program based on an Actigard/imidacloprid dual treatment was developed. Since 2000 there have been 266 trials where the dual treatment was evaluated. The mean level of control seen is 46.3 %. Actigard treatment frequently causes obvious early season slow growth, however trials in North Carolina showed no loss of yield associated with Actigard use. While Actigard/imidacloprid is the only effective treatment for reducing spotted wilt, year to year and farm to farm variation in quality of control has been seen. Some of the variation seems related to imidacloprid performance. Data does not suggest any loss of sensitivity to imidacloprid in the tobacco thrips vector of spotted wilt. It has been observed that tobacco set in fields where soil conditions were poor for active root growth experienced reduced spotted wilt control. Trials conducted in Georgia have found that soil temperature during the first two weeks after transplanting is related to spotted wilt control (R^2 = 0.31; p=0.0015). Higher soil temperature was related to better spotted wilt control.

AP 07

The *eIF(iso)4E-T* deficient *va* tobacco is superior to Virgin A Mutant in resistance to Potato Virus Y

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Potato Virus Y (PVY) infects tobacco (*Nicotiana tabacum*), inducing vein necrosis and thereby causing yield and quality losses. Tobacco strains with *va* locus (deletion of *Va* that includes *eIF4E1-S*), for example Virgin A Mutant (VAM) and TN90, show resistance to PVY. Actually, VAM shows the highest resistance among the *va* tobaccos. Although deletion mutants of *eIF4E1-S* resist PVY, resistance-breaking strains of PVY (RB-PVY) have appeared. Earlier studies demonstrated that the loss of function of a tobacco *eIF(iso)4E-T* gene reduces susceptibility to RB-PVY and that simultaneous inhibition of both *eIF4E1-S* and *eIF(iso)4E-T* functions confers enhanced resistance to both PVY and RB-PVY. This study clarifies whether the *eIF(iso)4E-T* deficient *va* tobacco (*eIF4E1-S* and *eIF(iso)4E-T* double mutant, designated as 'TN90-iso-t') shows higher resistance to PVY than VAM. PVY assay revealed that TN90 and VAM showed little or no symptom at 12 days post-inoculation (dpi), but 40 % (VAM), and 64 % (TN90) of plants showed symptoms at 34 dpi. The VPg sequences of PVY infecting TN90 at 28 dpi had RB-PVY type mutations (V108I or K105E) and these mutations were never detected in the inoculum and susceptible variety at 28 dpi, which suggest that the PVY genome was randomly mutated and eventually to allow the virus to adapt to infect TN90 lacking *eIF4E1-S*. By contrast, in TN90-iso-t, vein necrosis was observed for only one of 25 plants at 34 dpi and 54 dpi. ELISA revealed only slight PVY accumulation, even at 56 dpi in TN90-iso-t. Consequently, TN90-iso-t was near-immune to PVY. Therefore, we demonstrated that the *eIF(iso)4E-T* deficient *va* tobacco showed higher PVY resistance than VAM. Because the double mutation did not affect plant growth, TN90-iso-t is expected to be useful for the breeding of PVY-resistant and RB-PVY-resistant tobacco.

AP 08

Potential fungicides and biocontrol agents to manage target spot (*Thanatephorus cucumeris*) on flue-cured tobacco in Virginia

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Target spot (*Thanatephorus cucumeris*) remains a threat to flue-cured tobacco production in Virginia, and field experiments since 2001 have sought to identify effective fungicides for target spot control. Trials in 2017-2019 included fungicides from FRAC groups 4, 7, 11, and 44, and biocontrol agents from FRAC groups P01, P05 and P06. Plots were arranged in a randomized

complete block design with 5-6 replications, and consisted of one border row on either side of a 40 ft test row on 4 ft row centers. Percent leaf area damage (%LAD) was subjectively rated using a 0-100 % scale. Tobacco leaves were harvested as they ripened and cured as recommended. Data were evaluated via analysis of variance and the Waller-Duncan test (k-ratio=100; $P \leq 0.05$). Inpyrfluxam reduced %LAD in 2017 compared to the untreated control when other spray programs did not. Target spot damage in 2018 was also significantly lower where inpyrfluxam had been applied vs no treatment. Azoxystrobin alternated with *Bacillus mycoides* isolate J reduced %LAD on 23 July, but only on lower leaves. Azoxystrobin sprays at layby and topping reduced %LAD on lower leaves only on 6 August. Target spot damage just before harvest in 2019 was again significantly reduced by inpyrfluxam versus the untreated control, but not by any other fungicide or biocontrol treatments. Disease was significantly lower than the control on 7 August where inpyrfluxam, flutriafol, metconazole, azoxystrobin, azoxystrobin alternated with *Bacillus mycoides* isolate J, or mancozeb had been applied. Although fungicide treatments never increased total cured-leaf yield compared to the untreated control in these experiments, yields from plots receiving inpyrfluxam were often the highest in each trial, and were 282-342 lb/A higher than the untreated control in 2019.

AP 09

Multi-omics data revealed nov-miR1170 regulates nicotine biosynthesis of *Nicotiana tabacum*

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Nicotine is the main alkaloid in tobacco (*Nicotiana tabacum*), accounting for about 90 % of the total alkaloids. For this reason, tobacco is a good model plant for the study of nicotine in the fields of biosynthesis, transportation, accumulation and degradation. Most of the genes involved in nicotine biosynthesis (like QPT, PMT, ODC, ADC, A622 and so on) have been identified and characterized, while whether or not miRNAs are involved in this pathway is largely unknown. Here, the cultivated tobacco variety ZY100 and its high nicotine EMS mutant YJ1 (YJ1 leaves accumulate 2-fold higher nicotine than ZY100) were used to investigate miRNA's function by integration analysis of transcriptome, miRNAs and degradome data. Data analysis of transcriptome and miRNAs revealed 3,865 differentially expressed genes and 193 differentially expressed miRNAs. Using degradome sequencing and *in silico* approaches, 77 miRNA loci were predicted to target six nicotine biosynthetic and seven jasmonate pathway genes. One novel miRNA nov-miR1170 was validated by transient expression experiment *in vivo*. Over-expression of nov-miR1170 significantly reduced the expression of its target gene QPT (48 % - 67 %) and the nicotine content (42 % - 72 %). This study uncovers that nov-miR1170 can regulate nicotine biosynthesis by targeting QPT gene in tobacco.

AP 10

Evaluation of low-nicotine tobacco cultivars and agronomic production practices by non-destructive photonic sensing

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In the last few years both FDA and WHO have recommended the lowering of nicotine levels in cigarettes to reduce overall addiction. As a consequence, the interest in low nicotine cultivars and agronomic practices that can reduce nicotine accumulation in tobacco leaves has increased, but presently there are still open questions on the results for growers and industry, especially for quality and smoke flavour characteristics. This study aimed to apply a non-destructive photonic sensing method to the proximal detection of nitrogen (N) status at the stage of maximal plant N assimilation, to find any correlation with leaf nicotine content (%) at harvest.

A varietal test was carried out at Fattoria Autonoma Tabacchi (FAT - Città di Castello [PG], Italy), comparing standard and new low-nicotine cultivars of Virginia Bright tobacco (SLV, ITB697, MS K326 LA, K326) managed with local best practices (standard nitrogen fertilization [N] and topping) and with low-nicotine agronomic practices (reduced N [50 kg/ha] 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 the cultivars. 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. In the field, the NBI index at maximal plant N assimilation was shown to be correlated to leaf nicotine content (%) destructively measured at harvest.

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

AP 11

Analysis of the surface chemistry of high- and low-alkaloid Burley varieties/lines grown using standard agronomic practices and under low-nicotine field management

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Proposed FDA regulations may require significant reduction of the nicotine content of tobacco products. Lowering the nicotine content may impact tobacco leaf surface chemistry that affects flavour. The objective was to compare the leaf surface chemistry profiles of high- and low-alkaloid Burley varieties/lines grown under standard and low-nicotine field practices. Six varieties/lines, one check and five low alkaloid lines, were sampled shortly after topping. Leaf surface exudate was collected via solvent extraction and analyzed by GCMS. Qualitatively, chemical profiles of trichome exudate of four Burley lines (LA TN 90LC, ITB 1501, J14 and J29) and the check (TN 90LC) were highly similar, consisting of α- and β-cembratriene-diols (CBT-diol isomers), apparent diterpenoid oxidation products, sugar esters (SEs) (minor) and free sugars. The low-alkaloid French line ITB 259 produced labdane-type diterpenes *cis*-abienol and labdene-diol, but not cembranoid diterpenes. Such diterpene composition is unusual for a Burley tobacco. TN90 LC and LA TN 90LC had similar surface chemistry profiles and abundance of total compounds under standard field practices. Sugar esters and oxidized products were reduced in the six varieties/lines under the low nicotine field practices. The mutations leading to low-alkaloid variant of TN 90LC did not impact the leaf surface chemistry. Low-alkaloid field practices likely lowered the quality of Burley tobaccos.

AP 12

Reducing nicotine in Burley tobacco by combining low alkaloid varieties and agronomic practices – a very different second year

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In 2018, we did a study to test whether nicotine levels of 0.3 - 0.5 mg/g could be achieved if the currently available low alkaloid (LA) varieties were grown using all agronomic practices that are known to lower nicotine. One standard variety, TN 90LC (check), and three LA varieties, LA TN 90, ITB 1501 and ITB 259, were grown with standard recommended practices and also with practices designed to lower nicotine (no topping, close spacing, low nitrogen, early harvest, irrigation). When grown with the nicotine-reducing practices, only ITB 1501 had nicotine < 0.5 mg/g for the whole plant and in each stalk position except tips. The abnormally wet 2018 growing season resulted in atypically low nicotine levels. The study was repeated in 2019, with very different results. 2019 was very dry, and nicotine levels were unusually high. In the nicotine-

reducing practices, none of the varieties had nicotine < 0.5 mg/g. ITB 1501 had the lowest nicotine in the whole plant (1.5 mg/g) and in all stalk positions (1.6 mg/g in the primings, 1.5 mg/g in the lugs and leaf, and 1.4 mg/g in the tips). This variety has consistently had the lowest nicotine of all the LA varieties tested. The yield data were the inverse of those in 2018. In the very wet 2018 season, the yields in the standard practices treatment were acceptable, but in the nicotine-reducing practices treatment they were very low. In the very dry 2019 season, the yields in the standard practices treatment were low, because the tobacco was severely droughted, but the yields in the nicotine-reducing practices treatment were acceptable, because the tobacco benefitted from irrigation. These data illustrate how large an effect season has on alkaloid levels. Nicotine in the 2019 check was almost twice that in the 2018 check. The results from these two very different seasons show that it is impossible to consistently achieve a target nicotine level.

AP 13

Effect of tilling depth on bacterial community structure and enzyme activities of flue-cured tobacco rhizosphere soil in mountainous areas of Yunnan

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To explore the effects of different tillage depths on the soil microecological environment of mountainous tobacco fields in Yunnan, PRC, four treatments including 20 cm of rotary tillage (RT20, control), 30 cm of deep tillage (DT30), 30 cm (ST30) and 40 cm (ST40) of subsoiling tillage were set. The changes of bacterial community structure and enzyme activity of flue-cured tobacco rhizosphere soil were then analyzed. Results showed that the DT30, ST30 and ST40 measures significantly reduced the activity of catalase, and increased the activities of urease, acid phosphatase and cellulase, compared to RT20. The catalase activity with DT30 and ST40 measures decreased by 47.83 % and 68.16 %, acid phosphatase activity of ST30 and ST40 treatments increased by 652.77 % and 432.77 %, respectively, with significant differences ($P < 0.05$). Deep tillage and subsoiling tillage measures were beneficial to increase the diversity and richness of bacterial communities. The dominant bacterial communities and relative abundance under tilling treatments were significantly different at phylum and genus levels. Compared with RT20, the relative abundance of *Gemmatimonadetes* of DT30, ST30 and ST40 increased by 30.93 %, 20.97 % and 11.44 % respectively, and the relative abundance of *Nitrospirae* increased by 54.55 %, 22.73 % and 11.36 %, respectively. In addition, the relative abundance of *Nocardioides*, *Gemmatimonas*, *Sphingomonas* and other beneficial bacterium in DT30, ST30 and ST40 treatments were more than RT20. Correlation analysis showed that the distribution of bacterial communities under different tillage treatments was significantly

correlated with the soil enzyme activities. For example, *Marmoricola*, *Nocardioides*, and *Sphingobium* were significantly positively correlated with acid phosphatase activity, *Bradyrhizobium*, *Streptomyces*, *Mesorhizobium*, *Mycobacterium* were significantly negatively correlated with urease activity. This indicates that the change of cultivation methods has created different ecological niches of rhizosphere soil. The selection and adaptation of different microorganisms to the niches may be the main reason for the changes in microbial species composition and community structure.

AP 14

A comparison of traditional and alternative fertilizer programs for flue-cured tobacco production

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Previous fertilizer research in flue-cured tobacco has compared a wide range of nutrient programs, ultimately demonstrating the usability and function of numerous sources. The objective of this study was to investigate the influence of nitrogen (N) based sources on the assimilation of other macro and secondary nutrients. Research was conducted in two environments in 2019 to evaluate various combinations of basal and sidedress N sources. Basal treatments consisted of granular 6-6-18 or liquid 28-0-0 urea-ammonium nitrate (UAN) and potassium sulfate (0-0-50). Sidedress N sources were ammonium nitrate potassium (ANK), ammonium nitrate sulfate (ANS), or UAN, which were applied four weeks after transplanting. Each basal and sidedress source was paired to create a two x three factorial treatment arrangement. Four weeks after transplanting, the analysis of foliar tissue samples revealed higher concentrations of phosphorus and potassium in treatments containing 6-6-18 (0.36 and 3.31 %, respectively) relative to UAN and potassium sulfate (0.29 and 3.04 %, respectively). Foliar concentrations of macro and secondary nutrients were similar at flowering, with the exception of sulfur, which was lowest in the UAN + UAN treatment. After curing, foliar potassium was greatest in treatments containing 6-6-18 + ANK (2.12 %) relative to 6-6-18 + UAN (1.78 %), UAN + UAN (1.80 %), and UAN + ANK (1.57 %). Despite differences in foliar nutrient concentrations, cured leaf yield, quality, price, and value per hectare were similar, meaning that each of these fertility programs is likely suitable for use in tobacco production. This point is reinforced by the fact that nutrient concentration was deemed sufficient within each sampling interval, regardless of treatment. With the knowledge that practical differences are unlikely to be documented across the programs we evaluated, commercial farmers are encouraged to consider fertilizer price rather than performance when considering these nutrient sources.

AP 15

Nitrogen fertilizer source and the impact to flue-cured tobacco nutrient assimilation, yield, quality, value, and chemistry

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The impact of nitrogen (N) fertilizer sources to macro, secondary, and micronutrient assimilation at various growth stages and their impact to post-harvest measurements have not been reported in flue-cured tobacco. Research was conducted in 2016 and 2017 to test the effects of four N fertilizer sources (calcium nitrate, calcium ammonium nitrate, liquid urea-ammonium nitrate, and ammonium sulfate) on these parameters. Foliar concentrations of total N, phosphorus, magnesium, and chloride were not impacted by N source in green or cured leaf samples. In contrast, foliar nitrate, potassium, and sulfur concentrations were sometimes influenced by N treatment. Results were variable; however, visual nutrient deficiencies were not observed nor were they analytically identified, thus indicating that nutrient assimilation was sufficient. In contrast, foliar boron concentrations were identified as deficient in all treatments 3 weeks after transplanting and at layby. Applications of urea-ammonium nitrate reduced boron concentration by ≈ 2 to 4 mg kg^{-1} relative to other N sources two weeks after layby and at flowering, with only the latter below the established sufficiency minimum of 18 mg kg^{-1}. However, visual symptoms of boron deficiency were not observed. Calcium concentration was greatest in treatments comprised of calcium nitrate, which provided an additional 95-112 kg Ca ha^{-1}, depending on environment. Residual soil calcium was 10-times greater than what is required for maximized growth; therefore, the slight increase in calcium uptake has little practical value. Foliar calcium concentration was deficient two weeks after layby (< 0.75 %) and was borderline deficient at flowering, regardless of treatment. Cured leaf concentration was remarkably higher, thus indicating that deficient calcium concentrations are often transient and will recover post-topping. Overall, these differences are likely to be of little concern as cured leaf yield, quality, value ha^{-1}, price kg^{-1}, and total alkaloids and reducing sugars were similar among all N sources. Nitrogen fertilizer source appears to have little practical effect on the assimilation of macro, secondary, and micronutrients, regardless of N source. While nutrients, such as Ca and B, were deficient at different stages of growth, visual symptoms of deficiency were not observed nor were post-harvest measurements impacted. Based upon these results, commercial farmers have great flexibility in regards to N source selection.

AP 16

Nitrogen application programs for fine-textured soils of the North Carolina Piedmont

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Late-season nitrogen (N) assimilation can greatly impact the yield and quality of flue-cured tobacco, particularly in the fine-textured Piedmont soils of North Carolina. Research was conducted in three environments to evaluate the effects of N application rate and number of N applications to the yield, quality, value, and leaf chemistry of flue-cured tobacco. Liquid N (28 % urea-ammonium nitrate) was applied at 56, 78, and 101 kg N/ha. Each rate was either applied in two (50 % of the target rate 7–10 days after transplanting and 50 % of the target rate five weeks after transplanting) or three splits (50 % of the target rate 7–10 days after transplanting, 25 % of the target rate five weeks after transplanting, and 25 % of the target rate seven weeks after transplanting). Cured leaf N concentration was similar at 56 and 78 kg N/ha (2.58 and 2.61 %, respectively) but was increased in treatments receiving 101 kg N/ha (2.77 %). Additionally, three N applications (2.73 %) increased cured leaf N relative to two N applications (2.58 %). The same treatment parameters did not impact yield or value but reduced cured leaf quality in one growing environment due to prolonged N assimilation resulting from dry conditions. Within this environment, quality was greatest when 56 kg N/ha was applied in two applications. Results indicate that current recommendations for N application rates (56 kg/ha) and timings (split-applied twice in equal portions) are adequate to obtain maximum yield, quality, and value in fine-textured soils similar to those evaluated in this study.

AP 17

Establishing nitrogen fertility recommendations for the production of organic Burley tobacco

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As demand increases for organic tobacco products, growers transitioning to certified organic production systems require appropriate agronomic recommendations. Two studies were conducted at the Mountain Research Station in Waynesville (W), NC and the Upper Mountain Research Station in Laurel Springs (LS), NC in 2018 and 2019. Study one investigated the effects

of application methods (broadcast, split application, or side dress) of pelleted, hydrolyzed feather meal (Nature Safe® 13-0-0) and composted chicken poultry manure (Harmony 5-4-3). Study two compared application rates (168, 224, 280, and 336 kg N·ha^{-1}) of both organic fertilizers. A conventional control (SQM 12-0-46 + 28-0-0 liquid UAN) was split applied (112 kg N·ha^{-1} broadcast during field prep and 112 kg N·ha^{-1} side-dressed at layby). Results from 2018 indicated a slight advantage when using feather meal over poultry litter as the former increased leaf alkaloid content. Furthermore, split application resulted in higher yields compared to broadcast application at W (4353.5 kg·ha^{-1} vs 3763.5 kg·ha^{-1}, respectively). Fewer differences were observed from study one in 2019. Yields averaged 3747 kg·ha^{-1} and 2280 kg·ha^{-1} in LS and W, respectively, and were not affected by application method. No differences were observed in yield and leaf quality at the LS location for study two in 2019. Leaf yield from W showed a significant quadratic rate response, peaking at 2,500 kg·ha^{-1} with 280 kg N·ha^{-1}. Though differences among the application methods did not exist, it is generally recommended to split apply nitrogen source material to avoid potential loss from heavy rain events. Furthermore, we found no benefit to application rates above 280 kg N·ha^{-1}. The general lack of differences among the two organic fertilizer options and the conventional check indicate that both poultry litter and feather meal are appropriate nitrogen sources for organic Burley tobacco production.

AP 18

Effects of polyethylene mulches on pest management and yield in organic flue-cured tobacco

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Management of weed and insect pests in organic production of flue-cured tobacco is challenging due to lack of effective approved control options. Polyethylene plastic mulches are commonly used in vegetable and berry production to manage in-row weed populations, buffer soil temperatures, limit rain-induced soil loss, and maintain soil moisture. Mulch color has been shown to impact plant growth, soil temperature and insect pest populations in vegetable crops. The objective of this study was to evaluate the effects of different colors of polyethylene mulches in organic flue-cured tobacco production. Three trials were conducted in three environments with four colors of polyethylene mulch (red, white, black, and silver) with drip irrigation and bare ground with and without drip irrigation. Light reflectance, soil temperature, aphid population, plant growth, weed emergence, leaf yield and quality data were collected. In 2019, the silver mulch treatment maintained the lowest early season aphid counts (0.68 per plant) compared to bare ground with drip, red, black, and white mulches, which ranged from 4.07 to 6.17 per plant. In-row weed emergence in black, white and silver mulch treatments ranged from 16.7 to 70 seedlings per m^2, 170 seedlings per m^2 in bare ground without irrigation and 333.3 seedlings per m^2 in red mulch treatment. Maximum average soil temperature in bare ground treatments were consistently higher than the silver treatment throughout the entire

growing season. Tobacco yield and quality were not different among all treatments except for the red plastic mulch which showed a reduction in leaf yield and quality. Additional data from 2020 trials will also be presented. Under favourable conditions, there is no yield or quality benefit of utilizing polyethylene mulches in flue-cured tobacco production. This is consistent with research in other crops. Given increased weed or insect pest pressure or drought conditions, treatment differences may become more apparent.

AP 19

Impacts of conservation tillage and cover crop mulch on weed emergence and leaf yield and quality in organic flue-cured tobacco

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Intensive tillage in flue-cured tobacco production contributes to soil erosion and reduced water-holding capacity of soils. Conservation tillage minimizes soil disturbance by planting a crop directly into biomass residue of overwintered cover crop using specialized planting equipment. Reducing tillage has been shown to improve soil health (increased rainwater infiltration, improved water-holding capacity, reduced erosion), reduced production costs (fuel and labor) and improved weed management. Weed management is the biggest challenge in an organic production system. Given the lack of effective approved herbicides, cultivation is the main method of weed management but can be expensive and becomes difficult or impossible in a wet season. The objective of this study was to compare the effects of conservation and conventional tillage on weed emergence, yield, quality and cured leaf chemistry of flue-cured tobacco grown without herbicides. Split-plot randomized complete block trials with four blocks were conducted in three environments with raised bed and flat ground field preparation as the main plot and conventional and conservation tillage as the split plot treatment. A cereal rye cover crop was established in the fall and terminated in the spring, via tillage (conventional) or roller-crimper (conservation tillage). The tobacco cultivar NC 196 was grown in all plots. Cover crop biomass, weed emergence by species, soil compaction, yield, quality and cured leaf chemistry data were measured. In 2019, conservation tillage reduced weed emergence by 42.14 % compared to conventional treatments (P = 0.0003). Cured leaf value was 35.38 % higher under conservation tillage practices than conventional (P = 0.0171). Additional data from 2020 trials will also be presented. These results indicate that conservation tillage practices may be an effective weed management strategy, while improving yields in an organic production system.

AP 20

Winter cover crop management in the production of organic flue-cured tobacco

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The production of organic flue-cured tobacco requires costly nitrogen (N) management strategies, intensive tillage and bedding, and repeated cultivation for bed maintenance and weed control. This study was conducted to determine if cover cropping could mitigate the significant economic investment of N fertilization in organic tobacco. In 2018 and 2019, the effects of three winter cover crops: hairy vetch (*Vicia villosa* Roth); Austrian winter pea (*Pisum sativum* var. *arvense* L.); and crimson clover (*Trifolium incarnatum* L.), were compared to typical N management without a cover crop, and investigated for their potential to supplement N to a flue-cured tobacco crop in North Carolina. Tobacco yields were significantly greater under cover crop treatments in both years. In 2018, all cover crops improved yields by an average of 30 %, and hairy vetch increased yields at one location by 38 % (3,242 kg ha^{-1}) compared to no cover (2,357 kg ha^{-1}) in 2019. Crop value was significantly greater in legume cover crop treatments at one location in 2019 by \$2,985 ha^{-1}, and did not differ from the control in 2018. In 2019, in-season soil samples showed cover-crop N was removed from the system at topping, a critical marker of a necessary N-free period for the maturation of the leaves. Only hairy vetch at the Kinston location showed elevated nitrogen at topping compared to the control, however this did not have a negative effect on quality, yield, or value. Cover crops are not traditionally used in tobacco production due to concerns with unpredictability of nitrogen supply and extended N mineralization compromising the quality of the cured tobacco leaf. Our research did not support these concerns, and found evidence supporting the potential use of cover crop N as a cost-effective management strategy in the production of flue-cured tobacco.

AP 21

Impacts of lower-leaf removal timing, number, and nitrogen application to flue-cured tobacco

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The removal and exclusion of lower-stalk tobacco from harvest continues to be encouraged in North Carolina. Previous research efforts have been focused on the simultaneous practices of topping and leaf removal. Very little information has been reported that addresses the timing aspect of leaf-removal, specifically when it occurs late in the growing season. Research was

conducted in four North Carolina environments to evaluate each possible treatment combination of two lower-leaf removal programs (0 and 8 leaves plant^{-1}), three removal timings (two weeks before topping, at topping, and two weeks after topping), and two N application rates (0 and 11 kg N ha^{-1}). SPAD measurements consistently revealed a lighter leaf color in treatments consisting of leaf removal two weeks before topping, regardless of N application rate. Foliar cured leaf samples from upper-stalk positions also contained less total N when eight leaves (2.25 %) were removed relative to zero leaves (2.32 %). These results indicate that subsequent N fertilizer application did not supply N as efficiently as remobilization from lower, older leaves that were entering into a senescent stage of development. In the eight-leaf removal program, both cured leaf yield and value declined by 27 % relative to the zero leaf program. Despite significant losses in yield and value, the eight-leaf program completely eliminated lug grades of tobacco. Leaf removal timing and N application rate did not affect yield, quality, value, or grade distribution. Our results suggest that there is no agronomic benefit or cost to removing lower-leaves two weeks before or after topping; however, commercial farmers may find this information to be of use from a time management perspective, should they decide to remove lower-stalk leaves.

AP 22

Cigar wrapper tobacco production in western North Carolina

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Since 2000, Burley tobacco production has declined by more than 2,000 ha in the Appalachian mountain region of western North Carolina. The loss of Burley tobacco has left a void in the agricultural economy that has not yet been filled by another commodity. Cigar wrapper tobacco types, such as Pennsylvania seedleaf (PA41) and Connecticut Broadleaf, may be suitable replacements for Burley because of overlapping production practices and a favorable growing climate. Cigar tobacco has not been produced in western North Carolina; therefore, very little is known about the production system. The objective of our study was to quantify the days to flower removal, yield, and grade distribution of six cigar tobacco varieties in two separate environments. In each environment, three PA41 varieties (Eshbach, Grower's Choice, Welk's Pride) and three Connecticut Broadleaf varieties (B2, D1, and PAB) were compared. PA41 varieties consistently out yielded Connecticut Broadleaf varieties by 766 to 1,304 kg ha^{-1} and required an additional 10-12 days to reach full flower. PA 41 likewise produced a higher percentage of wrapper grades in one environment, while Connecticut Broadleaf produced more binder grades in another. Where statistical differences were observed within each tobacco type, PAB and Welk's Pride were the highest yielding varieties. The effect of variety was not significant for days to flower removal or wrapper grade production. In general, wrapper grade frequency was very low in this study (0-6 %), which was a result of reactive and insufficient

pesticide application, higher than recommended topping height, and aggressive plant handling. We believe that cigar tobacco can be successful in western North Carolina; however, it will not come absent of significant extension education and research investment. Future research is planned in North Carolina for both tobacco types.

AP 23

Industrial hemp: the benefits, concerns, and unknowns for North Carolina tobacco farmers

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North Carolina is the leader in flue-cured tobacco production in the United States; however, a growing reduction in demand has led growers to seek other sources of farm gate revenue. In 2014, the federal Farm Bill legalized state regulated growth of industrial hemp (*Cannabis sativa* L.) for research. The following year, North Carolina began a pilot program that afforded licensed growers the opportunity to produce industrial hemp, creating a new source of income for tobacco farmers – particularly in the biomass cannabidiol (CBD) market. Early indications are that flue-cured tobacco farmers have a distinct advantage with industrial hemp production systems, due to overlapping equipment needs, experience with greenhouse seedling production, approaches to pest management, product quality control, and drying/curing facilities. The integration of industrial hemp with flue-cured tobacco systems has not been absent of concern, due to the many knowledge gaps that currently exist because of the long-time illegality of the crop in the United States. This presentation will address some of the information that has been learned since 2015 and will focus on the potential benefits to integrating hemp with flue-cured tobacco systems. We will also address topics of concern as they pertain to disease, insect, nutrient, and weed management.

AP 24

Economic factors influencing lower leaf removal decisions

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With a current global over-supply of flue-cured tobacco, tobacco producers in North Carolina have been encouraged to remove the lowermost leaves prior to harvest due to their low value in manufactured products. In field trials, the removal of four or eight leaves per plant at topping reduced cured leaf yield by 644 kg/ha and 902 kg/ha, respectively, when compared to systems absent of leaf removal (2973 kg/ha). While cured leaf quality was not affected by leaf removal programs, per hectare value declined by 19 % to 24 % within the same treatments. Despite the negative impacts to yield and value, the four-leaf removal program did not impact crop throw. However, the eight-leaf removal program completely eliminated lug grades while increasing the portion of leaf and tip grades. Machine-harvest production budgets indicate that net economic return was reduced from approximately $US 2764/ha in programs absent of lower leaf removal to $US 982/ha and $US 671/ha in four- and eight-leaf removal programs, respectively. In hand-harvest programs, net return was reduced even further, with treatments absent of leaf removal being more profitable than the four- and eight-leaf removal programs ($US 2020, $US 301/ha, and $US 80/ha, respectively). Ultimately, cost savings are found in leaf removal programs; however, they are not large enough to offset the profitability reductions generated from significant yield losses. At present, these systems do not appear to be economically feasible or sustainable for US farmers without subsequent price increases ranging from $US 0.75/kg - $US 0.99/kg.

AP 25

Analysis of TSNAs and their relationship with alkaloids in cigar wrapper and filler tobaccos from different regions and varieties

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Cigar tobacco production is growing rapidly as cigar consumption has been increasing recently in China. In order to be clear about the current status of tobacco-specific nitrosamine (TSNA) contents of cigar wrappers and fillers from different regions of the world and from different varieties, 55 domestic and imported cigar samples were collected, and 16 cigar varieties were cultivated in Sichuan Province of China for cured sample collection and measurements. The correlation of TSNAs to their precursors, alkaloids and nitrate, was also analyzed. The results showed that the total TSNA content in cigar samples from different producing areas ranged from 1.46 to 25.75 μg/g, with NNN content being the highest among four individual nitrosamines, ranging from 0.63 to 20.91 μg/g, and varying the greatest among all the samples. The nicotine and nornicotine contents were 0.66 % ~ 10.02 % and 0.04 % ~ 0.79 %, respectively, with percent nicotine conversion being 2.07 % ~ 28.01 %. The total alkaloid level in fillers was higher than that in wrappers and binders. However, the contents of nicotine and nornicotine and the nicotine conversion rate varied greatly among samples from the same region and within the same cigar tobacco type, indicating that cigar tobacco from any region had the problem of nicotine to nornicotine conversion that resulted in decreased levels of nicotine and increased levels of nornicotine, which is the precursor of NNN. Correlation analysis showed that nornicotine in cigar tobacco leaves had a significantly positive correlation with NNN and total TSNAs. Results from variety test also showed that all the tested varieties had the problem of nicotine to nornicotine conversion at different degrees, with the percent nicotine conversion in bulk samples ranging from 5.03 % to 53.12 %. Correlation analysis also showed a significant positive correlation between nornicotine and NNN and total TSNAs. In conclusion, current cigar tobacco worldwide has the problem of nicotine conversion, which leads to increased levels of NNN and total TSNAs in both wrapper and filler cigar tobaccos. There is an urgent need to purify the cigar population so as to substantially lower TSNA contents in cigar tobacco and cigar products.

AP 26

Rapid screening of nicotine converters from tobacco seedling population

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Conversion of nicotine to nornicotine is a challenge for tobacco leaf production. A gas chromatography-mass spectrometry-based method was developed for the rapid screening of seedling nicotine converters which would convert a large portion of nicotine to nornicotine as they grow up and mature. Two strategies (with and without incubation) were developed and evaluated. The non-incubation strategy including sampling, extraction, and instrument analysis takes up about 5-6 min for one seedling sample. With this strategy, the complete removal of converters was achieved with the sacrifice of 9 % non-converters. For the incubation strategy, in-tube samples were cured at 37 °C for 12 h before the extraction and the instrument analysis. Complete separation of converters and non-converters was achieved using the incubation strategy. A pseudo percent nicotine conversion (PPNC) was introduced for the replacement of the normally used percent nicotine conversion (PNC) as the converter Adjudicator. The PPNC proved to be simpler and more effective than the PNC. The established method is a good choice for the fast and efficient identification of nicotine converters from a big number of seedling populations.

AP 27

Three-year N-nitrosonornicotine data for stable reduced converter (SRC) dark tobacco crop

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During the past several decades, substantial efforts have been made by the tobacco industry and academic institutions to reduce NNN levels and its precursor nornicotine in tobacco products. Research on the mechanism of nornicotine formation led to the identification of three tobacco genes (*CYP82E4*, *CYP82E5* and *CYP82E10*) encoding for cytochrome P450 nicotine demethylases that convert nicotine to nornicotine. Through conventional breeding, we developed dark tobacco varieties (Stable Reduced Converter (SRC) varieties) in which the three nicotine demethylase genes are non-functional. Tobacco varieties containing these non-functional nicotine demethylase genes, named ZYVERT® technology, were grown in different locations on field research tests for three years (2013, 2014 and 2015) and showed an average NNN reduction of 74 % compared to current industry standard low converter (LC) tobacco varieties. In 2017, 2018 and 2019 Altria Client Services contracted with growers in Kentucky and Tennessee for the on-farm production of dark air-cured and dark fire-cured SRC tobacco

varieties with ZYVERT® Technology. Tobacco bales from the SRC tobacco varieties and commercial LC varieties were sampled at delivery and analyzed for TSNAs. NNN reductions in the SRC crop averaged 52 %, 55 % and about 49 % for dark air-cured and 68 %, 68 % and about 64 % for dark fire-cured, for the 2017, 2018 and 2019 years, respectively, as compared to the LC crop.

AP 28

Correlation between post-curing TSNA increase and alkaloid and nitrite contents in cured leaves

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Alkaloids and nitrite in tobacco leaves are known to be involved in the formation of tobacco-specific nitrosamines (TSNA). The formation is affected greatly by environmental conditions during curing and post-curing stages such as storage. To elucidate the correlation between alkaloid and nitrite contents and the TSNA formation more clearly, cured leaf samples of Burley tobacco with different concentrations of alkaloid and nitrite contents were treated using high-temperature conditions to promote TSNA formation. First, six cured leaf samples with different concentrations of alkaloid and nitrite contents were produced experimentally using a selected variety and modifying cultivation and curing practices. Secondly, heat treatment (HT) at 70 °C for 14 days was applied to them to maximize TSNA formation. The contents of alkaloids, nitrite and TSNA post-curing, and TSNA post-HT were determined using the JT standard method. As planned, samples of six kinds were obtained as combinations of three degrees of alkaloid contents (Low, < 10 mg/g; Middle, 10–50 mg/g; High, > 50 mg/g) and two degrees of nitrite content (Low, < 1.0 µg/g; High, > 1.0 µg/g). All six samples showed low TSNA contents of less than 1.0 µg/g immediately after curing. No positive correlation was found between TSNA contents and alkaloid or nitrite contents. After HT, TSNA contents increased extremely in samples with Middle and High levels of alkaloid content. The relation between the increased amounts of TSNA contents after HT and alkaloid and nitrite contents before HT was indicated. As described above, alkaloid and nitrite contents of cured leaves were related to increasing of TSNA contents depending on post-curing environmental conditions. To elucidate the TSNA formation mechanisms, further investigation is needed.

2020 CORESTA CONGRESS ONLINE

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

POSTER PRESENTATIONS

APPOST 01

Study of fungicides effect of Vista and Nativo 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 purpose of this project was to carry out a performance survey of the fungicides Vista (active ingredient Tricyclazole 37.5 % + Thiophanate methyl 35 %, WP 72.5 %), Nativo (active ingredient Tebuconazole 50 % + Trifloxystrobin 25 %, WG 75 %) and Topsin M (active ingredient Thiophanate methyl WP 70 %) for tobacco collar rot disease management. The experiments were carried out in a completely randomized design with fungicides of Vista, Nativo and Topsin M at concentrations of 0, 50, 100, 150, 200 and 250 ppm with 16 treatments in four replications. Another experiment under float seedbed conditions was carried out on the K326 variety with fungicides Vista at concentrations of 400, 500 and 600 g/ha (1, 1.25 and 1.5/1000), Nativo at concentrations of 120, 160 and 200 g/ha (0.3, 0.4 and 0.5/1000), and Topsin M at concentrations of 800 g/ha (2/1000) and control (check) treatment spray with water with eight treatments and three in replications in a randomized complete block design at the Tirtash Research and Education Center in Northern Iran in 2019. Design data was analyzed with MSTAT-C software and mean comparison was done by LSD. The results of variance analysis showed there was a significant difference among the treatments at the 1 % probability level. At the laboratory, all treatments, except for Vista treatment, 50 ppm (93 %) and control (0 %) prevented 100 % mycelium growth of sclerotinia fungi. In the seedbed, the Topsin M fungicides with a concentration of 800 g/ha (2/1000), Nativo at concentrations of 200, 160 and 120 g/ha (0.5, 0.4 and 0.3 /1000), and Vista with concentrations of 600, 500 and 400 g/ha (1.5, 25.1 and 1/1000), respectively, showed 100 %, 100 %, 100 %, 96 %, 96 %, 93 % and 88 % control of tobacco collar rot disease. Therefore, fungicides of Vista with a concentration of 400 g/ha (1/1000) and Nativo with a concentration of 120 g/ha (0.3/1000) are recommended for the management of tobacco collar rot disease in the seedbed.

APPOST 02

Production of wettable powder biopesticide formulation from two superior *Bacillus thuringiensis* strains native to Northern Iran

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Biological pest control agents have many advantages such as low risk, low cost and specific effect. They can be an effective alternative to conventional chemical insecticides. *Bacillus thuringiensis* (Bt) is the most widely used biopesticide due to its efficacy to control pests. From an economic perspective, and to improve efficacy and persistance, development of suitable new formulations is always significant in the production and application of microbial biopesticides.

The aim of this study, was to produce a wettable powder (WP) formulation of two superior Bt strains (Bt-72) and (Bt-73) native of Northern Iran. In previous research, these strains were isolated, their genetic characteristics were identified and preliminary bioassays conducted. The two Bt strains were produced in a liquid medium culture with low-cost resources in the fermenter. The mixture of spore-crystals was recovered and dried. Pure powder of spore-crystals was obtained with final concentration around 2×10^{10} (CFU/mg). The formulation was prepared from mixing the 50 % spore-crystal mixture from each of the two strains together with 50 % adjuvants including different fillers, surfactants, materials enhancing durability, protective materials against ultraviolet light, suspensors, wetters, palatability materials, antimicrobial materials and insect larvae attractive agent.

Biological, physical and chemical tests of the prepared formulations based on the protocol CIPAC (1970) were done. Phytotoxicity was examined to evaluate which effective formulation could be selected. The results of these tests showed that the superior formulation, had 73 % suspensibility (25 S) for wetting time, 2×10^{10} spore-crystals CFU/mg), 920 mg/ml deltaendotoxin and high durability - about two years. The results of laboratory bioassays showed that this formulation had the highest mortality rate - about 95 %, and its LC_{50} value on second larvae of tobacco bulworm (*Helicoverpa armigera*) was 490 ng/cm^2 leaf. Economically, this formulation is recommended for commercialisation.

APPOST 03

Field evaluation of the efficacy of formulations produced from *Bacillus thuringiensis* (*Bt*) and nuclear polyhedrosis virus (NPV) isolates native to Northern Iran against *Helicoverpa armigera*

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Tobacco bud worm (*Helicoverpa armigera*) is one of the most destructive pests of tobacco that can cause severe reduction in both quality and yield. *Bacillus thuringiensis* (Bt) is an important and successful insect pathogen, with many advantages. Previous research and evaluation of the wettable powder (WP) formulation produced from Bt and nuclear polyhedrosis virus (NPV) superior strains native to Northern Iran, was conducted in the laboratory. The aim of this study was a field evaluation of the formulation to carefully assess the efficacy and sustainability to sunlight. The research was done at Tirtash Tobacco Research and Education Center of Iran. Trial outline was RCBD, seven treatments and three replications carried out in 2018. The treatments included the four superior native formulations treatments of Bt and NPV + commercial Bt formulation (Biolep®) + chemical insecticide (Avant®) + control (water).

Artificial contamination of plots in the field was done with larvae of *H. armigera*. Spraying was performed twice with different amounts of Bt and NPV native formulations. Counting the number of larval mortality was performed at 1, 3, 5, 7, 10 and 14 days after spraying. The mortality percent in the field was calculated by the Henderson & Tilton (1955) formula. The results showed that there was a good synergistic effect between Bt and NPV native formulations. The highest mortality rate observed in Bt72 (5000 ppm) + NPV (1250 ppm) and Bt73 (5000 ppm) + NPV (1250 ppm) treatments was 86.6 % and 80 %, respectively. The lowest mortality was in the control (water spraying) that had no lethal effects. Generally, the results of this research showed that the Bt produced native formulations alone and also in combination with NPV native formulations had good lethal effects on the larvae of *H. armigera* both in the laboratory and in the field.

Therefore they can be used as safe, effective and low-risk biopesticides, replacing chemical insecticides and used for IPM programs.

APPOST 04

Identification of a PVY isolate breaking down the resistance of *va*-genotye tobacco in China

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Recently, the potato virus Y (PVY) strains that could break down the resistance of *va*-genotype tobacco varieties emerged on cultivated tobacco constantly. In 2016, a *va*-resistance breaking-down PVY strain, which was named PVY-CJ, was isolated and was verified and identified in this study. Re-inoculation on the *va*-genotype tobacco varieties, multiple RT-PCR, segmented amplification of the whole PVY genome by RT-PCR, molecular phylogenetic analysis and the sequence alignment of VPg protein of PVY strains were adopted to confirm and identify the PVY-CJ strain. Results showed that all the three *va*-genotype tobacco varieties showed infection symptoms when they were inoculated with PVY-CJ inoculum. PVY-CJ was classified as a PVY^{NTN} strain according to the result of multiple RT-PCR. Sequence analysis showed that the whole genome of PVY-CJ contained an open reading frame of 9180 nt encoding a polyprotein of 3060 amino acids. The result of molecular phylogenetic analysis further classified PVY-CJ as a PVY^{NTN} strain. A substitution of lysine to glutamic acid happened on position 105 of the amino acid of VPg of the PVY-CJ strain according to the sequence alignment of PVY-CJ with other PVY^{NTN} strains. A PVY strain, which could break down the resistance of *va*-genotype tobacco varieties in mainland China, was reported and identified for the first time. It provided material for breeding new PVY-resistant tobacco varieties.

APPOST 05

Phosphorous acid effects on Pythium root rot in tobacco float bed

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Pythium damping-off and root rot of tobacco seedlings in float beds represent difficulties for cultivation worldwide. Sanitation management such as disinfestation of potting media and float trays as well as utilization of non-contaminated water and seedling equipment are implemented to control Pythium diseases. Pesticide application is another effective control measure. Several fungicides have been introduced in some countries to control Pythium disease in float beds. However, they present risks of adverse environmental effects. Some agricultural fertilizers, such as phosphorous acid and silicic acid, have been reported as effective to prevent diseases in other crops. Furthermore, phosphorous acid is reportedly effective against oomycetes, which include *Pythium* spp. The objective of this study was to verify phosphorous acid application effects on Pythium disease control in tobacco seedlings in a float bed without adversely affecting tobacco seedlings. Twenty-day-old tobacco seedlings were transplanted into seedling

trays. Then floating water was treated simultaneously with a *Pythium aphanidermatum* zoospore suspension and different doses of phosphorous acid fertilizer. After treatment, seedlings were kept at 25 °C in a greenhouse. For phytotoxicity testing, different doses of phosphorous acid fertilizer were applied. Phosphorous acid fertilizer showed control effects at concentrations greater than 0.1 mL/L. However, phosphorous acid fertilizer showed phytotoxicity at concentrations greater than 0.2 mL/L. It suppressed seedling growth at concentrations greater than 0.5 mL/L. Phosphorous acid fertilizer showed control effects at 0.1 mL/L without phytotoxicity, suggesting its utility as an eco-friendly alternative to currently used fungicides. Further investigation into phosphorous acid application timing, such as earlier applications, must be conducted to increase its effectiveness.

APPOST 06

Diagnosis of leaf curl and crooked tip diseases on tobacco plants in Tay Ninh Province, Vietnam

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Tobacco leaf curl and crooked tip disease causes regular damage in the Tay Ninh Province of Vietnam and resulted in serious tobacco yield and quality loss in 2019. To define exactly the cause of the disease, the Tobacco Institute collected seven disease samples from tobacco growing in the Tay Ninh Province for analysis by the Research Centre for Tropical Plant Pathology – Vietnam National University of Agriculture. The analyses was done by RT-PCR and gen sequence methods with the following pair of primes: TYKan-A-F1 and TYKan-A-R1 (to identify DNA-A of TYLCKanV), size: 698 bp; TYKan-B-F1 and TYKan-B-R2 (to identify DNA-B of TYLCKanV), size: 402 bp; Krusty and Homer (to identify DNA-A of Begomovirus), size 600 bp; BegoA-For1 and BegoA-Rev1 (to identify DNA-A of Begomovirus), size: 1200 bp. Results of the analyses determined two species of virus, which include tomato yellow leaf curl Kanchanaburi virus (TYLCKaV) and pepper yellow leaf curl Indonesia virus (PepYLCIDV). These cause the same disease symptoms on tobacco plants and belong to the Begomovirus genus and are spread by *Bemisia tabaci*. The pepper yellow leaf curl Indonesia virus was detected for the first time in Vietnam. This result is an important basis to decide proper management and reduce tobacco losses due to the disease in the 2020 season in Tay Ninh Province.

APPOST 07

Evaluation of the effects of organic and chemical fertilizer on quality and yield of flue-cured tobacco under irrigated and rainfed conditions

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Traditional agricultural practices reduce organic matter and soil fertility and also cause a reduction in quality and yield in many parts of the world. Therefore, attention to the preservation of soil organic matter seems necessary. Also, in order to create stability in soil fertilization, it is necessary to use renewable sources such as organic fertilizers. For this purpose, a completely randomized block design was performed with 12 treatments and four replicates under two rainfed and irrigated conditions for one year using the tobacco cultivar K326. The resulting data was analyzed using MSTAT-C software. Treatments were T_1 cow manure 10 tons per hectare; T_2 cow manure 20 tons per hectare; T_3 no fertilizer; T_4 100 % chemical fertilizer; T_5 enriched poultry manure 450 kg per hectare; T_6 enriched poultry manure 450 kg plus 200 kg potassium sulfate per hectare; T_7 enriched poultry manure 650 kg per hectare; T_8 enriched poultry manure 650 kg plus 150 kg potassium sulfate per hectare; T_9 enriched poultry manure 850 kg per hectare; T_{10} enriched poultry manure 850 kg plus 100 kg potassium sulfate per hectare; T_{11} Vermicompost 5 tons per hectare; T_{12} Vermicompost 10 tons per hectare. Variance analysis under the rainfed conditions showed that all quantitative traits (leaf length, width, thickness, green weight, dry weight, price, gross income, net income, leaf area index) were significant at the 1 % level. In the qualitative traits relative water content, cytoplasmic membrane, chlorophyll b, chlorophyll ab and carotenoids were significant at the 1 % level, chlorophyll a was significant at the 5 % level and the proline amino acid was not significant. The best treatment under rainfed conditions was T_8 enriched poultry manure 650 kg plus 150 kg potassium sulfate per hectare. In the variance analysis table of the treatment under irrigated conditions all quantitative traits (leaf length, width, thickness, green weight, dry weight, average tobacco price, gross income, net income, leaf area index) were significant at the 1 % level. In the qualitative traits, relative water content and cytoplasmic membrane were significant at the 1 % level, chlorophyll a and chlorophyll ab was significant at the 5 % level, proline amino acid, chlorophyll b and carotenoids were not significant. The best treatments under irrigated conditions were the T_8 enriched poultry manure 650 kg plus 150 kg potassium sulfate per hectare, the T_7 enriched poultry manure 650 kg per hectare and the T_4 cow manure 10 tons per hectare.

APPOST 08

Growth, nitrogen uptake and soil N_2O emissions in flue-cured tobacco as influenced by drip fertigation strategy

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Fertilizer strategy mainly includes the time and rate of fertilization, which is the most important content in the crop fertilization regime. Considering the rapid expansion of the application scope of drip fertigation technology, a scientific strategy for fertigation is highly needed in the guidance of flue-cured tobacco production, in favour of nutrient management with both enhancing benefits and mitigating soil N_2O emissions. A field experiment was carried out in flue-cured tobacco from 2018 to 2019 to study the effects of drip fertigation strategy on the growth, nitrogen uptake and soil N_2O emissions. Three treatments in drip fertigation strategy were established, namely conventional strategy (CDF), average strategy (ADF), and optimized strategy (ODF), based on the treatments of no fertilizer application (CK) and conventional fertilizer application (CF). The results showed that dry matter accumulation, nitrogen uptake and tobacco leaf yield increased in various degrees, compared with that of CK. Nitrogen use efficiency and tobacco leaf yield increased in various degrees, while nicotine content of tobacco leaf decreased in various degrees as compared to that of CF. The effects of drip fertigation strategy on the growth, nitrogen uptake and soil N_2O emissions was noticeable in various degrees. Dry matter accumulation rate at vigorous growing stage increased by 0.06 ~ 0.13 times, and on average, leaf area increased by 6.8 % ~ 86.4 %, nicotine content of tobacco leaf increased by 7.4 % ~ 16.5 %, nitrogen uptake of whole tobacco plant increased by 10.1 % ~ 19.8 %, nitrogen fertilization efficiency increased by 28.7 % ~ 66.8 %, nitrogen agronomic efficiency increased by 7.0 % ~ 94.1 %, total N_2O emissions in tobacco field decreased by 10.0 % ~ 38.4 % ($P < 0.05$), and N_2O emission factor decreased by 10.0 % ~ 37.8 % ($P < 0.05$), relative to that of CDF and ADF, respectively. Based on the nitrogen demands of flue-cured tobacco, the optimized operation promoted the formation of flue-cured tobacco leaf area and the absorption and utilization of nitrogen fertilizer. The strategy not only ensured the yield and nicotine content level of tobacco leaf, but also reduced the risk of soil nitrogen leaching and N_2O emissions from the tobacco field. It is an integrated nutrient management method for drip fertigation in flue-cured tobacco production.

APPOST 09

Dynamic change of soil pH, physi-chemical characters and enzymatic activity under restructuring arable layer

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To reveal the synergistic effect of deep vertical rotary tillage combined with inorganic, organic, biological and other materials on restoration of acid field, the dynamics of pH, physical properties, main nutrients and enzyme activities in the soil were studied after application of deep vertical rotary tillage and soil modification materials. Results show that: (1) After the arable layer of acidic soil was restructured, the soil pH increased rapidly, then decreased slowly and gradually came to be stable during the growth process. Soil bulk density firstly decreased then slightly rebounded. Soil porosity ascended and then descended slightly. Soil organic matter firstly rose and then fluctuated. Soil alkali-hydrolyzable nitrogen rose sharply and then dropped slowly. Soil available phosphorus increased rapidly and then tended to fall. Soil available potassium increased significantly first, then declined and finally rose steadily. Soil invertase, urease firstly rose, then dropped, and at last tended to be stable. (2) After tobacco transplanting at 120 days, under the application of combining deep vertical rotary tillage with lime, green manure and bio-organic fertilizer, compared to non-experimented soil, soil bulk density was reduced by 7.32 % ~ 8.45 %. However, soil pH, soil porosity, soil organic matter, soil alkali-hydrolyzable nitrogen, soil available phosphorus, soil available potassium, soil urease activity, and soil invertase activity were elevated respectively by 1.29 % ~ 1.62 %, 8.17 % ~ 9.23 %, 16.89 % ~ 27.72 %, 13.39 % ~ 18.81 %, 69.83 % ~ 245.17 %, 47.05 % ~ 100.91 %, 32.44 % ~ 99.44 %, and 79.22 % ~ 129.62 %. (3) Compared with deep vertical rotary tillage+lime+green manure treatment, the application of deep vertical rotary tillage+lime+green manure+biological organic fertilizer appeared to be more effective. Therefore, the combination of deep vertical rotary tillage with the mixture of lime, green manure, biological organic fertilizer, and other soil improving matters, can achieve synchronous improvement of soil acidity in soil top and subsurface, synchronous improvement and fertilization of soil acidity, and improvement of mountain acidic soil.

APPOST 10

Improved soil fertility and microbial diversity by microbial organic fertilizers in continuous tobacco cropping areas

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In order to study the effects of different fertilization methods on the microbial community structure and diversity of tobacco planting soils under continuous cropping conditions, Illumina Miseq high-throughput sequencing technology was used and Metagenomics analysis was conducted to analyse the soil microbial communities under different fertilizer treatments. The control was inorganic compound fertilizer for tobacco: the application amount was 600 kg/ha, and $N:P_2O_5:K_2O$ = 10:10:24. Based on the same rates as for the inorganic fertilizer, microbial fertilizers from different sources were added as other treatments. Xiangyun Guansheng organic fertilizer containing *Bacillus amyloliquefaciens* with an effective viable bacteria count of 0.02 B/g was used at a rate of 750 kg/ha. Midu Guofa Organic Fertilizer, whose main component is animal and plant compost, was also used. Fujian Sanju Solid Bacterium Fertilizer which contains *Bacillus amyloliquefaciens*, *Arthrobacter tabacum* and Bacillus jelly, with an effective viable bacteria count of 0.02 B/g, was used at a rate of 750 kg/ha; Fujian Sanju liquid bacteria fertilizer containing *Bacillus mucilaginosus* was used at a rate of 15 000 ml/ha.

Results showed that: (1) The amount of valid sequence data obtained after sequencing allegations was distributed between 37 417 - 390 002.75 reads, the average length was distributed between 259.8 - 271.7625 bp, and the number of operation classification units (OTUs) in each sample were distributed between 455.75 - 642.25. (2) The Wayne diagram showed that a total of 2143 OTUs were obtained, with a total of 88 OTUs. (3) Alpha diversity analysis showed that the richness of the microbial community in the soil treated with microbial fertilizer gradually increased according to the ACE index and Chao index, while no obvious changes were observed in the other treatments. This increase in the microbial community and diversity is more obvious according to the Shannon index and Simpson index. The diversity of microbial species in all the soil samples was increased. (4) Community structure analysis showed that the soil bacteria types in the sampled soil were categorized into 16 phyla, 44 classes, 45 orders, 45 families, 45 genera, and Ascomycota, Basidiomycota, and Zygomycota were in higher abundance. (5) The colony heat map showed that all the organic fertilization treatments increased the bacterial community composition in the soil compared with the control in which inorganic fertilizer was applied. The treated samples also had more bacterial diversity than that in the control. Conclusion: The bacterial structures of organic fertilizer groups were more closely aggregated than those of the inorganic fertilizer group, which indicated that the addition of microbial organic fertilizers could significantly change the bacterial structure of the soil in the case continuous tobacco cropping.

APPOST 11

Ensuring nicotine uniformity by measuring tobacco leaf using NIR spectrometer

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When designing tobacco products, the production and supply of tobacco leaves that have stable quality are important. Measuring the chemical constituents of the tobacco leaf provides uniform quality of processed lamina production. For this study, nicotine was selected as a major chemical constituent related to quality. The objective was to develop simple and cost-effective methods using a handheld and an on-line near-infrared (NIR) spectrometer for measuring the nicotine content in tobacco leaves in the field and in baled tobacco, respectively. Tobacco leaf surfaces were scanned using the handheld NIR spectrometer in the field, while bale surfaces were scanned using the on-line NIR spectrometer. They were subsequently analyzed using gas chromatography with a flame ionization detector (GC-FID) to ascertain a reference nicotine content. Calibration models for leaf and bale were established using partial least squares (PLS) regression in calibration sets. Prediction results of validation sets indicated root mean square errors of prediction of 0.52 % for the leaf and 0.45 % for the bale. Moreover, nicotine content of 47 batches from three grades of processed lamina were estimated based on that of tobacco bales obtained from the model for baled tobacco. The standard deviation in each grade was 1.5 to 2.0 as a relative value, with 100 representing the estimated content. The results suggest that nicotine measurement using the handheld NIR spectrometer in the field could be a simple method to predict a harvest time and stabilize nicotine contents in tobacco leaf. The results show that nicotine measurement of tobacco in bales using the on-line NIR spectrometer is a cost-effective method to produce lamina containing a uniform amount of nicotine. Therefore, these measurement methods using the NIR spectrometers could contribute to ensuring the quality of processed lamina.

APPOST 12

A study for inheritance of chemical components in dried leaves of tobacco varieties of different types and their F_1 progenies

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Investigations were made with five tobacco varieties (Prilep P-23, Prilep P 8-9/80, Floria FL-7, Samsun S-1 and Virginia MV-1) and their four F_1 hybrids (P-23 × MV-1, P 8-9/80 × MV-1, FL-7 × MV-1 and S-1 × MV-1), for mode of inheritance for chemical components in dry 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 objective of this study was to investigate the mode of inheritance of the chemical components and to detect possible heterotic effects, which will allow a selection of lines with the most favourable chemical composition, and not to reduce yields and other quality features. The results of the investigations indicate the fact that we obtained hybrids with reduced nicotine content, and favourable content of other chemical components. At the same time, we received valuable material for further selection activities. From the hybrids we singled it out S-1 × MV-1 as a cross with the best harmony of the chemical composition.

APPOST 13

Tobacco production programming in the Suwannee River Valley of North Florida

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The Suwannee River Valley of North Florida has been known for producing premium flue-cured tobacco since the 1920s. Recently, a Tobacco Extension Program Team was formed to assist local producers with current production practices, governmental regulations, and industry demands. Team goals were to (1) increase knowledge of improved cultural and production

techniques and (2) encourage producers to incorporate new tobacco cultivars and crop protection agents (CPA) into their management practices.

The Tobacco Extension Program, which consists of an annual tobacco production meeting, an on-farm trial, and the Georgia-Florida Tobacco Tour, provided tobacco producers the opportunity to gain knowledge of the most current and researched production methods. The success of this Extension program was due to the long-term use of traditional classroom trainings that were coupled with on-farm demonstrations, field consultations, and small group learning experiences. From 2016 to 2018, approximately 50 tobacco producers, farm managers, and stakeholders from North Florida and South Georgia attended the annual tobacco production events. Exit evaluations showed that 87 % of the attendees showed an increase in knowledge specific to disease management and other best management practices. At the conclusion of the Georgia-Florida Tobacco Tour, 94 % of attendees reported an increase in cultivar selection knowledge. The adoption of recommended cultivars has increased yield and reduced CPA applications. This has generated a savings of $US 123.50 ha^{-1} while increasing yields, thus resulting in a total savings of $US 55 000 in the Suwannee River Valley. It was also observed that producers attending the tour were more likely to interact with presenters when compared to the traditional classroom meetings. By executing our objectives through program activities, the Extension team was able to contribute to the sustainability of tobacco in this specialized region.

APPOST 14

The effect of silicate solubilizing bacteria and mycorrhizal symbiosis on potassium fertilizer requirements of tobacco (*Nicotiana tabacum* L.)

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Potassium (K) plays a vital role in increasing tobacco yield and controlling quality parameters such as leaf combustibility. Soil has rich reserves of K, among which only 1 - 2 % can be directly absorbed by plants. It may be more economically viable to transform the fixed slow-release K into available K. The ability of some microorganisms to dissolve soil K-bearing minerals is used in tobacco farming. The present study was conducted to screen the potassium-solubilizing bacteria (KSB) isolates from tobacco-cultivated soils and evaluate the effects of KSB isolates and arbuscular mycorrhiza fungi (AMF) on the K fertilizer requirement of tobacco. A study was conducted in factorial completely randomized design (CRD) with three factors with three replications. The potassium fertilizer factor included four recommended doses of fertilizer (RDF) for potassium (0, 50 %, 75 %, and 100 % RDF), the KSB factor included both KSB inoculants and no KSB inoculants, and the AMF factor included both AMF inoculant and no AMF inoculant. The nine KSB isolates were isolated, purified and evaluated. Some of the bacteria isolates studied included KSB20, KSB22, KSB30, KSB40, KSB42, KSB90, KSB92 and KSB10 isolates and these were more effective in releasing potassium from soil potassium-bearing minerals. In the

case of a low amount of potassium fertilizer application (0 and 50 % RDF), the AMF and KSB inoculants significantly increased the shoot and leaf dry weight of tobacco in comparison with the control. The KSB inoculant led to a 6 % increase in the tobacco shoot dry weight in comparison with control. KSB inoculation significantly increased leaf K concentration and AMF inoculation led to a 10 % reduction in leaf K concentration in comparison with the control. KSB can be used to increase the potassium concentration to about 3 % in leaf dry matter and improving tobacco leaf, and reduced applied potassium fertilizer application by about 25 %.

2020 CORESTA CONGRESS ONLINE

SMOKE SCIENCE and PRODUCT TECHNOLOGY

ORAL PRESENTATIONS

ST 01

Numerical simulation of cigarette smoking process: Effects of multifactor variations on cigarette burning behaviour and releases of tar and CO

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In order to study the effects of multifactor variations on cigarette burning behaviour and the release of tar and CO, a mathematical model of the physical and chemical mechanisms of cigarette smoking was used to simulate the influence of cigarette paper permeability, filter ventilation and cigarette filling density on the airflow velocity field, temperature field, char density field and the concentration fields of O_2, tar and CO. The results are presented as images, which intuitively reveal the working mechanism of each factor. With the increase of cigarette paper permeability, more air entered through cigarette paper instead of the combustion cone center, resulting in the slowdown of both tobacco pyrolysis and char combustion reactions and the decrease of tar and CO release. When filter ventilation is increased, the amount of air flowing into the combustion cone decreases and oxygen supply becomes insufficient, which lowers the solid phase temperature, decelerates pyrolysis and combustion reactions simultaneously, and decreases tar and CO release. If the cigarette filling density is increased, the resistance of airflow into the combustion cone increases, causing the slowdown of the combustion reaction and a decrease of solid phase temperature; however the pyrolysis reaction is accelerated due to the increasing amount of cut tobacco, which significantly increases the release of tar and CO. On the basis of theoretical research, a simplified model for predicting tar and CO yields was set up. By comparing our own and others' experimental data with the predicted values, the average relative deviations were below 7 %, which verified the accuracy of the model.

ST 02

CANCELLED

Paper filter – a market changer?

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ST 03

Method development for the analysis of mono-carbonyl compounds in e-vapor products by LC-MS

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At the 73rd Tobacco Science Research Conference (TSRC), we presented a novel method for quantitation by LC-MS of mono-carbonyl compounds in e-vapor product aerosol samples. The method is simple and robust with good recoveries. We have since extended the method validation to include e-liquid samples (recoveries 90.2 - 111.8 %) for the same mono-carbonyls (formaldehyde, acetaldehyde, acrolein and crotonaldehyde). In this presentation, we will discuss the extension to e-liquids. We will also present the steps we have taken during the method development process to optimize the method and to overcome challenges specifically related to the analytes and/or the matrix in question. Analysis of carbonyl compounds is challenging due to environmental contamination and relative instability of the analytes. E-vapor product aerosol and e-liquid matrices are problematic for instrument maintenance issues. Details will be provided for method development and optimization related to managing samples with no to low levels of analyte with a high environmental background and steps taken to optimize the conditions for the conversion of free carbonyls to 2,4-dinitrophhenylhydrazine (DNPH) derivatives for detection and analyte stability purpose; for improving sensitivity and selectivity and chromatographic baseline noise to optimize the method LOQs; and for ways to make the method robust enough for maintenance and linear dynamic range for use in a production lab. The method has a calibration range of 2 to 400 ng/mL for all four analytes, with limit of quantitation (LOQ) at 2 ng/mL, or 0.0029 µg/puff for aerosol, or 0.4 µg/g for e-liquids.

ST 04

Method optimization on analysis of TSNA in electronic cigarette liquids and N-nitrososarcosine (NSAR) in smokeless tobacco by UHPLC-MS/MS

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The main challenges for analysis of tobacco specific nitrosamines (TSNA) in electronic cigarette liquids (e-liquids) are matrix effects, especially for high nicotine samples. High nicotine content in e-liquids can have heavy ion suppression effects on TSNA analysis by current LC-MS/MS methods and thus significantly affect the method's sensitivity and accuracy. For N-nitrososarcosine (NSAR), the published LC-MS/MS method used only one MS/MS ion pair and offered less selectivity. The objectives of this work are: (1) to study the e-liquid sample matrix effects on TSNA detection and evaluate different LC mobile phases and UHPLC columns to separate TSNA from nicotine and reduce matrix effects; (2) to improve the selectivity of NSAR analysis by adding more MS/MS ion pairs in the method. Mass detection conditions were optimized for TSNA and NSAR, multiple MS/MS ion pairs were examined to increase the method's selectivity. For NSAR analysis in smokeless tobacco, both positive and negative MS/MS ion pairs were monitored in a single method by QSight Mass Spectrometer with fast polarity switching, which enhanced the selectivity of the method. The TSNA method was improved using the optimized LC and MS conditions and the TSNA could be well separated from nicotine and thus reduce matrix effects on TSNA analysis. The method was validated using an e-liquid sample and the method showed good linearity ranging from 0.01 to 100 ng/mL, with limit of quantification (LOQ) at sub ng/mL level. The TSNA method could be further improved by coupling an automated solid phase microextraction (SPME) sample preparation technique with UHPLC-MS/MS and the sensitivity could be easily increased 3-fold by this method.

ST 05

Determination of glycidol in e-liquids and emissions from e-cigarettes

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The direct analysis of trace levels of glycidol from e-liquids and e-cigarette emissions by GC-MS, is confounded by its size and the limited number of specific ions available for quantification. It is also susceptible to artificial formation resulting from either degradation or reactivity of the propylene glycol (PG) and vegetable glycerin (VG) matrix in the GC injection port. Therefore, a method was developed using derivitization to increase the selectivity and sensitivity for the quantification of glycidol in e-cigarette matrices.

Gas chromatography-mass spectrometry (GC-MS) in selected ion monitoring (SIM) mode, with deuterated glycidol-d5 as internal standard, was used to quantify trace amounts of glycidol in e-liquids and emissions from e-cigarettes. Solid phase extraction (SPE) was applied to reduce the impact of the PG and VG matrix on the derivization procedures. Two selective derivatizations were conducted to form the 3-bromo-1,2-propanediol-phenylboronic acid derivative of glycidol.

The calibration range was from 1 ng/mL to 500 ng/mL, with a test sample limit of quantitation (LOQ) equivalent to 66.7 ng/g for e-liquids and 20.2 ng/collection for e-aerosol. The accuracy and precision were determined using lab fortified blank (LFB) samples and lab fortified matrix (LFM) samples. LFB recoveries of glycidol spiked at 100 ng/mL, ranged from 98.4 % to 109 %. The fortified matrix recoveries ranged from 94.8 % to 120 % for samples spiked with 5 ng/mL glycidol, 95 % to 105 % for samples spiked with 100 ng/mL, and 101 % to 106 % for samples spiked with 450 ng/mL of glycidol. Stability and robustness was confirmed with acceptable recoveries of samples with slight modification on several critical parameters during sample preparation.

In conclusion, this novel approach was able to accurately determine trace amounts of glycidol in both e-liquids and e-aerosol.

ST 06

eHTP aerosol generation: mass balance method to evaluate the contribution of stick elements and device design

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The aerosol composition generated by electrically heated tobacco products (eHTPs) can vary depending on sticks and device designs. An aerosol constituents mass balance approach can help in the understanding of the contribution of the different stick elements (tobacco, cooling, filter) and the influence of the device design on aerosol deliveries.

The objectives of this work were to:

1) develop a simple method for the simultaneous analysis of aerosol constituents (nicotine, glycerol, propylene glycol, water), not only in aerosol, but also in stick elements before and after heating, which take into account a range larger than those used for aerosol,

2) assess the contribution of each eHTP stick elements to aerosol deliveries for different commercial eHTPs.

Health Canada Intense smoking regime was used to generate and collect eHTP aerosol on a linear Borgwaldt RM4 machine. Some modifications such as solvent type were applied in order to be able to systematically quantify the aerosol constituents level in all eHTP sticks parts before and after heating for various eHTP designs. Internal and external heating systems such as IQOS, LIL, PULZE, glo™, Ploom S were assessed.

Transfer rates from the tobacco substrate to the aerosol varied from minimum 6 to 14 % for glycerol to maximum 38 % for nicotine. Constituents which did not transfer to the aerosol either remained in the tobacco substrate (up to 73 %) or deposited on the filter/cooling elements (up to 36 %), the distribution was dependent on the eHTP design tested.

These results suggest that stick design and the heating system both have strong impacts on the transfer of constituents from sticks to the aerosol. The method developed can help optimize the transfer of constituents to the aerosol.

ST 07

Investigation on HTP aerosol release dynamics with a new puff by puff HTP vaping machine

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Heated tobacco products (HTPs) heat a tobacco substrate at a temperature below that required to initiate combustion. However, switching from smouldering and active combustion to an internal or external heating system is also modifying the aerosol dynamics. The objectives of the work were to better understand the aerosol release dynamics of different HTP systems and to evaluate the release performance of different HTP reconstituted tobacco substrates.

A smoking machine was developed to automatically and successively collect each puff of the aerosol collected matter (ACM). The ACM coming from the same puff from a plurality of devices is collected on a unique filter pad dedicated to one puff set. In order to do so, a circular sample cartridge holder containing a plurality of sample cartridges is used. It rotates the filter pad holders between each puff. Nicotine, humectants, and water puff by puff profiles were analyzed by gas chromatography with flame ionization and thermal conductivity detection. The analytical methods previously developed for HTP total aerosol were modified to measure lower amounts. A repeatability and reproducibility study was conducted on 5 × 10 Heets® Amber smoked puff per puff in three replicates by six operators under Health Canada Intense (HCI) conditions with a 10 s puff interval. The mean relative error (Ře) achieved is 13 % for ACM, 10 % for nicotine, 17 % for glycerin, and 16 % for PG.

Unsurprisingly, the ACM, nicotine, and glycerin puff-by-puff profiles differ from one HTP system to another. Likewise, profiles obtained from different reconstituted tobacco substrate prototypes show significant differences. In conclusion, the machine developed method allows us to compare different puff-by-puff release profiles with good repeatability and reproducibility. Being able to easily characterize HTP products puff by puff profile can be helpful to improve reconstituted tobacco substrate performance across the vaping experience.

ST 08

Development and validation of a routine method for the determination of carbonyl compounds in heated tobacco products (HTPs) by UPLC-MS

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Mainstream smoke is a complex mixture containing > 4800 compounds. Within this mixture there are various carbonyls which are also present on the FDA's abbreviated and extended harmful and potentially harmful constituents (HPHC) list, such as formaldehyde, acetaldehyde, acrolein, and crotonaldehyde. Advances in tobacco science have produced new products aimed at lowering the potential exposure to HPHCs. Among these innovations are heated tobacco products (HTPs), also called heat-not-burn, which eliminate combustion to produce an aerosol. HTPs typically reduce combustible HPHC yields by < 90 %, which limits the usefulness of traditional mainstream smoke methods such as CORESTA Recommended Method (CRM) 74. Due to the lower aerosol yields, HTP methods require better sensitivity or improved stability to allow for the collection of more matrix.

Here, we report the development of a new method for the trapping and analysis of twelve carbonyl compounds (formaldehyde, acetoin, acetaldehyde, diacetyl, acetone, acrolein, propionaldehyde, furfural, pentanedione, crotonaldehyde, methyl ethyl ketone, and butyraldehyde) using UPLC-MS. Method validation included an assessment of selectivity, precision, accuracy, stability and trapping efficiency. Presented here is an improved collection process that traps carbonyls on a Cambridge filter pad followed by a single impinger containing acetonitrile/isopropyl alcohol at -35 °C, thereby eliminating *in situ* trapping in DNPH. By collecting at low temperatures, we are able to extend stability of the compounds within this solution for up to two hours, allowing for the collection of more matrix. Additionally, by not collecting in DNPH, we are able to minimize degradation of acid sensitive hydrazones, which allows for longer collection times and results in improved recoveries for acrolein and crotonaldehyde (107 % - 110 % and 101 % - 102 % respectively). Complete resolution among all analytes is achieved over a 12.5-minute injection utilizing UPLC-MS operating in SIR mode. The calibration curves were quadratic with 1/x weighting and had a range of 0.01 µg/mL to 8 µg/mL for each analyte except for formaldehyde (up to 4 µg/mL) and acetone (0.05 µg/mL to 4 µg/mL) with r^2 = 0.995.

ST 09

Influences of tobacco powder particle size on the reconstituted tobacco slurry process for electrically heated tobacco products

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In order to investigate the influence of tobacco powder of different particle sizes on the quality of tobacco materials in electrically heated tobacco products (eHTPs), slurry process reconstituted tobacco was prepared by using tobacco powder of different particle sizes (180 - 380 mesh) on the basis of a home-made experiment platform for slurry process reconstituted tobacco. The surface microstructure and thermo-gravimetric characteristics of the prepared reconstituted tobacco were characterized by scanning electron microscope and simultaneous thermal analyzer, respectively. The smoke release amounts of eHTPs with tobacco materials made from tobacco powder of different particle sizes were qualitatively analyzed by e-cigarette smoke analyzer. The results showed that: 1) When the particle size of tobacco powder was larger than 280 mesh, the reconstituted tobacco featured uniform tobacco powder distribution and compact microstructure. 2) The thermal weight loss characteristics of the reconstituted tobacco could be analyzed by the thermochemical analysis method within the range of 0 to 600 °C. The major thermo-gravimetric stage was from 40 to 370 °C, wherein the mass loss of reconstituted tobacco was higher than 50 %. 3) In the heating process of reconstituted tobacco, the smoke release amount closely negatively correlated to the particle size of tobacco powder, namely the release amount increased with the decrease of the particle size. The results of this study provide a technical support for tobacco material preparation and new product development of heated tobacco products.

ST 10

Modular new product development in highly dynamic markets

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The implementation of a new approach always poses some organizational challenges but can also offer unrecognized opportunities. The Integrated PKT-approach for Development of Modular Product Families has been successfully implemented to reduce the existing variance-induced complexity within many modularization projects in industry by reducing the internal component variety and process complexity. To support the new development activities based

on new technologies, the modularization approach has been tailored to initially structure a product family instead of just single products and to also consider future user and customer diversity. An additional increase in complexity and uncertainty is induced by the increasing share of digital parts within products since they move towards IoT-systems that organizations need to cope with.

This study investigates the opportunities and challenges of implementing the modular New Product Development (NPD) approach for the development of New Generation Products (NGP) (e.g. e-cigarettes, HNB devices) in the highly dynamic fast-moving consumer goods (FMCG) markets. On the example of a use case, we show the integration of standardized modules into customer specific NGPs with the help of appropriate product design models. The risks and benefits of the modular NPD approach are evaluated and discussed based on two product examples. The results of the study show that the used modularization approach enables an organization to develop the components and modules simultaneously and independently by defining the interfaces before the development activities start, thus reducing development lead times.

Risk and challenges during implementation as well as the benefits within application are evaluated conclusively. The learnings from the study are distilled to provide recommendations for initial product design. The study shows that organizations can benefit from modular product development already in early development phases. The parallel development of modules diversifies the risk and accelerates the product development timeline.

ST 11

Extractable and leachable testing of electronic nicotine delivery systems

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Many electronic nicotine delivery systems (ENDS) contain multiple components which are known to contain substances which have the potential to migrate from the components and potentially be exposed to the user. Thus, the assessment of extractables and leachables (E&L) is an important part of evaluating consumer safety. Due to the nature and variety of components used in ENDS, analytical techniques need to be capable of detecting a wide range of compounds with different physiochemical properties. Some of these compounds can be highly toxic, therefore analytical methodologies need to also be capable of detecting analytes at very low parts per million levels. In addition, mass spectrometry is typically employed to support the identification of detected compounds. The purpose of this study was to determine the substances which migrate from different polymers using solvents. This data was then used to target substances in e-liquid over several timepoints. A combination of headspace gas chromatography (GC) mass spectrometry (MS), direct injection GC-MS, liquid chromatography time of flight MS and inductively coupled plasma MS, was used to screen for substances in extraction solvents and target substances in e-liquids. In addition, an untargeted screen for leachables was also performed on e-liquids. Different polymers and different grades of polymer

extracted under the same conditions exhibit a widely differing extractable profile. Targeted analytes were observed at levels below toxicological concern. The untargeted leachable screen identified a number of additional compounds. Substances from polymeric materials used in ENDS can migrate into e-liquids, therefore it is important to understand the toxicity of these compounds and the potential impact on the user.

ST 12

Thermal behaviour of tobacco pads for aerosol generation

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Aerosol generation by heating tobacco to temperatures well below the burning point is one attempt to reduce the health risk of tobacco products. However, due to the poor thermophysical properties of tobacco, it takes a considerable amount of time and energy to heat the full volume of tobacco to the desired temperature. This paper is a summary of an effort to design an innovative type of a tobacco heating device with modified heat transfer behaviour.

Tobacco pads with increased heat conductivity were formed by pressing a mixture of tobacco powder with particles of aluminum oxide. The temperature rise-time through the pad was decreased three to four times faster and therefore the power consumption compared to pure tobacco was also decreased. Moreover, a vaping device was designed and prototyped for characterization of these pads.

This study was repeated for various types of pads with different layout and thicknesses, different particles sizes of aluminum oxide, and different mass ratios of the aluminum oxide to tobacco. In addition, the experimental results were compared with the simulation results of the heating processes in order to make a more accurate estimation of the pads' thermal characteristics (heat capacitance, heat transfer rate, and thermal diffusivity). The measurement setup for this study consists of a regulated heater plate providing a constant temperature, an IR camera for temperature measurement, and a profilometer to measure the exact volume and thickness as well as the surface properties of the pads such as surface roughness. Considering the abundance and relatively low price of the aluminum oxide particles, the low power requirement of vaping cycles potentially paves the way for production of cheaper and faster tobacco heating devices.

ST 13

In vitro biological assessment of nicotine administration efficiency of a prototypic ENDS with a novel volumetric heater

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Due to the growing awareness of the adverse health effects of smoking tobacco and repeated recommendations from health experts, smokers try to quit using nicotine replacement therapies. This leads to an interest in electronic nicotine delivery systems (ENDS). To be an effective smoking-cessation method, the nicotine delivery and its alveolar absorption associated with ENDS must be equal to or better than that of tobacco cigarettes, while delivering fewer harmful and potentially harmful substances. Our approach was to develop a novel, non-metal volumetric heater to address these concerns. Its chemical inertia, ability to be precisely regulated in temperature applied to the e-liquid and digital features may present a promising solution for ENDS users' needs and expectations in quitting tobacco smoking effectively and efficiently.

In this study, we aimed to characterize the nicotine administration efficiency of a volumetric heater comparatively with other ENDS with other heater technologies (metal coil, metal mesh, ceramic heater) currently available on the market.

Coupling an LM1E smoking machine (Borgwaldt KC, Hamburg, Germany) with an RFS compact aerosol exposure unit (Cultex Technology, Hannover, Germany), aerosols generated by ENDS from the same e-liquid onto SmallAir (Epithelix, Geneva, Switzerland) and Calu-3 cell cultures were applied. Aliquots of the basolateral medium were taken and the absorbed nicotine concentration was then measured by HPLC/UV.

Initial results showed that our volumetric heater administered 118 % more nicotine in average than an ENDS equipped with a coil heater. No significant difference was observed regarding trans-epithelial electrical resistance. These findings held true for both cell culture systems used.

Future experiments will include the characterization of the volumetric heater technology with regards to physical, chemical and biological parameters. Further experiments are also being conducted currently to evaluate the potential of this heater to administer active substances other than nicotine.

ST 14

In vitro assessment of acute respiratory toxicity

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Efficient and effective assessment of inhaled substances using human-relevant approaches is important for product development and risk management. The aim of this project is to assess acute inhalation toxicity of chemicals using an *in vitro* system, relevant biological markers, realistic test concentrations, and appropriate controls.

BEAS-2B cells (a human bronchial epithelial cell line) were used to assess the portal-of-entry (POE) effects of silanes on the human respiratory tract. The cells were exposed at the air-liquid interface to various concentrations (0.72 ppm, 25 ppm, 85 ppm) of triethoxysilane (TES) vapour using a VITROCELL 6/4 exposure system. Exposure to nitrogen dioxide and clean air were used as positive and negative controls, respectively. Lactate dehydrogenase cytotoxicity and resazurin-based cell viability assays revealed a concentration-dependent increase in toxicity 30 minutes post-exposure and decrease in viability 24 hours post-exposure. A significant increase in secretion of inflammatory mediators [interleukin IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and tumor necrosis factor-alpha (TNF-a)], was observed after exposure to 25 ppm TES using Meso Scale Discovery technology. Because of the high cytotoxicity, inflammatory mediators were not measured for 85 ppm. Future experiments will include testing additional controls (Triton X-100 and sodium chloride) and silanes (differing in carbon-chain lengths) to assess the advantages of using a 2D cell line versus a 3D human reconstructed tissue model (MucilAir™, Epithelix). These results will evaluate the ability of an *in vitro* system to predict the likelihood of a chemical to cause POE effects on the human respiratory tract and could be a useful approach to rank chemical toxicity.

ST 15

Characterization of on!® nicotine pouches – Part 2: Nicotine dissolution release profiles

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on!® is an oral tobacco-derived nicotine pouch product that does not contain cut, ground, powdered or leaf tobacco. In order to issue market authorization, the U.S. Food and Drug Administration (FDA) must determine whether the on!® nicotine pouches are appropriate for the protection of public health (APPH). The nicotine release profiles for the portfolio of on!®

nicotine pouches to inform the determination of APPH were characterized. Evaluating nicotine release profiles through dissolution testing is valuable for product assessment and for product-to-product comparisons. A robust dissolution method to study the *in vitro* release of nicotine from on!® products into artificial saliva using the U.S. Pharmacopeia flow-through cell dissolution apparatus 4 (USP-4) was used. Additionally, a UPLC-UV method for the determination of nicotine in dissolution fractions was validated. Nicotine release profiles were compared by calculating the difference factor (f_1) and similarity factor (f_2) by adopting methodology referenced in Guidance for Industry from FDA's Center for Drug Evaluation and Research (CDER).

on!® nicotine pouches are marketed in a variety of flavors and nicotine strengths. Nicotine release rates, based on percent released, were comparable across nicotine strengths and flavor variant. Furthermore, nicotine release rate for on!® nicotine pouches was found to be equivalent to Skoal Bandits™ (a traditional pouched moist smokeless tobacco product) based on the FDA's criteria.

ST 16

Harm reduction opportunities with a portfolio of oral tobacco-derived nicotine containing pouches

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There is overwhelming scientific evidence supporting a risk continuum with combustible products such as conventional cigarettes presenting the highest risk and non-combustible tobacco products presenting relatively lower risks. Millions of adult cigarette smokers and smokeless tobacco (ST) consumers (including dual users of cigarettes and smokeless tobacco [ST] products) are seeking alternatives to their current products. Oral tobacco-derived nicotine (OTDN) products is a rapidly evolving category that may play a role in tobacco harm reduction. Our OTDN products, currently marketed under the brand name on!®, are available in a portfolio of seven flavor varieties, with offered in five nicotine levels (1.5 mg, 2 mg, 3.5 mg, 4 mg and 8 mg). These products do not contain cut, ground, powdered or leaf tobacco and only contain tobacco-derived nicotine and non-tobacco ingredients.

Results from various studies showing that completely switching to the on!® nicotine pouches holds the promise for tobacco harm reduction were presented. The levels of harmful and potentially harmful constituents (HPHCs) in on!® pouches, except for nicotine, are substantially lower or not detectable compared to higher risk tobacco products (e.g., cigarettes and ST products). It was also demonstrated that HPHCs (e.g. NNK, NNN, cadmium and arsenic) associated with major tobacco-related diseases are not measurable in these products. Sustained reduction in exposure to most HPHCs will likely lower tobacco-related disease risks when completely switching to the on!® nicotine pouches relative to continued use of cigarettes or ST products. The scientific evidence presented indicates that the on!® products are appealing

to adult smokers and ST users, many replace their current products with on!® and a reasonable proportion (~ 27 %) even switch completely from cigarettes in an actual use study. Nonusers of tobacco products on the other hand have little interest in such products. Therefore, the on!® products can be considered to have the potential to reduce the harm from combustible tobacco products among those who are unable or unwilling to quit cigarettes.

ST 17

The dos and don'ts of non-targeted screening by LC–HRAM-MS for chemical characterization of smoke-free products

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Liquid chromatography coupled to high-resolution accurate-mass spectrometry (LC–HRAM-MS)-based non-targeted screening (NTS) is a key methodology for characterizing the chemical composition of complex matrices. It enables simultaneous identification and semi-quantification of a large number of compounds by using an unbiased approach. However, a single analytical method and compound identification strategy is not enough to cover the chemical space that is typically present in such matrices. This presentation introduces a comprehensive workflow of LC–HRAM-MS-based NTS from sample generation, compound identification, semi-quantification to the final report. Particular emphasis is placed on the analytical method requirements and pitfalls encountered during compound identification for in-depth chemical characterization and comparison of aerosol from Tobacco Heating System 2.2 (brand name IQOS®) and smoke from the reference cigarette, 3R4F. A set of four chromatographic/ionization techniques combined with simultaneous full-scan and first-order fragmentation (MS^2) data acquisition was applied. Compound identification was performed by using mass spectral libraries and *in silico*-predicted fragments from multiple integrated databases. Of the four complementary analytical approaches employed, reversed-phase LC–heated electrospray ionization MS in positive mode demonstrated the greatest coverage, with more than 50 % of the identified compounds present in the IQOS aerosol. A total of 67 % of the identified compounds were confirmed against a reference standard. In the elaboration of differences between the IQOS aerosol and 3R4F-derived smoke, a high coverage of chemical space was also achieved owing to the employed complementary compound identification strategies. In a subset of 331 identified compounds in 3R4F smoke, a total of 50 compounds were not present in an application-oriented in-house database, but could be identified by *in silico* prediction of MS^2 spectra based on the ChemIDplus, HMDB, and FDA databases. Altogether, this comprehensive and innovative approach has general applicability and a huge potential benefit for analysis of any complex matrix.

ST 18

Computer-assisted structure identification (CASI) for high-throughput identification of small molecules by GC×GC–HRAM-TOFMS

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Compound identification is widely recognized as a major bottleneck in modern metabolomic approaches and high-throughput non-targeted characterization of complex matrices. An automated platform CASI (computer-assisted structure identification) was developed, designed to accelerate and standardize the identification of compound structures by comprehensive two-dimensional gas chromatography with time-of-flight unit-resolution and accurate-mass spectrometry (GC×GC–[HRAM]TOFMS). Smoke samples from 3R4F reference cigarettes and aerosol samples from a heat-not-burn product, Tobacco Heating System (THS) 2.2, commercialized as IQOS®, were analyzed by GC×GC–TOFMS (Pegasus® IV, LECO) and GC×GC–HRAM-TOFMS (Pegasus® GC-HRT 4D, LECO). Structural proposals for the complete dataset were derived from CASI as an integral part of our non-targeted workflow. CASI considers mass-spectral database matching and matching of chromatographic data to quantitative structure–property relationships (QSPR)-derived prediction models for first and second dimension separations and boiling point, and it ranks proposals according to a scoring function. ACD/MS Fragmenter is used to predict fragmentation features for the CASI proposals to reconstruct a theoretical accurate-mass spectrum, which is then compared with the fragment ions from experimentally determined accurate-mass spectra by using NIST MS Search v.2.2. The resulting spectral "FIT", termed "fragmentation score", is directly linked to the proportion of determined fragment ions matching those predicted for the candidate structure. A linear combination of fragmentation scores and CASI scores is used to strengthen the candidate selection process and further increase the confidence for CASI-derived structural proposals. Among the more than 350 compounds found in THS 2.2 aerosol at concentrations above 100 ng/item by using GC×GC-TOFMS, a subset of 30 compounds was selected—considering their structural diversity and specificity of mass spectra—to test the performance of the enhanced structural identification workflow by using either mass-spectral database searches (a true positive rate of 100 % was achieved) or compound databases as an entry point (performance was compared with MetFrag).

ST 19

Non-targeted chemical characterization of complex matrices by nominal- and high-resolution accurate-mass GC×GC–TOFMS

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Comprehensive chemical characterization is an important element in the development of heat-not-burn products. Beyond the challenge of developing powerful analytical methods with sufficient chromatographic and spectral resolution, it is essential to have an automated data evaluation process in place that integrates structural identification, semi-quantification, and statistical comparison. The non-targeted screening (NTS) approach with two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC–TOFMS) presented here uses three separate analytical methods to maximize the chemical space coverage. The structural identification process is streamlined with a computer-assisted structure identification (CASI) platform, which improves the confidence level for compound identification, delivers semi-quantitative information for all compounds, and facilitates the detection of novel compounds and concentration differences. Each analytical method contains a dedicated set of retention index markers and stable isotope-labeled internal standards, which are representative of the anticipated range of chemical species present within the defined separation space. Advanced data processing of acquired raw data is followed by a sequence of automated data evaluation steps for data consolidation, retention time prediction, compound identification, semi-quantification, verification, and reporting. GC×GC coupled with high-resolution accurate-mass (HRAM) TOFMS further strengthens the confidence of structural proposals derived from CASI (e.g. by comparing experimental accurate-mass spectra with reconstructed mass spectra generated by *in silico* fragmentation of candidate structures proposed by CASI).

The performance of the existing GC×GC–TOFMS workflow and novel aspects of integrating accurate mass into the new NTS GC×GC–HRAM-TOFMS workflow are demonstrated by applying both techniques for chemical characterization of aerosol from the Tobacco Heating System 2.2 (brand name IQOS®).

ST 20

Non-targeted analysis using gas chromatography mass spectrometry for evaluation of chemical composition of e-vapor products

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The Premarket Tobacco Product Applications (PMTA) guidance issued by the FDA for electronic nicotine delivery systems (ENDS) recommends that in addition to other stability information, manufacturers submit chemical changes (e.g. aerosol constituents) to support the product's shelf-life. Although aerosols from e-vapor products are considerably less complex than aerosols from heat-not-nurn (HNB) or mainstream smoke from cigarettes, there are still challenges that arise from the chemical composition of the matrix, variety of flavors used, and the potential for chemical interactions to occur during storage and heating. There is additional complexity associated with efficiently collecting both volatile and semi-volatile compounds delivered in the aerosol from e-vapor products.

This non-targeted analysis (NTA) method is a semi-quantitative approach to evaluate the chemical composition of e-vapor products and can be used to characterize the aerosol and changes over time. We have developed a gas chromatography-mass spectrometry (GC-MS) based non-targeted analysis method for evaluation of volatile and semi volatile compounds. The data processing includes an automated data analysis that includes both Agilent MassHunter Unknowns Analysis software for mass spectral deconvolution, peak detection, and library searching and reporting. This method was able to accurately identify > 99 % of known compounds using mass spectral matching with a custom library. Our library contains over 1100 unique compounds, with approximately 600 confirmed with reference standards, to aid in identification. The performance characteristics of our method were validated for accuracy, precision, repeatability, and selectivity. This semi-quantitative method provides estimated concentrations which are 0.5 to 2.0-fold compared to the actual value. A limit of detection was established at approximately 0.7 ppm. This approach is applicable for the evaluation of volatile and semi volatile compounds in e-vapor products allowing for chemical characterization of e-vapor aerosol and e-liquids to support development of reduced risks products.

ST 21

Untargeted chemical characterization of the aerosol generated by a heated tobacco product

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In contrast to quantitative analysis, where chemical constituents of interest are targeted to the exclusion of all others, an untargeted approach considers indiscriminate determination of all analytes relevant to a specific chemical space. A portfolio of methods, based upon comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS) and liquid chromatography with high-resolution accurate-mass spectrometry (LC-HRAM-MS) was applied for characterization of the aerosol generated by a heated tobacco product. The methods used were semi-quantitative in nature, and concentrations for a large number of compounds were estimated versus a limited number of stable isotope-labeled reference standards of known concentrations by using an internal standardization approach. A reporting threshold of 100 ng/item was applied, which served to optimize the proportion of substances identified relative to a practicable amount of effort required for their identification.

Applying the Health Canada Intense smoking regimen, a total of 529 chemical constituents (excluding water, glycerin, and nicotine) were identified to be present in the mainstream aerosol of the Tobacco Heating System (THS) 2.2, a heated tobacco product developed by Philip Morris Products S.A. and commercialized under the brand name IQOS®. The majority of constituents were present in the particulate phase (n = 402), representing more than 80 % of the total mass estimated by untargeted screening. The gas-vapour phase was represented by 166 constituents, with a proportion of these constituents being present in both particulate and gas-vapour phases (39 compounds). The identities of 80 % of all chemical constituents (representing > 96 % of the total determined mass) were confirmed by using authentic analytical reference materials.

All 529 constituents identified in THS 2.2 aerosol were also present in the mainstream smoke of the 3R4F reference cigarette.

ST 22

Can liquid chromatography scan techniques be as useful a tool for e-liquids as gas chromatography scan techniques have been for cigarette tobaccos?

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Gas chromatography (GC) scan techniques have been very useful in profiling volatile and semi-volatile constituents of cigarette tobaccos before and after fabrication and packaging. Such scan techniques are even more valuable when the chromatographic profiles can be compared with those of tobaccos known to have been manufactured and packaged correctly. However, when such techniques have been applied to e-liquids, the results have been less useful as they are often dominated by the major components of the e-liquids: glycerol (VG) and propylene glycol (PG). Constituents of low volatility may also be missed. Consequently, we evaluated liquid chromatography (LC) using three different Cogent TYPE-C™ Silica columns (Bidentate C18, Phenyl Hydride, Amide; 250 mm x 4.6 mm, 1 mL/min flow rate) using acetonitrile-water mobile phases (isocratic or binary gradient) with UV detection at several wavelengths (including 195 nm for analytes that do not have absorptivity at higher wavelengths). While satisfactory chromatographic separations were obtained under ANP, NP, or RP conditions, scan techniques require a "product map" of analytes that are usually present in properly formulated e-liquids as well as analytes that have been reported in e-liquids that do not perform as expected. Over 100 formulations have been evaluated including commercial e-liquids, e-liquids formulated from commercial flavor concentrates and nicotine solutions (in PG or VG), as well as e-liquids formulated in-house from flavors and ingredients sold for other purposes. Substances known to degrade e-liquid performance were added to known good e-liquids at approximately the reported amounts to show that the LC approach was valid and could be done with readily available commercial LC instrumentation.

ST 23

New measures for assessing the abuse liability of connected ENDS

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This study describes the new opportunities that connected electronic nicotine delivery systems (ENDS) offer with regard to deterring abuse. The study also describes the challenges posed by using existing measurements and outlines new dependence measures that are pertinent to assessing abuse liability of the next generation of connected ENDS.

The current method for assessing the abuse liability of e-cigarettes is based on the nicotine dependence measures for tobacco cigarettes. Using a common instrument enables scientists

and regulators to easily compare tobacco and electronic cigarettes, however, not all symptoms of tobacco addiction are valuable indicators of e-cigarette dependency. For example, "refraining from use where prohibited" is a valid symptom of tobacco cigarette dependency, however, it is less significant to e-cigarette users, because there are fewer restrictions on where e-cigarettes may be used. The dissimilar importance of symptoms explains some of the variability in dependence risk between tobacco and e-cigarettes. Recent studies show that characteristics and patterns of use are additional determinants of the prevalence and severity of e-cigarette dependence.

As e-cigarettes become digital products, their characteristics and operation will change rapidly and vary so greatly that comparisons between e-cigarettes and tobacco cigarettes will be tenuous. The current method for assessing abuse liability will become inadequate. For example, the current wave of e-cigarettes gives users the ability to remotely control and monitor vaping devices using a companion mobile app installed on their smartphones. Smartphones and mobile apps are also addictive products and several methods exist to measure their risk. In order to accurately assess the abuse liability of an e-cigarette that is remotely controlled with a mobile app, a new method combining indicators for both products will be required.

ST 24

Dos and don'ts in the design of indoor air quality studies on smoke-free products

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Indoor air quality (IAQ) studies on smoke-free products (SFPs), such as electrically heated tobacco products (EHTPs) and e-vapour products (EVPs), have demonstrated substantial reduction of environmental emissions relative to cigarette smoking. IAQ studies on these products include measurement of common airborne markers (carbonyls, volatile organic compounds, particulate matter, and CO) together with specific tracers such as nicotine. Indoor environments are typically free of these specific tracers. However, depending on the ambient pollution levels in building surroundings, emissions from interior materials, and human occupancy and activities, indoor environments naturally contain certain levels of the general markers. Accordingly, assessment of environmental aerosols emitted by SFPs requires evaluation of all these confounding pollution sources. In addition, control measures should be taken to ensure compliance of the study participants with the experimental protocol. The results of previous studies have shown that prolonged human presence and activities indoors lead to an increase in the levels of several airborne constituents. This has reinforced the necessity for specific experimental requirements for IAQ assessment of SFPs. Thus, simply using "empty room air" as a background is insufficient; it is essential to use "room air" obtained in the presence of the same number of panelists doing the same activities as those during the sessions with SFPs. When experiments with SFPs are run in real-life conditions (restaurant, bars, etc.),

careful monitoring of activities during measurement (drinking, cooking, and serving hot food) and levels of constituents in outdoor air is obligatory for proper interpretation of the results. Furthermore, the consumption of SFPs should be verified, for example, by weighing the EVPs before and after use or by measuring the nicotine content in EHTP filters. Finally, the levels of aerosol retention by the individual panelists should be assessed.

ST 25

Human abuse liability assessment of tobacco and nicotine products: considerations to meet current regulatory recommendations

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The U.S. Food and Drug Administration (FDA) recommends that tobacco product manufacturers provide information regarding the abuse liability of tobacco and nicotine-containing products to support pre-market tobacco, modified risk tobacco product and substantial equivalence applications. While many methods exist, no standard tobacco product abuse liability assessment methodology has been established and no formal guidance document for such testing has been issued. The CORESTA Product Use Behaviour (PUB) Sub-Group reviewed the FDA's recommendations, published literature and studies related to the abuse liability of tobacco, and information from other authoritative bodies to identify the most promising approaches for abuse liability assessment of tobacco products. The current regulatory recommendations around abuse liability assessments as well as strategies to apply traditional abuse liability testing approaches for pharmaceutical products to tobacco and nicotine-containing products will be discussed. Considerations include the key study endpoints, subject population, study design, relevant comparator products, and product use approaches. Additionally, insights drawn from regulatory reviews of abuse liability information for a variety of tobacco product applications will be summarized. Recommendations for abuse liability testing of tobacco products and for synthesis and interpretation of findings from these assessments will be shared. Finally, global considerations for abuse liability assessments of tobacco and nicotine products for pre-marketing applications/regulatory review will be presented.

ST 26

Comparative risk assessment of heated tobacco product and electronic cigarette aerosols with cigarette smoke based on cancer potency and margin of exposure

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An average reduction of more than 90 % in concentrations of harmful and potentially harmful constituents (HPHCs; among given lists) has been observed in the aerosols of commercial heated tobacco products (HTPs) and electronic cigarettes (ECs) relative to the corresponding concentrations in smoke from the 1R6F reference cigarette or commercial cigarettes. Recently, quantitative risk assessment has been performed to better characterize the impact of the decrease in individual HPHC levels in ECs and HTPs on health risk when compared with cigarettes. Different methodologies have been published based on incremental lifetime cancer risk or cancer potency values for estimating the cancer risk and based on hazard quotient or margin of exposure (MOE) for evaluating the non-cancer risk. Our analysis combined MOE and cancer potency values with the goal of estimating the cancer and non-cancer risks associated with exposure to HPHCs from a range of commercial HTPs and ECs in comparison with those from reference and commercial cigarettes on the basis of available compound specific toxicological threshold references from official regulatory agencies. HPHCs were measured in HTP aerosols from eight brands and in cigarette mainstream smoke from 273 brands. The cancer potency related to product emissions was then calculated and translated into mean lifetime cancer risk. MOEs were defined as the ratios of human inhalation exposure limits to the estimated human exposure levels for the considered HPHCs. Compared with cigarettes, the relative cancer risk from lifetime exposure was 0.039 for HTPs and 0.009 for ECs. Compared with cigarettes, the relative MOE was 55.45 for HTPs and 107.57 for ECs. HTPs and ECs showed a large decrease in estimated cancer and non-cancer risks relative to the predicted cancer and non-cancer risks from cigarettes.

ST 27

Cardiovascular, carcinogenic and reproductive effects of nicotine exposure: a narrative review of the scientific literature

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The emergence of new tobacco heating products and electronic nicotine delivery systems (ENDS) is changing the way humans are exposed to nicotine. The purpose of this narrative review is to provide a broad overview of published scientific literature with respect to the effects of nicotine on three key health-related areas: 1) cardiovascular risk, 2) carcinogenesis

and 3) reproductive outcomes. These areas are known to be particularly vulnerable to the effects of cigarette smoke, and in addition, nicotine has been hypothesized to play a role in disease pathogenesis. Acute toxicity will also be discussed.

The literature until February 2019 suggests that there is no increased cardiovascular risk of nicotine exposure in consumers who have no underlying cardiovascular pathology. There is scientific consensus that nicotine is not a direct or complete carcinogen, however, it remains to be established whether it plays some role in human cancer propagation and metastasis. These cancer progression pathways have been proposed in models *in vitro* and in transgenic rodent lines *in vivo* but have not been demonstrated in cases of human cancer.

Further studies are needed to determine whether nicotine is linked to decreased fertility in humans. The results from animal studies indicate that nicotine has the potential to act across many mechanisms during fetal development. More studies are needed to address questions regarding nicotine exposure in humans, and this may lead to additional guidance concerning new ENDS entering the market.

ST 28

Transfer of aroma components in slim cigarettes flavoured by different methods

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It is of great significance to systematically study the transfer of aroma components in slim cigarettes flavoured by different methods for increasing the aroma quantity of slim cigarettes. In this study, a gas chromatography-tandem mass spectrometry method was established for simultaneously quantitatively determining the alcohols, aldehydes, ketones and esters in cut filler, mainstream smoke particulate matter and the filter, and the influence of four flavouring methods (cut tobacco, breakable capsule, tow and cotton thread flavouring) on the transfer of aroma components before and after smoking were investigated. The results showed that: 1) After storage, the distribution of aroma components in cut filler and the filter differed significantly among the cigarettes flavoured by different methods, and the transfer rates of aroma components were higher in tobacco-flavoured samples. 2) The transfer rates to mainstream smoke particulate matter of aroma components in tow-flavoured samples were similar to those in thread-flavoured samples, and those of aroma components with higher boiling points in tobacco-flavoured samples were higher than those in tow-flavoured samples and thread-flavoured samples and significantly higher than those in capsule-flavoured samples. 3) With the increase of boiling point, the transfer rates to mainstream smoke particulate matter of aldehydes, ketones and esters in tobacco-flavoured, tow-flavoured and thread-flavoured samples increased, while those in capsule-flavoured samples increased first and then decreased; and alcohols slightly differed from the other aroma components in the relationship between transfer rate to mainstream smoke particulate matter and boiling point. 4) The filter retention rates of the four kinds of aroma components in tow-flavoured samples were similar

to those in thread-flavoured samples. Capsule flavouring resulted in the highest filter retention rate of components with higher boiling points, while tobacco flavouring resulted in the lowest. This study provides a reference for the flavouring methods and the transfer of flavours in slim cigarettes.

ST 29

Comparison of reference cigarette variability, smoke yields, and filler HPHC content of 1R6F and 3R4F

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Reference cigarettes have a long history of use in the cigarette industry as quality control monitors, model cigarette systems, and analytical method development tools. The University of Kentucky's Center for Tobacco Reference Products 3R4F has been commonly used since its manufacture in 2006 but is being replaced by 1R6F due to limited quantities. The two reference cigarettes across multiple smoke collection and chemical analyses to establish control limits for 1R6F prior to our laboratory's transition were compared.

The two cigarettes were compared on 14 filler analytes and 20 smoke analytes, under both non-intense (ISO 3308) and intense (ISO 20778) smoking conditions. Overall, the day-to-day variability of 1R6F was slightly less than 3R4F under non-intense conditions. However, the day-to-day variability of the two reference cigarettes was comparable under intense conditions. The within day variability was comparable for both reference cigarettes under both smoking regimes.

There were numerous statistical differences in the smoke and filler chemistry. Notably 3R4F NNN levels averaged about 20 % more in both filler and smoke and NNK levels averaged 20 % more in filler and 28 % more in smoke.

The certified uncertainty of reference products is often misunderstood, and we will discuss the proper interpretation of the 1R6F certified uncertainty and compare our average 1R6F results to the University of Kentucky certified values. All the average 1R6F results within our lab were within the certified value limits for 1R6F except for crotonaldehyde under non-intense conditions.

Because variability was comparable or less for 1R6F relative to 3R4F, it is reasonable to substitute 1R6F for 3R4F as a laboratory monitor, though laboratory control limits will need to be adjusted after the transition.

ST 30

Dokha tobaccos – does their chemistry follow their growth in popularity?

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Dokha tobaccos reportedly are increasing in popularity. They are high-alkaloid Oriental tobaccos, grown under dry conditions in Middle Eastern countries and designed to be smoked in a narrow pipe known as a medwakh. However, they can also be smoked in a filter tube. They are typically sold with the designations of "Cold", "Warm" and "Hot", referring to their nicotine content. Some dokha tobaccos are added to shisha tobaccos to increase the nicotine content. However, little has been reported about their chemistry. Several samples of dokha tobacco were obtained from Internet-based retailers. Their chemical properties obtained using both routine chemical measurements (obtained from a commercial tobacco laboratory) and liquid chromatography analyses. Results were compared with those for the University of Kentucky RT3 Ground Oriental Research Tobacco. The following data (mean of two determinations) were obtained and presented in the order of RT3, "Cold", "Warm", and "Hot" and are on a dry-weight-basis. Alkaloids (nicotine): 0.63, 3.76, 7.08, 8.12; total sugars: 4.01, 6.92, 4.70, 4.51; reducing sugars: 3.44, 6.09, 4.37, 3.96; quick oven volatiles: 14.33, 12.97, 10.46, 10.21. Tobacco pH was determined using CORESTA Recommended Method (CRM) 69. Values were: 5.11, 4.72, 4.80, and 4.97. Two types of LC analyses were done. The first was based on polyphenol methods using 60/40 MeOH/H_20 extraction of the tobacco followed by analyses on a 250 × 4.6 mm YMC Triart C18 column using several mobile-phase and UV-detection conditions. The results for the polyphenols showed much similarity between the RT3 and dokha tobaccos. The second was based on 87/13 ACN/H_2O extraction followed by analyses of a Cogent Amide column with RI detection under conditions used for tobacco sugars. The main finding was the differences among the profiles for the dokha samples and the RT3. Smoke from the "Hot" dokha packed in a filter tube was very mild given its high nicotine content.

2020 CORESTA CONGRESS ONLINE

SMOKE SCIENCE and PRODUCT TECHNOLOGY

POSTER PRESENTATIONS

STPOST 01

An algorithm for inspecting breakable capsules with "Off-Centre" defects based on machine vision technology

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There is a multitude of quality defects for breakable capsules. The "Off-Centre" defects refer to the phenomenon that the content of breakable capsule is not centered due to uneven thickness of the shell material. Aiming at the low efficiency of manual selection for breakable capsules with "Off-Centre" defects, an "Off-Centre" defect inspection algorithm based on machine vision technology was developed to automatically sort breakable capsules for cigarettes. Capsules were orderly fed into a specially designed fixture, and then the fixture was rotated to a high-speed camera that was used to acquire the images of the capsules. An edge detection algorithm based on the Laplace operator and Canny operator was adopted to extract features of the obtained image, recognize edges, locate the capsule position and calculate the capsule eccentricity. According to dozens of images of a capsule, the algorithm could determine whether the capsule had an "Off-Centre" defect. The results of comparative experiments between the inspection algorithm and manual inspection showed that, the recognition rate of the algorithm was above 98 %, its speed was 100 times that of manual inspection, and the algorithm could accurately distinguish the capsules with black spots from the capsules with "Off-Centre" defects. The application of the algorithm to capsule inspection remarkably improves the inspection efficiency for "Off-Centre" type defects and the quality of breakable capsules.

STPOST 02

Analysis of highly volatile flavouring compounds in cigarettes smoke

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A method for the analysis of highly volatile flavouring compounds in cigarette smoke is proposed. The extraction and concentration of these compounds is challenging because of the complexity of the smoke matrix, and the high vapour pressure and low boiling point of the target compounds. Established analytical methods are based on trapping the smoke vapour phase in cryogenically cooled impingers containing methanol. This dilutes the sample and retains many other components of the smoke. An alternative is collection of smoke in Tedlar® bags, and sampling of volatiles by solid phase microextraction (SPME) and GC-MS using a cryo-cooled GC. The proposed method uses the new Supel-Inert® foil bags fitted with a Thermogreen septum for gas sampling followed by HS-SPME-GC-MS analysis without cryo-cooling. A mixture of flavouring compounds (including ethyl acetate, isobutanol, 2-pentanone, isobutyraldehyde and ethyl isobutyrate) was injected into cigarette filters. The cigarettes were smoked (8 puffs, ISO 3308) and the mainstream smoke was collected in Supel-Inert foil bags. After smoke collection, the bags were fortified with the internal standard toluene-d8 and incubated in an oven at 105 °C for 5 min. A DVB/CARB/PDMS SPME fibre was inserted through the septum to extract the flavouring compounds for five minutes prior to GC-MS analysis. Compounds were quantified by standard addition using five points analysed in triplicate. Optimisation of method parameters (e.g. time of extraction, temperature, stability of the vapour phase) will be discussed. To the best of our knowledge, this method has not been applied to the analysis of these types of compounds in cigarette smoke and has the potential to be applied to other highly volatile flavouring compounds in aerosols.

STPOST 03

Conversion of glycerin in cigar smoke to formaldehyde in DNPH trapping solution

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Following the 2016 deeming of cigars by the Food and Drug Administration (FDA), there has been an increased interest in cigar science, including ways to accurately measure the harmful and potentially harmful constituents (HPHCs) found within mainstream cigar smoke. Included in this list are various carbonyls including formaldehyde, acetaldehyde, acrolein, and crotonaldehyde. For ease of analysis, carbonyls are traditionally trapped in an acidic DNPH solution to convert the carbonyls to their respective hydrazone adducts.

During the analysis of mainstream cigar smoke for carbonyls, it was observed that following sample preparation, the levels of formaldehyde in select cigars would continue to increase over time with a % increase in the relative response of ~ 50 % to 80 % over 17 hours. In the production of cigars, glycerin is typically added as a humectant and previous work has shown that under the correct conditions, glycerin can contribute to the creation of formaldehyde through oxidation and subsequent retro aldol reaction. This has led us to suspect that high levels of glycerin in some cigars may contribute to the increase in observed formaldehyde levels under the sample conditions similar to CORESTA Recommended Method (CRM) 74. To examine this, six aliquots of glycerin-d8 were mixed with DNPH trapping solution, three of which were then quenched with pyridine and the other three were not. All six samples were repeatedly injected overnight using an LCMS and monitored for the formation of formaldehyde-d2-DNPH. Over 12 hours, there was a steady rise in the observed levels of formaldehyde-d2-DNPH in samples which were quenched when compared to unquenched samples (no added pyridine), which remained relatively constant. Overall, there was a three-fold increase in the levels of formaldehyde-d2-DNPH produced in quenched samples as opposed to unquenched samples. Because of this, it is recommended that when assessing samples with a high glycerin content, they be analyzed as quickly as possible to mitigate the overall effect of the production of formaldehyde from glycerin.

STPOST 04

Assessing the sources of smoke variability in machine-made cigars

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Cigar smoking presents a higher level of variability than what is typically seen in cigarette smoking. In our experience, increased variability is observed for all cigar types including machine-made, mass produced cigars. To understand the sources of variability, this study assesses select HPHC in machine-made cigars in both the tobacco and smoke matrices. Fifty individual cigars were weighed and ground and the resulting tobacco was analyzed for nicotine, ammonia, NNN, NNK, and benzo[a]pyrene content. Separate portions of the same product were analyzed for weight, resistance to draw and the yield of nicotine, ammonia, NNN, NNK, and benzo[a]pyrene in the mainstream smoke.

Tobacco results, on a per gram basis, were more consistent than smoke yields. Tobacco results, on a per cigar basis increased by 5-10 % versus the per gram results. Weight and pressure drop values for the brand of cigar tested returned RSD values of approximately 20 %.

Nicotine variability on a per cigar basis remained relatively consistent between matrices at approximately 14 % RSD. The variability observed for ammonia in smoke was 22.4 % RSD while the tobacco matrix showed only 13.8 % RSD. Smoke RSD values for B[a]P, NNN, and NNK were 12.5 %, 14.0 %, and 15.2 %, respectively, which was lower than the variability observed in tobacco for these compounds at 17.4 %, 20.2 %, and 19.6 %.

Total cigar weight was the main factor of the increased variability observed in the per cigar ground filler results. Resistance to draw and cigar weight, not blend variability, were the main factors for variability observed for nicotine, ammonia, NNN, NNK, and benzo[a]pyrene in the mainstream smoke.

STPOST 05

A screening method by gas chromatography–mass spectrometry for the quantitation of 33 aerosol constituents from a heat-not-burn tobacco product

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This screening method allows the quantitation of 33 compounds [phenol, *o*-cresol, *m*-cresol, *p*-cresol, catechol, resorcinol, hydroquinone, 1,3-butadiene, isoprene, benzene, acrylonitrile, toluene, pyridine, styrene, propylene glycol, menthol, acrylamide, naphthalene, nicotine, acetamide, quinoline, triacetin, glycerin, carbon disulfide, furan, diacetyl, 2,3-pentanedione, acetol, glycidol, furfural, 2-furanmethanol, caprolactam, and 5-(hydroxymethyl) furfural] in the aerosol generated by an electronically heated tobacco system with a single aerosol collection and single analytical method, where the same extract is analyzed by three different gas chromatography–mass spectrometry methods. This method is aimed at drastically decreasing the workload from four aerosol collections to one and from five analytical methods to one, involving three instrumental methods.

The aerosol generated by electronically heated tobacco, in compliance with the Health Canada (HC) smoking regimen, was collected by using two microimpingers containing a cooled solvent mixture, connected in series after a glass fiber Cambridge filter pad (CFP). After aerosol collection, the impinger solutions and CFP were combined for extraction. Then, the same aerosol extract was split into three aliquots, where 21 compounds were analyzed on a DB-WAX capillary column, ten on a DB-FFAP, and three on a DB-624, after derivatization.

Linearity was demonstrated for all compounds ($R^2 > 0.995$). The recovery from spiked aerosol extracts was between 82.1 % and 113.8 %. For individual aerosol collections, the coefficients of variation of repeatability (CV_r) for the whole process (aerosol collection and analytical methods) ranged between 3.6 % and 17.7 % and those of intermediate precision (CV_{IP}) ranged between 3.8 % and 19.9 %. For homogenized aerosol extracts, the CV_r for the analytical methods ranged between 0.9 % and 6.2 % and the CV_{IP} ranged between 0.9 % and 10.4 %. The method was validated in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines and shown to be selective, precise, and accurate compared to the tested concentration ranges.

STPOST 06

Assessment of filter pre-treatment for metal analysis in e-vapour aerosol

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Quartz filter pads are commonly used to collect e-vapour aerosol for metal analysis. Detecting and quantifying trace metals is sometimes difficult using this method, because quartz filter pads contain some metal elements at detectable and variable levels. Although recent studies have reported that electrostatic precipitation can be applied instead of filters to analyse metals at low background levels, this method requires dedicated equipment, such as glass collection tubes and high-voltage generators. Therefore, filter pad collection remains advantageous in terms of operational efficiency. In this research, we have developed an approach to reduce the metal background by treating quartz filter pads with acid. The background level of eight metal elements in treated filter pads under several treatment conditions, including different acids (nitric, hydrochloric, and mixed), acid concentrations (10 % - 50 %), and numbers of treatment cycles (once, twice, and three times), was quantified. As a result, an optimum treatment procedure to reduce the metal background from filter pads was successfully established. The background level was equivalent to that of the blank in the electrostatic precipitation method. Furthermore, it was confirmed that filter treatment with acid did not influence the ability of the filter to collect e-vapour aerosol. The results indicate that treated quartz filter pads can be used as a collection method for metal analysis.

STPOST 07

New developments in vacuum photoionisation TOF-MS technique to analyse smoking products on-line and in real time

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Photo ionisation-time of flight mass spectrometry (PI-TOFMS) has been established for on-line analysis of complex gas mixtures. Cigarette smoke, e-cigarette vapour and the vapour of tobacco heating products provide good examples for such complex gas mixtures. Many toxicants, such as butadiene, acetaldehyde, naphthalene, phenol or polycyclic aromatic hydrocarbons (PAH), can be detected with single puff-resolution in the smoke or vapour of smoking products.

However, new developments in vacuum PI-TOFMS enable new insights into the analysis of smoke/vapour constituents. The parallel use of Single Photon Ionisation (SPI) and Resonance Enhanced Multi Photon Ionisation (REMPI) enable a more specific separation between general

organic compounds and aromatic structures as well as in specific cases also an on-line separation of isobaric compounds. New laser SPI sources can improve the sensitivity and selectivity. Using a F_2 excimer laser achieves higher photon densities and more generated ions. The respective ionisation energy is approx. 7.9 eV, which suppresses smaller and highly abundant constituents to set a new focus on the on-line investigation of the composition of smoke/vapour mixtures).

As a third new development, the usage of a Fast-GC approach coupled to a PI-TOFMS can additionally improve the results. Every single puff can be separated using the fast-GC to add a further data dimension and increase the reliability of the achieved data.

STPOST 08

Determination of α-tocopherol acetate (vitamin E acetate) in e-liquids and cannabis liquids samples - a comparison between HPLC-DAD and LC-MS/MS methods

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The determination of vitamin E acetate in the liquids of electronic cigarettes is important due to its possible connection to lung injury associated with the use of vaping products. These issues have been predominantly linked to the use of illicit cannabis products where vitamin E acetate has been found in the liquids in the range of 4 % to 40 %. However, it is critical to develop accurate and reliable analytical methods, sensitive enough to demonstrate vitamin E acetate has not been added to any e-cigarette or vaping products.

Two analytical methods for the analysis of vitamin E acetate in e-liquids were developed using a simple dilution of the e-liquid with methanol. The reversed-phase HPLC-DAD (Diode Array Detection) approach offered an applicable range of 10 to 1000 µg/g of e-liquid. The lab fortified blank (LFB) and lab fortified matrix (LFM) recoveries, ranged from 99.3 % to 101 % and 82.1 % to 114 %, respectively. Although this simple and cost-effective method was suitable for quantifying vitamin E acetate in samples when used as a principle component of the liquid, it lacked selectivity and may suffer a positive bias due to interference from matrix components. Therefore, a more sensitive and selective LC-MS/MS method was required.

The LC-MS/MS method provided an applicable range of 100 ng/g to 50 µg/g of e-liquid, using up to 5 MRM transitions. This made it suitable for the analysis of complex liquids, including cannabis liquid matrices. However, characteristics or properties of the compound provided many challenges. The deuterated (-D9) vitamin E acetate internal standard allows for the correction of potential matrix effects with the accuracy and precision from LFM recoveries ranging from 84.5 % to 108 % across a range of fortification levels.

In conclusion, the LC-MS/MS method can accurately quantify trace amounts of vitamin E acetate in both e-liquids and cannabis liquid matrices.

STPOST 09

Study of thermal decomposition of Iranian Virginia tobacco components

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The object of this study was to investigate the thermal decomposition of different parts of Virginia tobacco. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) curves resulting from thermal decomposition of tobacco components at 10 °C per minute heating rate were recorded under the air atmosphere and argon. Thermal decomposition began at approximately 70 °C, 50 °C, 80 °C and 80 °C and major weight changes were between 280 °C - 540 °C, 270 °C - 580 °C, 280 °C - 580 °C and 260 °C - 580 °C temperatures with two stages of weight loss that occurred for tobacco midrib, tobacco scrap (lamina), tobacco stem and tobacco root, respectively. Study of the results of thermal analysis of plant tissues showed that removal of the moisture occurs at 30 °C - 120 °C and, the destruction of hemi cellulose at 120 °C - 200 °C, cellulose degradation at 200 °C - 400 °C, and carbonization occurs at 200 °C - 400 °C temperature range, respectively. The various chemical components of the plant, such as lignin, hemicellulose, and cellulose, are broken down to form compounds such as carbon dioxide, carbon monoxide, methane, methanol, and tar. In the 400 °C - 600 °C temperature range, pyrolysis of lignin and carbonization of cellulose occur.

STPOST 10

Characterization of on!® nicotine pouches – Part 1: HPHCs

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on!® is an oral tobacco-derived nicotine pouch product that does not contain cut, ground, powdered or leaf tobacco. In order to issue market authorization, the U.S. Food and Drug Administration (FDA) must determine whether the on!® nicotine pouches are appropriate for the protection of public health (APPH). We characterize the levels of harmful and potentially harmful constituents (HPHCs) for the portfolio of on!® nicotine pouches to inform the determination of APPH.

FDA has not issued specific guidance for reporting HPHCs for oral tobacco-derived nicotine products, such as on!® nicotine pouches. Absent specific guidance from FDA, the abbreviated list of HPHCs in on!® nicotine pouches was measured according to the guidance for smokeless tobacco products, recognizing that these products do not meet the statutory definition of a smokeless tobacco product. The HPHCs evaluated included nicotine, NNN, NNK, B[a]P, acetaldehyde, formaldehyde, crotonaldehyde, cadmium and arsenic. The objective of this work

was to determine HPHCs in on!® nicotine pouches and compare those results to commercially available tobacco products such as cigarettes, smokeless tobacco including snus, and an oral nicotine replacement therapy (NRT) product. Except for nicotine, it was observed that there were either no detectable levels or significant reductions in HPHCs when compared to traditional combustible and smokeless tobacco products, including snus, and comparable results relative to the NRT.

STPOST 11

Factors influencing pyrolysis and smoke release characteristics of tobacco particles at low temperature

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In order to reveal the rules of pyrolysis and smoke release of tobacco particles under low temperature heating conditions, the effects of glycerol content, moisture content, and size of tobacco particles on their thermal conductivity, pyrolysis and smoke emission were investigated using a thermal conductivity tester, a thermal gravimetric analyzer (TGA) and a cone calorimeter (CONE). The results indicated the following: 1) The thermal conductivity of samples presented a rising trend with the increased content of glycerol and moisture and the decrease of particle size. The specific heat capacity showed a growth trend with the increase of moisture content and reduction of particle size, while it increased first then decreased with the rising of glycerol content, 2) The addition of glycerol dramatically increased the percentage of mass loss of tobacco particles at low temperatures ranging from 140 °C to 300 °C, which became the major mass loss stage. The influence of moisture content on the mass loss rate of tobacco particles weakened gradually with the rise in temperature. The moisture content mainly affected the emission of small molecules, such as dissociative water and glycerol, at low temperature. The main mass loss process of samples slightly shifted to low temperature ranges with the decrease of tobacco particle sizes, 3) The increase of glycerol content (within 30 %) was beneficial to shortening the initial release time of tobacco smoke, accelerating the smoke production rate, and raising the total smoke rate. The decrease of moisture content or particle size also had similar effects.

STPOST 12

A rapid quantitative optimization method for technological parameters of threshing based on uniform experimental design

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During the redrying and processing of tobacco leaves, there are numerous processing parameters for each threshing and separation unit. The influences of processing parameters on the size distribution of tobacco strips are coupled mutually with each other, which results in the difficulty of quantitative control for threshing quality. This paper proposed an optimization method to determine the processing parameters of threshing and separation units rapidly and quantitatively. Firstly, an importance evaluation method based on neural network was used to study the importance of processing parameters in the first and second threshing and separation units. Ten processing parameters that have the greatest impacts on the threshing quality were selected as the test factors. Secondly, a stepwise polynomial regression model was established on the basis of historical threshing data, and the predictive performance of the regression model was evaluated via uniform experimental design. Furthermore, due to the multiple structure indexes of tobacco leaves, a new comprehensive evaluation index was proposed to assess the threshing quality. Finally, the optimization schemes of processing parameters were proposed based on the score of the comprehensive evaluation index. The effectiveness of the proposed method was verified by an on-site test in Chenzhou Tobacco Redrying Factory. The real quality indexes of tobacco leaves were basically consistent with the prediction values with the relative errors of percentage of large-sized strips and percentage of large- and medium-sized strips less than 5 %. By using this method, only a simple parameter adjustment is needed to make the strip structure of tobacco meet the threshing requirements.

STPOST 13

Analysis of menthol optical isomers in tobacco products

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Menthol can exist in four enantiomeric pairs, each of which has D-type and L-type separately. Different menthol isomers possess different sensory properties. The establishment of configuration analysis technology for the menthol isomer is of great value for the quality evaluation and application of menthol in tobacco. Based on the detailed study of the separation efficiency of eight menthol optical isomers by different non-chiral and chiral capillary columns, a method for separating eight menthol optical isomers from tobacco products by tandem chiral capillary columns and gas chromatography-mass spectrometry was established. The eight menthol optical isomers were successfully separated by CycloSil-B + BGB-175 tandem chiral capillary column. The chromatographic peaks of eight menthol optical isomers all met the separation requirements, and the shapes of all peaks were sharp and symmetrical. The response value and retention time had good interday stability, and the stationary phase loss was small under separation conditions, which had no significant influence on menthol detection. The limit of quantification (LOQ) of the established method was lower than 72.9 µg/L with the recoveries ranged from 92.1 % to 109.5 % at three spiked levels. The method was successfully applied to the separation and quantitative detection of menthol optical isomers in traditional cigarettes and heated cigarettes. The results showed that the dominant form of menthol in the tested samples was L-menthol, and traces of other menthol isomers were detected in most samples as well.

STPOST 14

Mode-of-action analysis of the induction of micronuclei by a flavouring compound *in vitro*

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A compound showing a positive result on the *in vitro* micronucleus test (MNT), one of the standard battery of genotoxicity tests, must be followed-up with *in vivo* MNT. However, not uncommonly, some *in vivo* MNT results are negative, indicating so-called “misleading positives” on *in vitro* MNT. In this study, the issue was investigated by developing a way to analyse the mode of action (MoA) of micronucleus (MN) induction. The approach includes two assays: ToxTracker®, a reporter gene assay with outstanding specificity and sensitivity; and a high content analysis (HCA), which directly detects various molecular events in a cell. To demonstrate the feasibility of the approach, propenyl guaethol, a flavouring compound, which

has shown misleading positive results of MNT with an unknown MoA, was assessed. In the ToxTracker® assay, the reporter cell lines for DNA damage and oxidative stress responded to the compound. Moreover, co-treatment of an antioxidant with the compound suppressed these responses. The HCA assay directly detected accumulations of γH2AX and intracellular reactive oxygen species (ROS) by the compound. These results suggest that the misleading MN positive result for the compound is attributable to a secondary mechanism induced *in vitro* by the accumulation of intracellular ROS. To test this suggestion, the compound was assessed in *in vitro* MNT with pretreatment of an antioxidant, which significantly suppressed the induction of MN. These results show that one of the MoAs of the misleading positive induced by a flavouring compound was explained, which demonstrates the utility of this approach.

STPOST 15

The comparative analysis of cytokine production by a human 3D airway tissue model following exposure to traditional cigarette smoke, tobacco-heated product and e-cigarette aerosol

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The battery of regulatory assays currently used to assess the toxicity of aerosol exposure are limited in their ability to identify changes at the cellular and molecular level. This has prompted a shift towards a more holistic systems biology approach when assessing the effects of exposure to potential toxicants. For example, analysis of the inflammatory mediators produced may provide information on the toxicity-related mechanisms associated with such exposure.

In this study, the V-PLEX® human cytokine kit (MesoScale Diagnostics, LLC) was used to analyse a panel of 30 disease biomarkers following acute exposure of a human airway 3D tissue model (MucilAir™, Epithelix Sarl, Switzerland) at the air-liquid interface (ALI) using a Vitrocell® VC10® to cigarette (3R4F) smoke, tobacco-heated product (THP) and e-cigarette aerosol. Following exposure, post-exposure and recovery (24 hour) medias were collected and biomarker levels quantified. Changes were observed for a number of biomarkers in both the post-exposure and post-recovery media including; IL-1β, IL-8, IL-10, IL12p70, IFN-γ and VEGF. For example, IFN-γ demonstrated ~ two-fold increase in the post-recovery media following exposure to all test articles, VEGF was decreased following exposure to the THP and e-cigarette products but not 3R4F and IL-1β levels were increased following exposure to 3R4F but decreased following exposure to THP and e-cigarette.

Our observations demonstrate the potential of the MSD V-PLEX® human cytokine kit in assessing the effects of aerosol exposure on cytokine production, providing an insight to the different biological pathways affected by different commercially-available nicotine delivery systems.

STPOST 16

Comparison of *in vitro* cytotoxicity and genotoxicity of condensates derived from electronic-cigarettes and reference combustible cigarette

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In recent years, electronic nicotine delivery systems (ENDS) including electronic cigarettes (e-cigarettes) have emerged as potential reduced-risk alternatives to conventional cigarettes for adult smokers. The U.S. Food and Drug Administration (FDA), in their guidance for Premarket Tobacco Product Application (PMTA) for ENDS, recommends evaluating the toxicological profile of ENDS products using *in vitro* genotoxicity and cytotoxicity assays, in comparison to other commercially available products in the same category as well as conventional combustible cigarettes. In this study, whole aerosol condensates (e-condensate) were generated from 14 cig-a-like e-cigarettes (Test) and were subjected to the standard *in vitro* assay battery: neutral red uptake (NRU) for cytotoxicity, Salmonella mutagenicity (Ames), and micronuclei (MN) for genotoxicity. The e-condensates were also characterized for specific formulation ingredients as well as carbonyls immediately upon generation and after eight weeks in -70 °C storage. The results of Test condensates were compared with those from six U.S. commercially marketed cig-a-like e-cigarettes (Comparator) and 3R4F reference cigarette condensates. The 3R4F (smoke) condensate was cytotoxic in the NRU assay (IC_{50} of 0.044 ± 0.003 mg/Ml total particulate matter (TPM); 2.04 ± 0.16 µg/mL nicotine), mutagenic in the Ames assay (strains TA1537 + S9 and TA98 + S9), and genotoxic in the MN assay. In contrast, e-condensates from all Test and Comparator e-cigarettes did not show cytotoxicity or genotoxicity when tested at much higher concentrations (maximum concentrations tested up to: NRU [0.248 mg/mL TPM]; Ames [4.95 mg/mL TPM]; MN [0.495 mg/mL TPM]). The measured formulation ingredients and carbonyls detected in e-condensates were stable for up to eight weeks at -70 °C. In summary, the results from the current study support that e-cigarette aerosols have substantially lower biological activity in these assays than smoke from combustible cigarettes.

STPOST 17

Comparison of smoking machines for sample preparation for *in vitro* assays

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For *in vitro* assays of tobacco products, total particulate matter (TPM) samples can be prepared by collection of tobacco smoke/aerosol on a glass fiber filter and then extraction with a dimethyl sulfoxide solution. Two types of smoking machines, linear and rotary, are widely used to collect aerosol from heated tobacco products (HTPs). Recent studies have shown that losses occur during collection of TPM using rotary smoking machines because of condensation of the aerosol in the flow path. The objective of this research was to investigate the chemical and biological characteristics of TPM samples produced from HTPs by linear and rotary smoking machines. TPM samples from the two types of machines were weighed and analyzed by gas chromatography to quantify glycerin, propylene glycol, nicotine, water, and carbonyl compounds. Ames, micronucleus (MN), and neutral red uptake (NRU) tests were conducted to examine the mutagenicity and cytotoxicity of the TPM samples. The TPM yield from the rotary machine was lower than that from the linear machine because water from the aerosol condensed in the flow path. Consequently, compared with the linear-machine TPM sample, the rotary-machine TPM sample was more concentrated and contained higher concentrations of all main components, except for water. Furthermore, the TPM sample obtained from the rotary machine had higher cytotoxicity in the NRU assay than that from the linear machine. However, the Ames and MN test results for the rotary and linear TPM samples were not significantly different. Our results show that some characteristics differ between TPM samples obtained by the two types of smoking machines. Therefore, it is important to consider the difference in the test results by the type of smoking machine when conducting *in vitro* assays of HTPs.