Cancun • Mexico

CORESTA AP 2023

AGRONOMY & LEAF INTEGRITY
PHYTOPATHOLOGY & GENETICS

JOINT STUDY GROUPS CONFERENCE

PROGRAMME & ABSTRACTS

Cancún, Mexico

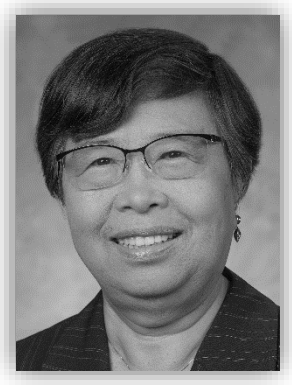
15–19 October 2023

Cooperation Centre for Scientific Research Relative to Tobacco

CONTENTS

WELCOME MESSAGE

ON BEHALF OF CORESTA



Dongmei XU
President of the CORESTA Scientific Commission

Dear Colleagues

I am delighted to extend a warm welcome to the Joint Conference for the Agronomy & Leaf Integrity and Phytopathology & Genetics Study Groups of CORESTA in 2023. I want to express my gratitude to the CORESTA Secretariat for hosting us and for choosing such a stunning and lively location for our gathering.

This conference is a momentous occasion as it marks our first in-person meeting after a prolonged period of remote meetings due to the COVID-19 pandemic. With over 50 presentations and 30 posters scheduled, as well as three workshops on "Sustainability", "ESG" and "Cigar Production," we are excited to showcase our programme.

As we come together, all Sub-Groups and Task Forces will report their achievements and progress. I extend my appreciation to the Coordinators and Secretaries for their commitment and dedication to ensuring that all working units align with our organizational vision and meet the expectations of our members.

CORESTA remains committed to providing credible science and best practices related to tobacco and its associated products. It continues to serve as a platform for engaging with regulators and a place where participants can exchange ideas, discuss their research, and foster new relationships.

We encourage all attendees to take advantage of the opportunity to network, engage in meaningful discussions, and learn from each other. The AP conferences aim to foster a collaborative community and provide a platform for professionals to exchange ideas and best practices.

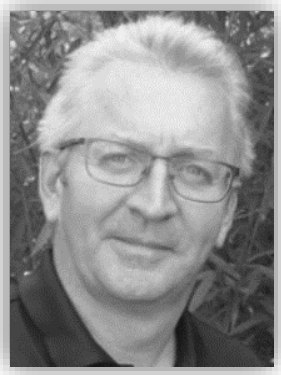
On behalf of the organizing committee, I would like to thank our sponsors, partners, and volunteers for their support in making these conferences possible. We also extend our gratitude to all attendees for their participation and contributions to this event.

We look forward to welcoming you to the AP conferences in 2023 and wish you a productive and enjoyable experience.

Dongmei XU
Altria Client Services LLC
Richmond, VA, U.S.A.

WELCOME MESSAGE

ON BEHALF OF THE ORGANISERS



Stéphane COLARD
Secretary General CORESTA

Welcome!

After three years of virtual Conferences and Congresses, it is a real pleasure to welcome you to the CORESTA Agro-Phyto Conference (AP2023) in Cancún, Mexico. Since 2020, the CORESTA Secretariat successfully met the challenge of organising virtual events, but the time has come to meet again in person.

The mission of CORESTA is to promote and facilitate international cooperation and best practices in scientific research relative to tobacco and its derived products. Its vision is to be recognised as an authoritative source of publicly available, credible science and best practices. The mission is fulfilled by enabling our Members to cooperate together in working groups, by offering all scientists, CORESTA Members or not, the possibility to present their results in an open forum, and by publishing reports and presentations on our CORESTA website.

The objective of the CORESTA AP2023 Conference is to provide again the scientific community with opportunities for discussions with international specialists leading to fruitful collaborations, and multiple occasions for interactions and networking. We strive to offer again scientists from all around the world, the chance to share their knowledge and experience. The CORESTA Agronomy & Leaf Integrity and Phytopathology & Genetics Study Groups prepared an attractive scientific programme with high level scientific presentations and thematic workshops. The quality of the programme is fully aligned with the CORESTA vision.

The organising committee thank the Scientific Commission and all presenters who are contributing to the success of this Conference. We also thank all participants who are attending in an open and cooperative spirit, and we are encouraging them to interact with each other.

We are confident that the venue and the programme will meet your expectations, and we welcome you in this unique environment, to work and share together, and to advance science and knowledge for the benefit of the broad scientific community.

Enjoy the Conference!

Stéphane COLARD
Chairman of the AP2023 Organising Committee
Paris, France

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2022-2024

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University of Kentucky	U.S.A.	Anne FISHER

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Stéphane COLARD

CORESTA, 11 rue du Quatre Septembre, 75002 Paris, France

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and
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2022-2024

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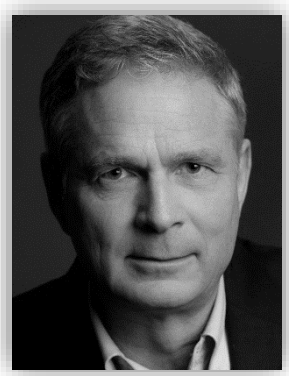
CORESTA PRIZE



Marco PRAT

Marco PRAT has a MSc in Agronomy and worked for many years as an agronomist for Universal Leaf Tobacco in Italy. In 2002, he joined the JT International (JTI) Scientific and Regulatory Department with responsibility for the crop protection agent (CPA) residue control programme. Marco held the position of Global Product Integrity Director within JTI Global Supply Chain Agronomy until his retirement end of April 2022.

Within CORESTA, Marco was active for many years. He was a member of Agrochemical Advisory Committee (ACAC) from 2003-2022, becoming Vice-Chairman in 2007 and Chairman in 2009. He has served on the Scientific Commission as President of the Agronomy Study Group from 2010-2012, as Vice-President of the Commission from 2012-2014 and as President from 2014-2016.

Marco was involved in the Sub-Groups Integrated Pest Management (IPM) and Proficiency Testing for Detection of Transgenic Tobacco (GMO). He was also a member and initiator of the Agrochemical Residue Field Trials (RFT) Sub-Group. He was a pivotal member of the Agrochemicals Analysis (AA) Sub-Group, of which he was also Coordinator from 2006-2016. He also participated in the now disbanded Sustainability (SUST) and Blue Mould (BM) Sub-Groups.

In addition to presenting papers at CORESTA conferences, he has organised, co-organised and participated in several Workshops: Sustainability Workshop in 2011, Tobacco Integrity Workshop in 2012, Conservation of Natural Resources Workshop in 2013, Collaborative Studies Workshop in 2014, Extension and Training Workshop in 2016 and Sustainable Tobacco Production Workshop in 2017.

Within the CORESTA framework, Marco has been instrumental in efforts to reduce CPA residues in the tobacco crop through the continued development of CPA Guidance Residue Levels (GRLs). He was active in organising and promoting research work on the reduction of CPA residues on cigar dark air-cured tobaccos with the setting up of field trials around the world, in cooperation with other companies involved in cigar production, which led to the publication of C-GRLs in the CORESTA Guide No. 21.

[Marco was not able to be present at the award ceremony in June 2022 in Paris, France, so the opportunity was taken to present him with the CORESTA Prize at the AP2023 Conference in Cancún, Mexico.]

CORESTA AP2023 CONFERENCE

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Stewart LIVESAY

Matthew VANN

Dongmei XU

Limeng ZHANG

CORESTA AP2023 CONFERENCE

SUB-GROUP / TASK FORCE MEETINGS

Meetings Venue

The Pyramid at Grand Oasis Cancún
Blvd. Kukulcan 5-Km 16, Zona Hotelera
77500 Cancún, Q.R., Mexico

Date	Time	Meeting	Room
Saturday, 14 October	9:00-11:00	GTS – Green Tobacco Sickness Task Force	Mérida
	11:00-12:00	BIO – Efficacy of Biological and Eco-Friendly CPAs Sub-Group	Mérida
	14:00-15:00	BKS – Collaborative Study Black Shank Sub-Group	Mérida
	15:00-16:00	LNTP – Collaborative Study of Low Nicotine Tobacco Agronomic Production Practices Task Force	Mérida
Sunday, 15 October	8:00-12:00	ACAC – Agrochemical Advisory Committee	Querétaro
	14:00-15:00	IPM – Integrated Pest Management Sub-Group	Mérida
	15:00-16:00	TSNA – TSNA in Air-Cured and Fire-Cured Tobacco Sub-Group	Mérida
	16:00-17:00	GMO – Proficiency Testing for Detection of Transgenic Tobacco Sub-Group	Mérida
	17:00-18:00	RFT – Agrochemical Residue Field Trials Sub-Group	Mérida

CORESTA AP2023 CONFERENCE

GENERAL INFORMATION

Conference Venue

The Pyramid at Grand Oasis Cancún
Blvd. Kukulcan 5-Km 16, Zona Hotelera, 77500 Cancún, Q.R., Mexico
https://oasishoteles.com/en/hotels/the-pyramid-at-grand-oasis

Registration

Saturday, 14 October, 11:00-14:00
Sunday, 15 October, 11:00-18:00

The Pyramid at Grand Oasis Cancún (Guadalajara Room).

Presentations

PowerPoint files should be brought to the Conference Office (Tulum Room) at The Pyramid at Grand Oasis Cancún the day before the scheduled presentation.

Posters

Posters are to be brought to the Conference Office (Tulum Room) at The Pyramid at Grand Oasis Cancún on Monday, 16 October. Posters will be mounted on Monday and displayed until after the poster session on Tuesday, 17 October. Posters can be taken back at the end of the poster session, or collected from the Conference Office on Wednesday and Thursday, 18 and 19 October.

The Poster Session is on Tuesday, 17 October, 14:00-16:00 (Mérida Room).

SOCIAL EVENTS

Welcome Reception

Sunday, 15 October, 19:00-22:00
The Pyramid at Grand Oasis Cancún, “The Zocalo” Terrace

Closing Dinner

Thursday, 19 October, 20:00 ~ 23:00
The Pyramid at Grand Oasis Cancún, “The Zocalo” Terrace
Dress code: Business casual

CORESTA AP2023 CONFERENCE

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

PROGRAMME

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

MONDAY 16 OCTOBER

SESSION 1: Opening

Chair: Dongmei XU

Yucatán Room

8:30-8:50		**Welcome and Opening Remarks** XU Dongmei *Altria Client Services, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*
8:50-9:30		**Invited Speaker:** TURRENT A. *Casa Turrent, 317 local B, Col. Polanco Av. Presidente Masaryk 11550 México City, Mexico*
9:30-9:50	**IG 01**	**CORESTA Tobacco Harm Reduction Workshop Overview** STEVENS R.(1); FLORA J.(2) *(1) RAI Services Company, 401 North Main St, Winston Salem, NC 27101, U.S.A.* *(2) Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*
9:50-10:30	**Prize**	**CORESTA Prize:** **Holistic approach to CPAs** PRAT M. *Retired Global Product Integrity Director, JT International GmbH, Diedenhofener Strasse 20, D-54294 Trier, Germany*
10:30-10:50		**COFFEE**

MONDAY 16 OCTOBER

SESSION 2: CPA Management 1

Chair: Fabienne LALANDE

Yucatán Room

10:50-11:10	**AP 01**	**A comparison of Crop Protection Agents (CPAs) usage in tobacco and other crops** POCHUCHA A.; LALANDE F.; PRAT M. *JT International GmbH, Diedenhofener Str. 20-30, D-54294 Trier, Germany*
11:10-11:30	**AP 03**	**Effects of application timing and methods on chlorantraniliprole residues in flue-cured tobacco** VANN M.C. *North Carolina State University, Department of Crop & Soil Sciences, Campus Box 7620, Raleigh, NC 27695, U.S.A.*
11:30-11:50	**AP 44**	**Field evaluation of fungicides for management of frogeye leaf spot caused by *Cercospora nicotianae* on Burley tobacco** MARTINEZ-OCHOA N.; PEARCE R.C. *University of Kentucky, Plant and Soils Department, 1401 University Drive, Lexington, KY 40546, U.S.A.*
11:50-12:10	**Report**	**Sub-Group Agrochemicals Analysis (AA)** POCHUCHA A. *JT International GmbH, Diedenhofener Strasse 20, D-54294 Trier, Germany*
12:10-12:30	**Report**	**Agrochemical Advisory Committee (ACAC)** SCOTT L. *Universal Leaf Tobacco Co., Inc., P.O. Box 25099, Richmond, VA 23260, U.S.A.*
12:30-14:00		**LUNCH**

*[**AP 02** – Withdrawn]*

MONDAY 16 OCTOBER

SESSION 3: Sustainability – GAP Training

Chair: Stewart LIVESAY

Yucatán Room

14:00-14:20	**AP 04**	**Crop protection agent container disposal / recycling project** BADEMCI E. *JTI TR Leaf Tobacco, Çapak, No:12, 2561. Sk., 35860 Torbali/Izmir, Turkey*
14:20-14:40	**AP 06**	**Individual meeting with farmers: a tool for good agricultural practice training** FRANCESCHETTI L.; TEDESCO C.; MILLI G. *Trasformatori Tabacco Italia (TTI) & FAT, I-06012 Città di Castello, PG, Italy*
14:40-15:00	**AP 08**	**Addressing livelihoods within small-scale tobacco grower base** ROUSSOS R.; ROBBIE C.; JACKSON A. *Premium Tobacco Group, Plot W, Jumeirah Business Center 5, JLT, Dubai, U.A.E.* *Premium Active Tanzania Limited, Morogoro, Tanzania*
15:00-15:20	**AP 26**	**An investigation into the influence of climate smart agriculture on the soil properties, yield and quality of Burley tobacco in maize and groundnut rotation in Malawi - a comparison of different cultivation techniques in a small-scale model** MTONGA Y.(1); THORNEYCROFT (1); NGWIRA R.(1); TAYLOR D.(1); MPHEMBERA A.(2); INSAURRALDE C.(3) *(1) Pyxus Agriculture Ltd / Alliance One Tobacco Ltd, Research and Development, P.O Box 30522, Lilongwe, Malawi* *(2) Agricultural Research and Extension Trust (ARET), Private Bag 9, Lilongwe, Malawi* *(3) Alliance One International, Inc., P.O. Box 450, Wilson, NC 27894-0450, U.S.A.*
15:20-15:40	**AP 29**	**Effect of intercropping on crop productivity and quality of flue-cured tobacco in Malawi** CHIPETA T.; MWALE H.; MAIDENI F.; CHANGAYA A.G. *Agricultural Research and Extension Trust (ARET), Private Bag 9, Lilongwe, Malawi*
15:40-16:00		**TEA**

*[**AP 05, AP 07** – Withdrawn]*

MONDAY 16 OCTOBER

SESSION 4: Sustainability Workshop

Chair: Matthew VANN

Yucatán Room

16:00-16:20	**WS 01**	**Use of Sentek soil probes in tobacco research** REED T.D. *Virginia Tech, Southern Piedmont Agricultural Research and Extension Center, 2375 Darvills Road, Blackstone, VA 23824, U.S.A.*
16:20-16:40	**WS 02**	**Enhancing sustainable tobacco production practices in Zimbabwe** DIMBI S.; CHINHEYA C.; MASUKWEDZA R.; MUKOYI F. *Tobacco Research Board (TRB), Kutsaga Research Station, Airport Ring Road, P.O. Box 1909, Harare, Zimbabwe*
16:40-17:00	**WS 03**	**A decade of the Agrochemical Advisory Committee's (ACAC) achievements and their impact on sustainability** SCOTT L. *Universal Leaf Tobacco, Inc., 9201 Forest Hill Ave, Richmond, VA 23235, U.S.A.*
17:00-17:20	**Report**	**Task Force *Nicotiana* Germplasm Collection (NGPC)** LEWIS R.S. *Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, U.S.A.*
		Discussion / Q&A

TUESDAY 17 OCTOBER

SESSION 5: Breeding & Genetics 1

Chair: Simon GOEPFERT

Yucatán Room

8:30-8:50	**AP 09**	**Use of mixed model for evaluation and selection of Burley tobacco** BATISTA E.C.(1); DE OLIVEIRA E.(1); FRANTZ E.L.(1); TEODORO BRUZI A.(2); PULCINELLI C.E.(1) *(1) Alliance One International, Global Center of Research, Development & Deployment, Brazil* *(2) Federal University of Lavras, Brazil*
8:50-9:10	**AP 10**	**Development of an alternative system for seed-based tobacco haploid plant generation** MOORE S.; KERNODLE S.P.; LEWIS R.S. *Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, U.S.A.*
9:10-9:30	**AP 11**	**Use of principal component analysis to cluster flue-cured Virginia tobacco genotypes** BATISTA E.C.(1); DE OLIVEIRA E.(1); SANTOS F.W.(1); FRANTZ E.L.(1); FERNANDES S.(2); PULCINELLI C.E.(1) *(1) Alliance One International, Global Center of Research, Development & Deployment, Brazil* *(2) University of Arkansas, Crop, Soil and Environmental Sciences Department, U.S.A.*
9:30-9:50	**AP 12**	**Genome-wide association analysis combined with quantitative trait loci mapping reveal the genetic loci of leaf traits in cigar tobacco** LIU Guo Xiang(1); ZHANG Xing Wei(1); JI Yan(1); YAN Si Fan(1); JIANG Xun(1); LI Yuan(1); WANG Jun(2) *(1) Institute of Tobacco Research, Chinese Academy of Agricultural Sciences, 11 Keyuan Jingsi Road, Laoshan District, Qingdao City, Shandong Province 266101, China* *(2) Deyang Company of Sichuan Provincial Tobacco Corporation, Deyang 618400, Sichuan, China*
9:50-10:10	**AP 13**	**Improving the efficiency of tobacco breeding using multivariate statistical methods** BATISTA E.C.; DE OLIVEIRA E.; SANTOS F.W.; FRANTZ E.L.; PULCINELLI C.E. *Alliance One International, Global Center of Research, Development & Deployment, Brazil*
10:10-10:30	**AP 14**	**Genetic control of enantiomeric specificity of nicotine biosynthesis in tobacco** ALLEN Z.; KERNODLE S.P.; STEEDE T.; DEWEY R.E.; LEWIS R.S. *Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, U.S.A.*
10:30-10:50		**COFFEE**

*[**AP 15** – Withdrawn]*

TUESDAY 17 OCTOBER

SESSION 6a: Biological Processes

Chair: Matthew VANN

Querétaro Room

10:50-11:10	**AP 16**	**Isolation, identification of a lytic bacteriophage PQ43 for *Ralstonia pseudosolanacearum* and its application in biocontrol of bacterial wilt** YI Ke(1); LIU Min(1); HUANG Binbin(2); JING Yongfeng(1); LIU Lijia(1); TAN Ge(1); LIU Qingshu(2); CHEN Wu(3); GE Long(4); PAN Qiang(4) *(1) China Tobacco Hunan Industrial Co., Ltd., Changsha 410019, China* *(2) Hunan Institute of Microbiology, Changsha 410009, China* *(3) College of Plant Protection, Hunan Agricultural University, Changsha 410128, China* *(4) Qingdao Phagepharm Bio-tech Co., Ltd., Qingdao 410009, China*
11:10-11:30	**AP 17**	**Evaluation of sunhemp treated with *Trichoderma* in short tobacco rotations for control of root-knot nematode (*Meloidogyne javanica*) and disease complexes** MAHERE T.S.; MARUNDA M.; CHINHEYA C.; DIMBI S. *Plant Health Services Division, Tobacco Research Board (TRB), Kutsaga Research Station, Airport Ring Road, P.O. Box 1909, Harare, Zimbabwe*
11:30-11:50	**AP 18**	**Use of symbiotic nitrogen fixation endophytes in tobacco seedling production** CHINAMO D. *Tobacco Research Board (TRB), Kutsaga Research Station, Airport Ring Road, P.O. Box 1909, Harare, Zimbabwe*
11:50-12:10	**AP 19**	**Improvement of the output value, chemical and processing quality of flue-cured tobacco by application of humic acid solution** PU Yi(1); DAI Kuai(2); GU Xinghui(3); LIU Meiju(4); JI Qinglin(1); LI Jiangzhou(2); ZHAO Changhua(3); ZHANG Limeng(2); LIN Shan(1) *(1) China Agricultural University, College of Resources and Environment, Beijing 100193, China* *(2) Yunnan Tobacco Company Yuxi Branch, Yuxi 653100, China* *(3) Yunnan Tobacco Leaf Company, Kunming 650200, China* *(4) Yunnan Agricultural University, College of Resources and Environment, Kunming 650204, China*
12:10-12:20	**Report**	**Sub-Group Proficiency Testing for Detection of Transgenic Tobacco (GMO)** FISHER C.R. *University of Kentucky, 1401 University Drive, Lexington, KY 4 0546-0312, U.S.A.*
12:20-12:30	**Report**	**Sub-Group Efficacy of Biological and Eco-Friendly CPAs (BIO)** MAVUKA R. *Tobacco Research Board (TRB), Kutsaga Research Station, Airport Ring Road, P.O. Box 1909, Harare, Zimbabwe*
12:30-14:00		**LUNCH**

TUESDAY 17 OCTOBER

SESSION 6b: Flavour & Aroma

Chair: Chengalrayan KUDITHIPUDI

Yucatán Room

10:50-11:10	**AP 20**	**Identification and manipulation of trichome specific controlling enhanced flavor and aroma in tobacco** LIU Hai(1); RABARA R.C.(1,2); KUDITHIPUDI C.(2); TIMKO M.P.(1) *(1) Department of Biology, University of Virginia, Charlottesville, VA 22903, U.S.A.* *(2) Altria Client Services LLC, Center for Research and Technology, Richmond, VA 23219, U.S.A.*
11:10-11:30	**AP 21**	**Computational-based genomic, transcriptomic, and metabolic analyses of biosynthetic gene clusters in tobacco** RABARA R.C.(1,2); LIU Hai(1); KUDITHIPUDI C.(2); TIMKO M.P.(1) *(1) Department of Biology, University of Virginia, Charlottesville, VA 22903, U.S.A.* *(2) Altria Client Services LLC, Center for Research and Technology, Richmond, VA 23219, U.S.A.*
11:30-11:50	**AP 22**	**Colchicine treated tobacco – changing biochemical composition of the leaves** DELLA VECCHIA M.G.; ZHOU Ancheng; RABARA R.C.; QI Dong; SHEN Yanxin *Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*
11:50-12:10	**AP 23**	**Study on the volatile organic compounds of flue-cured tobacco with different sweet aroma intensity by flavoromics approach** LIN Yechun; LI Jianwei; LIANG Guilin; ZHANG Changyun; LI Hongxun; SUN Zhenchun; WANG Feng *Guizhou Academy of Tobacco Science, Guiyang 550081, China*
12:10-12:30	**AP 24**	**Map-based cloning of the gene responsible for solavetivone accumulation in *Nicotiana sylvestris*** TAKEUCHI T.; UDAGAWA H.; MAGOME H.; TAKAKURA Y. *Japan Tobacco Inc., Leaf Tobacco Research Center, 1900, Idei, Oyama, Tochigi 323-0808, Japan*
12:30-14:00		**LUNCH**

*[**AP 25** – Moved to Thursday 19 Oct, Session 15]*
*[**AP 26, AP 29** – Moved to Monday 16 Oct, Session 3]*
*[**AP 28** – Moved to Wednesday 18 Oct, Session 11]*
*[**AP 27** – Withdrawn]*

TUESDAY 17 OCTOBER

SESSION 7: Posters

Mérida Room

14:00 – 16:00

IGPOST 01 **CORESTA strategy, cooperation and achievements**

LINDHOLM J.(1); STEVENS R.(2); DIGARD H.(3); COLARD S.(4)

(1) Swedish Match AB, Box 17037, SE-104 62 Stockholm, Sweden
(2) RAI Services Company, 401 North Main Street, Winston Salem, NC 27101, U.S.A.
(3) BAT Investments Limited, R&D Centre, Southampton, U.K.
(4) CORESTA, 11 rue du Quatre Septembre, 75002 Paris, France

APPOST 02 **Mancozeb application in tobacco: a revival of an older chemistry?**

STAINBACK C.B.; VANN M.C.; MACHACEK J.L.; CHEEK J.A.

North Carolina State University, Department of Crop & Soil Sciences, Campus Box 7620, Raleigh, NC 27695, U.S.A.

APPOST 03 **Flutriafol residues in non-traditional Burley tobacco**

VANN M.C.; WHITLEY D.S.; CHEEK J.A.; MACHACEK J.L.

North Carolina State University, Department of Crop & Soil Sciences, Campus Box 7620, Raleigh, NC 27695, U.S.A.

APPOST 04 **Estimation and prediction of genetic parameters and breeding values through REML/BLUP approach**

BATISTA E.C.(1); DE OLIVEIRA E.(1); SANTOS F.W.(1); FRANTZ E.L.(1); FERNANDES S.(2); PULCINELLI C.E.(1)

(1) Alliance One International, Global Center of Research, Development & Deployment, Brazil
(2) University of Arkansas, Crop, Soil and Environmental Sciences Department, U.S.A.

APPOST 05 **Estimating genetic variability and correlation of agronomic traits in Burley tobacco for selection purposes**

BATISTA E.C.; DE OLIVEIRA E.; FRANTZ E.L.; PULCINELLI C.E.

Alliance One International, Global Center of Research, Development & Deployment, Brazil

APPOST 06 **Functional identification of tonoplast membrane potassium channel gene *NtTPK* in tobacco**

JIAO Fangchan; WANG Bingwu; GAO Yulong; WU Xingfu; FENG Zhiyu; CHEN Xuejun; ZHAO Lu

Yunnan Academy of Tobacco Agricultural Sciences (YATAS), No. 33, Yuantong Str., Kunming, Yunnan, China

APPOST 07 **Chromosome-level genomes of the *Nicotiana tabacum* progenitors, *Nicotiana sylvestris* and *Nicotiana tomentosiformis***

SIERRO N.; OUADI S.; AUBERSON M.; DULIZE R.; GUEDJ E.; IVANOV N.V.; GOEPFERT S.

PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland

APPOST 08 **Exploring the microbial diversity and composition of three cigar product categories**

JOSHI S.; PHAM K.; MOE L.; McNEES C.R.

University of Kentucky, KTRDC, 1401 University Drive, Lexington KY 40546, U.S.A.

APPOST 09 **Deep tillage enhances the spatial homogenization of bacterial communities by reducing deep soil compaction**

HU Ruiwen(1); ZHENG Bufan(1); LIU Yongjun(2); PENG Shuguang(2); GONG Jia(1); LI Junhui(1); QIN Tian(1); LIANG Jingsong(1); XIONG Kunlong(1); ZHENG Zhongyi(1); HU Yajun(1); YI Zhenxie(1); ZHOU Qingming(1); LI Juan(1)

(1) College of Agronomy, Hunan Agricultural University, Changsha, China
(2) Hunan Branch of China National Tobacco Corporation (CNTC), Changsha, China

APPOST 10 **A multi-county survey of plant-parasitic nematodes in North Carolina tobacco fields**

BONYAK H.C.(1); GORNY A.M.(1); VANN M.C.(2); LEWIS R.S.(2); DAVIS E.L.(1)

(1) North Carolina State University - Department of Entomology and Plant Pathology, Raleigh, NC, U.S.A.
(2) North Carolina State University - Department of Crop and Soil Sciences, Raleigh, NC, U.S.A.

APPOST 11 **Screening of insecticides for controlling *Myzus persicae* in cigar leaf fields and safety evaluation of *Rhynocoris fuscipes***

CHEN Dexin(1); XIA Changjian(1); SUN Ranfeng(2); MA Guangxiang(2); DENG Haibin(3); WANG Xinggao(2); SHAO Yu(4)

(1) Haikou Cigar Research Institute of Hainan Branch of CNTC, No. 120, Hongchenghu Road, Fucheng Sub-District Office, Qiongshan District, Haikou 571100, Hainan, China
(2) College of Plant Protection, Hainan University, No. 58, Renmin Avenue, Meilan District, Haikou 570228, Hainan, China
(3) Guangdong Institute of Tobacco Science, West Tower of Chengtou Business Building, No. 69 Binjiang Road, Wujiang District, Shaoguan City, Guangdong Province 512000, Guangdong, China
(4) Danzhou Branch of Hainan Tobacco Company, Block 33, Nada Kong, south of the middle section of Nada Zhongxing Street, Danzhou City, Hainan Province 571700, Hainan, China

APPOST 12 **A new index "root-knot density" was created which can objectively evaluate the damage of root nematodes on tobacco**

FAN Miaomiao(2); DAI Kuai(1); LIU Meiju(3); ZHANG Limeng(4); LIN Shan(2); LI Jiangzhou(1)

(1) Research Center of Biological Control Engineering for Tobacco Diseases and Insect Pests of China Tobacco/Yuxi Branch of Yunnan Tobacco Company, Yuxi 653100, China
(2) College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China
(3) College of Resources and Environmental Sciences, Yunnan Agricultural University, Kunming 650201, China
(4) Yuxi Zhongyan Tobacco Seed Co., Ltd, Yuxi 653100, China

APPOST 13 **Photosynthetic performance as a tool in detecting infection in tobacco (*Nicotiana tabacum*)**

MATEVA K.I.; MUKOYI F.; RUZANE R.T.; MAGAMA F.; DIMBI S.

Tobacco Research Board (TRB), Kutsaga Research Station, Airport Ring Road, P.O. Box 1909, Harare, Zimbabwe

APPOST 16 **Fungicide and starter fertilizer placement at transplanting**

STAINBACK C.G.; VANN M.C.; MACHACEK J.L.; CHEEK J.A.; WHITLEY D.S.

North Carolina State University, Department of Crop & Soil Sciences, Campus Box 7620, Raleigh, NC 27695, U.S.A.

APPOST 18 **Study on hyperspectral multivariate linear prediction model of tobacco leaf nitrogen content**

GUO Ting; LI Wujin; XIAO Xi; LI Hongguang; LI Wen

Chenzhou Tobacco Company, No. 61 Yanquan North Road, Beihu District, Chenzhou City, Hunan Province, China

APPOST 19 **Automatic discrimination planting areas of flue-cured tobacco based on near-infrared spectroscopy technology and support vector machine improved by whale optimization algorithm**

QIU Changgui(1,2); LIU Ze(3); QI Lin(4); YANG Jingjin(4); WANG Xianguo(4); WENG Ruijie(4); LIU Jihui(4); WEI Qing(1); LIU Jing(1,2); YANG Panpan(1,2); LI Siyuan(4)

(1) Yunnan Reascend Tobacco Technology (Group) Co., Ltd., Kunming 650106, China
(2) Yunnan Comtestor Co., Ltd., Kunming 650106, China
(3) China Tobacco Yunnan Industrial Co., Ltd., Kunming 650231, China
(4) HongyunHonghe Tobacco (Group) Co., Ltd., Kunming 650231, China

APPOST 20 **Design and testing of sinusoidal curve variable frequency control system for circulating fan in bulk curing barn**

WANG Jianan(1); YIN Guangting(2); CHEN Xiaolong(2); SHEN Hongtao(2); DUAN Weidong(2)

(1) College of Tobacco Science / Tobacco Harm Reduction Research Center, Henan Agricultural University, Zhengzhou 450002, China
(2) China Tobacco Henan Industrial Co., Ltd., Zhengzhou 450000, China

APPOST 21 **"Di@gnoPlant Tabaco" for Brazilian tobacco stakeholders**

MARIGNAC E.

CORESTA, 11 rue du Quatre Septembre, 75002 Paris, France

APPOST 23 **Multiresidue method for the determination of pesticides in *Nicotiana tabacum***

ACAYABA R.; VALESE A.C.; CARDOSO V.H.R.; SALERNO G.; ELIAS V.O.

Eurofins do Brasil, Rodovia Engenheiro Ermênio de Oliveira Penteado, s/n km 57,7, Condomínio Industriale, Indaituba, 13337-300, SP, Brazil

APPOST 24 **Complete sequencing of the *Nicotiana tabacum* genome and transcriptome as a tool for discovering new resistance markers and gene functionalities**

BEZERRA R.S.(1); SILVA E.(1); MORGATHO V.(1); GOULART R.(2); DORO M.R.(2); PEREIRA A.M.(2); CARRARA G.(2); ELIAS V.O.(3); ACAYABA R.(3)

(1) Eurofins | Centro de Genomas, Rua Bittencourt Sampaio 105, São Paulo, 04126-060, SP, Brazil.
(2) Eurofins | Agroscience, Rodovia Engenheiro Ermênio de Oliveira Penteado, s/n km 57,7, Condomínio Industriale, Indaituba, 13337-300, SP, Brazil
(3) Eurofins do Brasil, Rodovia Engenheiro Ermênio de Oliveira Penteado, s/n km 57,7, Condomínio Industriale, Indaituba, 13337-300, SP, Brazil

APPOST 25 **Physiological and molecular analysis on colour mutant lines reveals relationship between chlorophyll and nitrate and TSNA content in flue-cured tobacco**

FENG Yuqing; SHI Hongzhi; ZHAO Yuanyuan; LI Yujing

College of Tobacco Science / Tobacco Harm Reduction Research Center, Henan Agricultural University, Zhengzhou 450002, China

*[**APPOST 01, APPOST 14, APPOST 15, APOST 17, APPOST 22** – Withdrawn]*

TUESDAY 17 OCTOBER

SESSION 8a: TSNA

Chair: Dongmei XU

Querétaro Room

16:00-16:20	**AP 30**	**NNN detection using monoclonal antibody** QI Dong; SHEN Yanxin *Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*
16:20-16:40	**AP 32**	**Diamine oxidase enzyme with respect to tobacco alkaloids and tobacco specific nitrosamines formation** KIM T.; DELLA VECCHIA M.G.; SHEN Y. *Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.*
16:40-17:00	**AP 33**	**Tobacco leaf transcriptomic analysis unveils differential effects of potassium chloride and potassium sulphate on formation of tobacco specific nitrosamines** SINGH S.K.(1); PATTANAIK S.(1); WEBB A.B.(2); BAILEY W.A.(2); PEARCE R.C.(2); LING Yuan(1,2) *(1) Kentucky Tobacco Research and Development Center, and (2) Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546, U.S.A.*
17:00-17:20	**Report**	**Sub-Group TSNA in Air-Cured and Fire-Cured Tobacco (TSNA)** FISHER C.R. *University of Kentucky, 1401 University Drive, Lexington, KY 4 0546-0312, U.S.A.*

*[**AP 31** – Converted to Poster (APPOST25)]*

TUESDAY 17 OCTOBER

SESSION 8b: Technology

Chair: Leo CARUSO

Yucatán Room

16:00-16:20	**AP 34**	**Effect of the carbon nanoparticles on the tobacco growth by modulating the rhizosphere microbiome** CHENG Lingtong; TAO Jiemeng; LU Peng; LIANG Taibo; MENG Lijun; SU Huan; ZHANG Jianfeng; CAO Peijian; JIN Jingjing; MU Wenjun *Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, China*
16:20-16:40	**AP 35**	**Evaluation of the performance of shisha tobacco under Zimbabwean growing conditions** RUZANE R.T.; MUKOYI F.; MATEVA K.I.; MAGAMA F.; DIMBI S. *Tobacco Research Board (TRB), Kutsaga Research Station, Airport Ring Road, P.O. Box 1909, Harare, Zimbabwe*
16:40-17:00	**AP 37**	**Capturing data electronically: modern problems require modern solutions** MACHACEK J.L.; VANN M.C.; CHEEK J.A. *North Carolina State University, Department of Crop & Soil Sciences, Campus Box 7620, Raleigh, NC 27695, U.S.A.*
17:00-17:20	**Report**	**Sub-Group Extended Diagnostic Expert System (XDES)** MARIGNAC E. *CORESTA, 11 rue du Quatre Septembre, 75002 Paris, France*

*[**AP 36** – Withdrawn]*

WEDNESDAY 18 OCTOBER

SESSION 9: Pest & Disease Control

Chair: Limeng ZHANG

Yucatán Room

8:30-8:50	**AP 38**	**IPM and pest epidemics: *p*R = pr_le^{prl} and all that** FISHER C.R. *Kentucky Tobacco Research and Development Center, University of Kentucky, Lexington, KY, U.S.A.*
8:50-9:10	**AP 39**	**Diversity of *Fusarium* species infecting tobacco in Zimbabwe** KARAVINA C.(1); MARUNDA M.(1); CHINHEYA C.(1); SAGONDA T.(1); JERE J.(2); ZVOBGO G.(3); MARWA T.(2); DIMBI S.(1) *(1) Plant Health Services Division, Tobacco Research Board (TRB), Kutsaga Research Station, P.O. Box 1909, Harare, Zimbabwe* *(2) Department of Biological Sciences, University of Zimbabwe, P.O. Box MP32 Mt Pleasant, Harare, Zimbabwe* *(3) Molecular Biology Services, Tobacco Research Board (TRB), Kutsaga Research Station, P.O. Box 1909, Harare, Zimbabwe*
9:10-9:30	**AP 40**	**Correlation of management practices and environmental conditions on incidence of angular leaf spot in dark fire-cured tobacco** WEBB A.B.; BAILEY W.A. *University of Kentucky, Research & Education Center, 348 University Drive, Princeton, KY 42445, U.S.A.*
9:30-9:50	**Report**	**Sub-Group Integrated Pest Management (IPM)** FISHER A.M. *University of Kentucky, Kentucky Research & Development Center, 202B KTRDC Building, 1401 University Drive, Lexington, KY 40546-0236, U.S.A.*
9:50-10:10	**Report**	**Sub-Group Pest and Sanitation Management in Stored Tobacco (PSMST)** WINEGARDNER M.(1); BARTHOLOMEW E.(2) *(1) Universal Leaf Tobacco Co., Inc., P.O. Box 25099, Richmond, VA 23260, U.S.A.* *(2) Alliance One International, Inc., 1868 Unit A Cambria Drive, Greenville, NC 27834, U.S.A.*
10:10-10:30		**COFFEE**

WEDNESDAY 18 OCTOBER

SESSION 10: CPA Management 2

Chair: Fabienne LALANDE

Yucatán Room

10:30-10:50	**AP 41**	**Transplant water application: investigations of in-furrow & sidedress systemic insecticide placement** STAINBACK C.G.(1); VANN M.C.(1); HUSETH A.(2) *(1) North Carolina State University, Department of Crop & Soil Sciences, Campus Box 7620, Raleigh, NC 27695, U.S.A.* *(2) North Carolina State University, Department of Entomology & Plant Pathology, Campus Box 7613, Raleigh, NC 27695, U.S.A.*
10:50-11:10	**AP 42**	**Flue-cured tobacco response to sub-lethal doses of HPPD-inhibiting herbicides** VANN M.C.; CAHOON C.; CHEEK J.A.; MACHACEK J.L. *North Carolina State University, Department of Crop & Soil Sciences, Campus Box 7620, Raleigh, NC 27695, U.S.A.*
11:10-11:30	**AP 43**	**Assessment of Romex 22.15 SC (Lambda-cyhalothrin 9.5 % and Thiamethoxam 12.6 %) efficacy for pest control in tobacco crop** LISUMA J.B.; MBWAMBO A.F. *Tobacco Research Institute of Tanzania (TORITA), Street 2 Dr. Malya, 45120 Tumbi, Box 431, Tabora, Tanzania*
11:30-11:50	**AP 45**	**Fungicide evaluations for foliar leaf spot disease management** STAINBACK C.B.; VANN M.C.; SHORT M.M.; CHEEK J.A.; MACHACEK J.L. *North Carolina State University, Department of Crop & Soil Sciences, Campus Box 7620, Raleigh, NC 27695, U.S.A.*
11:50-12:10	**Report**	**Sub-Group Agrochemical Residue Field Trials (RFT)** SEBATA M. *Japan Tobacco Inc., 1900, Idei, Oyama, Tochigi 323-0808, Japan*
12:10-14:00		**LUNCH**

*[**AP 44** – Moved to Monday 16 Oct, Session 2]*

WEDNESDAY 18 OCTOBER

SESSION 11: ESG

Chair: Stewart LIVESAY

Yucatán Room

14:00-14:20	**AP 46**	**Supply chain due diligence for compliance and sustainability of the tobacco industry in Malawi** CHIWAYA P.G.; CHANGAYA A.G.; MSANGOSOKO K.R.; CHAMANGO A.M.Z.; MAIDENI F. *Agricultural Research and Extension Trust (ARET), Private Bag 9, Lilongwe, Malawi*
14:20-14:40	**AP 47**	**Farmer Advantage Card for improving growers' livelihood in Turkey** CETINTAS E.; TUNCAY D. *Sunel Tobacco Company, Kemalpasa Mah, Pinar Cad, No: 89, Izmir, Turkey*
14:40-15:00	**AP 49**	**Evaluation of the potential of greenhouse gas emissions in tobacco producing properties in the southern region of Brazil** FRANTZ M.L.(1); MULLER R.L.(1); SHAEFER V.P.(1); KÖHLER A.K.(2); SCHNEIDER R. DE C. DE S.(2); SILVA R.M.(2) *(1) Premium Tabacos Brazil, Av. Felisberto Bandeira Moraes, 2405, Distrito Industrial, Santa Cruz do Sul, 96835-900, RS, Brazil* *(2) Universidade de Santa Cruz do Sul (UNISC), Av. Independência 2293, Universitário, Santa Cruz do Sul, 96815-900, RS, Brazil*
15:00-15:20	**AP 28**	**Use of *Chrysopogon zizanioides* (L.) Roberty (Vetiver) to deliver ESG projects throughout the tobacco leaf supply chain** CARRILLO A. *Nueva Matacapan Tabacos, Catemaco, Veracruz, Mexico*
15:20-15:40	**Report**	**Task Force Green Tobacco Sickness (GTS)** SCOTT L. *Universal Leaf Tobacco Co., Inc., P.O. Box 25099, Richmond, VA 23260, U.S.A.*
15:40-16:00		**TEA**

*[**AP 48** – Withdrawn]*

WEDNESDAY 18 OCTOBER

SESSION 12: ESG Workshop

Chair: Dongmei XU / Fabienne LALANDE

Yucatán Room

16:00-16:20	**WS 04**	**STP overview - accelerating positive impacts on social and environmental footprints in the tobacco industry** IRVING B.(1); KUMLIN A.(2) *(1) Altria Client Services LLC, Procurement ESG, 601 East Jackson Street, Richmond, VA 23219, U.S.A.* *(2) Sustainable Tobacco Program (STP), Secretariat Coordinator, Peterson Projects S.A., Rue Blavignac 10, 2nd Floor, 1227, Carouge, Switzerland*
16:20-16:40	**WS 05**	**Exploring different ways to enhance the livelihoods of tobacco growing communities and further help contracted growers and their families achieve and sustain a living income** QUATKE C. *JT International SA, 8 rue Kazem Radjavi, Geneva 1202, Switzerland*
16:40-17:00	**WS 06**	**A farmers' perspective on ESG and future opportunities** RENN J.; GRIFFIN S.; BOYD G. *Tobacco Growers Association of North Carolina, 3901 Barrett Drive #202, Raleigh, NC 27609, U.S.A.*
17:00-18:00		**Discussion / Q&A**

THURSDAY 19 OCTOBER

SESSION 13: Cigar Tobacco Production

Chair: Simon GOEPFERT

Yucatán Room

8:30-8:50	**AP 55**	**Developing nitrogen and potassium fertilizer rate recommendations for cigar wrapper tobacco in North Carolina** JAMES M.S.; VANN M.C.; CHEEK J.A.; MACHACEK J.L.; WHITLEY D.S. *North Carolina State University, Department of Crop & Soil Sciences, 101 Derieux Street, Raleigh, NC 27695, U.S.A.*
8:50-9:10	**AP 56**	**Agrochemical programs for cigar wrapper tobacco: impacts on cured leaf residues** VANN M.C.(1); LALANDE F.(2); PRAT M.(2); HARTLEY M.(3); GREEN B.(3) *(1) North Carolina State University, Department of Crop & Soil Sciences, Campus Box 7620, Raleigh, NC 27695, U.S.A.* *(2) JT International GmbH, 54294 Trier, Germany* *(3) Lancaster Leaf Tobacco Company, Lancaster, PA 17603, U.S.A.*
9:10-9:30	**AP 57**	**Effects of cooking rice water on chemical composition and aroma quality of fermented cigar tobacco leaves** ZHAO Yuanyuan(1); SHI Hongzhi(1); ZHANG Bingfeng(1); QIN Yaqing(2); REN Mengjuan(1); ZHANG Ruina(2); ZHANG Lanyue(1) *(1) College of Tobacco Science / Tobacco Harm Reduction Research Center, Henan Agricultural University, Zhengzhou 450002, China* *(2) Sichuan Provincial Tobacco Company, Chengdu 600041, China* *(3) Deyang Branch of Sichuan Provincial Tobacco Company, Deyang 618400, China*
9:30-9:50	**AP 58**	**Effects of aging with characteristic media on the sensory quality, chemical composition and microbial community of cigar** HU Wanrong(1); CAI Wen(1); JIA Yun(1); LIU Lulu(1); YANG Zhen(1); JIA Yuhong(2); LIU Yuanfa(3); LI Dongliang(1) *(1) Center of Technology Innovation for Cigar, China Tobacco Sichuan Industrial Co., Ltd., Chengdu 610101, Sichuan Province, China* *(2) Industry Efficient Utilization to Domestic Cigar Tobacco Key Laboratory of Sichuan Province, China Tobacco Sichuan Industrial Co., Ltd., Shifang 618400, Sichuan Province, China* *(3) School of Food Science and Technology, Jiangnan University, Wuxi 214112, Jiangsu Province, China*
9:50-10:10	**AP 59**	**Identification and application evaluation of functional microorganisms in cigar fermentation** JIA Yun(1,2); LIU Lulu(1); HU Wanrong(1); JIA Yuhong(2); FAN Jingyuan(1); HU Xi(2); LI Dongliang(1) *(1) Center of Technology Innovation for Cigar, China Tobacco Sichuan Industrial Co., Ltd., Chengdu 610100, Sichuan, China* *(2) Industry Efficient Utilization to Domestic Cigar Tobacco Key Laboratory of Sichuan Province, China Tobacco Sichuan Industrial Co., Ltd., Shifang 618400, Sichuan, China*
10:10-10:30		**COFFEE**

THURSDAY 19 OCTOBER

SESSION 14: Cigar Tobacco Workshop

Chair: Leo CARUSO

Yucatán Room

10:30-10:50	**WS 07**	**Challenges for cigar tobacco production** RAMOS R. *ITG Brands LLC, 420 N. English Street, Greensboro, NC 27405, U.S.A.*
10:50-11:10	**WS 08**	**Cigar tobacco production in Pennsylvania** GREEN B.; HARTLEY M. *Lancaster Leaf Tobacco Company, PO Box 25099, Richmond, VA 23260, U.S.A.*
11:10-11:30	**WS 09**	**Cigar tobacco leaf: a critical agricultural raw material for EU manufacturing of cigar and cigarillo products** VARAKAS P. *European Cigar Manufacturers Association (ECMA), Rue Véronèse 73, 1000 Brussels, Belgium*
11:30-11:50	**WS 10**	**Perspectives on crop protection agent analysis in cigars** ANSPACH T. *Eurofins Dr. Specht International GmbH, Am Neuländer Gewerbepark 2, 21079 Hamburg, Germany*
11:50-12:10		**Discussion / Q&A**
12:10-13:30		**LUNCH**

THURSDAY 19 OCTOBER

SESSION 15: Breeding & Genetics 2

Chair: Albert CHAMANGO

Yucatán Room

13:40-14:00	**AP 50**	**Resistance risk and resistance mechanism of *Ralstonia solanacearum* to SYP-14288** MU Wenjun(1); HU Liwei(1); HUANG Lei(2); WANG Jianlin(3); MA Xiaojing(1); QIAN Ni(3); SONG Jizhen(1) *(1) Zhengzhou Tobacco Research Institute of CNTC, No. 2 Fengyang Street, Zhengzhou 450001, China* *(2) China Tobacco Guangdong Industrial Co., Ltd., No. 62 Chiyan Street, Guangzhou 510310, China* *(3) China Tobacco Chongqing Industrial Co., Ltd., No. 2 East Nanping Street, Chongqing 400060, China*
14:00-14:20	**AP 51**	**Eukaryotic elongation factor (*eIF4*) targeted sequence analysis of PVY tolerant Kutsaga accession to determine nucleotide responsible for PVY resistance and CRISPR/Cas9 mutagenesis of a Kutsaga line for PVY resistance** ZVOBGO G.; MAGAMA F.; MAVUKA R.; DIMBI S.; KASHANGURA C. *Tobacco Research Board (TRB), Kutsaga Research Station, Airport Ring Road, P.O. Box 1909, Harare, Zimbabwe*
14:20-14:40	**AP 52**	**PIF1, a phytochrome-interacting factor, negatively regulates drought tolerance and carotenoids biosynthesis in tobacco** LIU Shaohua(1,2); ZHANG Yinchao(1); PAN Xuhao(1); YANG Qing(1); YANG Changqing(1); WU Fengyan(1); YANG Aiguo(1); LI Yiting(1) *(1) Key Laboratory of Tobacco Genetic Improvement and Biotechnology, Tobacco Research Institute, Chinese Academy of Agricultural Sciences, Qingdao 266100, China* *(2) R&D Department, Shenzhen Yupeng Technology Co., Ltd, Shenzhen 518110, China*
14:40-15:00	**AP 25**	**Developing and deploying climate-resilient tobacco (*Nicotiana tabacum*) varieties in marginal areas of Zimbabwe: stability and adaptability analysis in new tobacco varieties** MUKOYI F.; MATEVA K.I.; MAGAMA F.; DIMBI S. *Tobacco Research Board (TRB), Kutsaga Research Station, Airport Ring Road, P.O. Box 1909, Harare, Zimbabwe*
15:00-15:20	**Report**	**Sub-Group Collaborative Study Black Shank (BKS)** DING Wei(1); FISHER C.R.(2) *(1) Southwest University, 216 Tian Sheng Road, Bei Bei, Chongqing 400715, China* *(2) University of Kentucky, 1401 University Drive, Lexington, KY 4 0546-0312, U.S.A.*
15:40-16:00		**TEA**

*[**AP 53, AP 54** – Withdrawn]*

THURSDAY 19 OCTOBER

SESSION 16: Low Nicotine

Chair: Colin FISHER

Yucatán Room

16:00-16:20	**IG 02**	**Potential ultra-low nicotine limit in tobacco – can we meet it?** FISHER A.M.(1); FISHER C.R.(1); YANG S.(2); PATRA B.(1); SLONE S.(3); JI H.(1); KINNEY J.(1) *(1) Kentucky Tobacco Research and Development Center, University of Kentucky, 1401 University Drive, Lexington, KY 40546, U.S.A.* *(2) United States Department of Agriculture, 1616 Albrecht Blvd N, Fargo, ND 58102, U.S.A.* *(3) Dr. Bing Zhang Department of Statistics, University of Kentucky, 725 Rose Street, Lexington, KY 40536, U.S.A.*
16:20-16:40	**AP 60**	**Low nicotine flue-cured tobacco: influences of cultivar selection and agronomic management** CHEEK J.A.; VANN M.C.; MACHACEK J.L. *North Carolina State University, Department of Crop & Soil Sciences, Campus Box 7620, Raleigh, NC 27695, U.S.A.*
16:40-17:00	**Report**	**Task Force Collaborative Study of Low Nicotine Tobacco Agronomic Production Practices (LNTP)** KUDITHIPUDI C. *Altria Client Services LLC, 600 E. Leigh Street, Richmond, VA 23112, U.S.A.*
17:00-17:20	**AP 61**	**Effect of low-nicotine tobacco / eggplant grafting on nicotine reduction and origin of low nicotine in grafted tobacco leaves** SHI Hongzhi(1); ZHANG Mengyue(1); BAI Yafan(1); ZHAO Yuanyuan(1); SHI Sujuan(2) *(1) College of Tobacco Science / Tobacco Harm Reduction Research Center, Henan Agricultural University, Zhengzhou 450002, China* *(2) Shanghai Tobacco Group Co., Shanghai, China*
17:20-17:40	**AP 62**	**Understanding the molecular mechanism underlying the poor leaf quality of the low alkaloid tobacco varieties** PATRA B.; FISHER C.R.; SINGH S.K.; KINNEY J. *Kentucky Tobacco Research and Development Center, University of Kentucky, 1401 University Drive, Lexington, KY 40546, U.S.A.*
17:40-18:00	**AP 63**	**Development and evaluation of newly-generated ultra-low nicotine tobacco lines and hybrids** LEWIS R.S.; STEEDE T. *Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, U.S.A.*

CORESTA AP2023 CONFERENCE

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

ABSTRACTS

ORAL PRESENTATIONS

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

IG 01

CORESTA Tobacco Harm Reduction Workshop Overview

STEVENS R.(1); FLORA J.(2)

(1) RAI Services Company, 401 North Main St, Winston Salem, NC 27101, U.S.A.
(2) Altria Client Services LLC, 601 East Jackson Street, Richmond, VA 23219, U.S.A.

In June 2022, CORESTA held a Science Day in Paris, France. Presenters included experts in tobacco harm reduction (THR); Karl Fagerström, Riccardo Polosa, Clive Bates, Luca Rossi, and Lea Scott. Topics covered included the role of nicotine in harm reduction, misperceptions on the harm of nicotine, the challenges and opportunities of harm reduction substantiation, innovation and harm reduction of alternative products, and supply chain integrity. Through guidance from the CORESTA Board and with what we learned from Science Day, a workshop was developed and executed in April 2023, in Antibes, France, to develop insights into how CORESTA can advance the science related to THR. A diverse group of participants were selected by the CORESTA delegates (approximately 70 participants attended), and the workshop was designed to address the following objectives: 1) Identify key areas where CORESTA can advance the science related to THR; 2) Create actionable objectives to advance the key areas of science related to THR; and 3) Develop a recommendation for the infrastructure by which to accomplish the actionable objectives. In this presentation, we will provide an overview of the outcomes of the THR Workshop, progress to date, and next steps.

Notes

IG 02

Potential ultra-low nicotine limit in tobacco – can we meet it?

FISHER A.M.(1); FISHER C.R.(1); YANG S.(2); PATRA B.(1); SLONE S.(3); JI H.(1); KINNEY J.(1)

(1) Kentucky Tobacco Research and Development Center, University of Kentucky, 1401 University Drive, Lexington, KY 40546, U.S.A.
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The FDA has sought comments on a possible nicotine limit of 0.3-0.5 mg/g in the filler of cigarettes. Over the years, we have tested the conventional low alkaloid (LA) nic1nic2 mutants, agronomic practices known to lower alkaloids, and a combination of LA lines and agronomic practices. Neither the LA lines nor the agronomic practices alone lower nicotine to anywhere near this limit. In the trial combining LA varieties and agronomic practices, even in a very wet year, none of the varieties met this limit in all stalk positions, although several varieties did meet it in some stalk positions. In a dry year, the lowest level measured was 1.4 mg/g nicotine+nornicotine. We are now testing two gene-edited ultra-low alkaloid lines and two F4 lines combining a novel low alkaloid gene and the nic1nic2 mutants. We used the Burley 21 (Bu21) alkaloid series as checks. The nicotine+nornicotine range across stalk positions for Bu21 was 33.2-46.3 mg/g and for LA Bu21 was 2.60-3.42 mg/g). For the two F4 lines, the range was 2.19-2.80 and 1.80-2.71: significantly lower than LA Bu21 only in the primings, but consistently lower in the other stalk positions. For the two gene-edited lines, the range was 0.513-0.679 and 0.546-0.703, significantly lower than LA Bu21 in all stalk positions. Only the two gene-edited lines had significantly lower yields (2,774 and 2,794 kg/ha) than the Bu21 check (3,602 kg/ha). The poorest quality was in the two F4 lines (grade indices of 35 and 36), consistently but not significantly lower than LA Bu21 (53), which was significantly lower than the check, Bu21 (82). The two gene-edited lines had grade indices consistently but not significantly higher than the F4 lines and lower than LA Bu21. We conclude that we cannot consistently meet a 0.5 mg/g nicotine limit, not at this time, not with our current knowledge; although nicotine can be reduced to a level lower than the LA lines, albeit with yield and quality penalties.

Notes

AP 01

A comparison of Crop Protection Agents (CPAs) usage in tobacco and other crops

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The use of crop protection agents (CPAs) for tobacco production and its impact on people and environment has been largely scrutinized by global governing institutions, non-governmental organizations, and the media. Accordingly, this has become a pressing issue and a key focus area within the scope of Supply Chain Due Diligence.

There is little evidence of the real situation concerning the usage of CPAs for tobacco and other crop production. The limited number of studies available mainly take into consideration broad aspects such as land use statistics for crops and sales data of commercial CPA products. This methodology has a one-sided and perhaps a biased representation of the actual situation due to the limited land use for tobacco growing vs. other crops and consequently, lower sale volumes of CPAs for tobacco use.

To present a fair and objective comparison, a study has been conducted by collecting, through several affiliations, the representative crop protection programs for tobacco and other crops from various tobacco producing countries. This includes determining the number of commercial products used per crop protection program, application rates, active ingredients and active ingredient composition in the commercial product, with the aim to calculate the active ingredient quantities applied. In addition to the calculation of the amount of active ingredient used, the active ingredients have been classified in terms of hazard based on "The WHO Recommended Classification of Pesticides by Hazard: Guidelines to Classification - 2019" that focuses on acute oral and dermal toxicity. A hazard coefficient per crop has been derived based on two parameters: the WHO Hazard Class of the active ingredients and the amount of active ingredients per hectare applied in each crop protection program. Crop protection programs for tobacco and other crops have been compared by taking into consideration the amount of active ingredient per hectare applied and the hazard coefficient ranking. This methodology allows a more detailed and objective CPA usage comparison between tobacco and other crops by integrating one of the key factors when discussing about CPAs – Toxicity/Hazard.

Notes

AP 03

Effects of application timing and methods on chlorantraniliprole residues in flue-cured tobacco

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Chlorantraniliprole is a narrow-spectrum insecticide that offers good to excellent control of tobacco budworm (*Heliothis virescens*) and Tobacco Hornworm (*Manduca sexta*). Due to its long residual control, narrow-spectrum activity, safety, and the loss of other insecticides, chlorantraniliprole has gained popularity among commercial tobacco farmers in North Carolina. As a result, cured leaf residues are reported to have increased. To further understand chlorantraniliprole application and residues, research was conducted in three growing environments to evaluate treatments delivered in transplant water, in transplant water + foliar at layby, foliar at layby, the elongated button stage, one day before first harvest, and one day after first harvest. Chlorantraniliprole application was 0.55 L/ha (0.11 kg ai/ha) within each treatment. Treatments applied at the elongated button stage, one day before harvest, and one day after harvest were delivered in solution volumes of 187 and 467 L/ha to simulate over-the-top applications or those that could be tank-mixed with suckercides. Within the lug stalk position group, chlorantraniliprole residues were highest in the transplant water + foliar at layby (12.54 mg/kg), foliar at layby (9.63 mg/kg), and one day before harvest application timings (7.82 - 10.55 mg/kg). Residues in the lug stalk position group ranged from <LoQ to 5.34 mg/kg for all other treatments. Among the cutter, leaf, and tip stalk position groups, residues were highest in the one day before (6.67 - 12.11 mg/kg) and after first harvest treatments (6.14 - 14.97 mg/kg). A secondary analysis revealed no significant differences in residues between the 187 and 467 L/ha carrier volumes. Among the treatments evaluated, chlorantraniliprole residues were generally lowest when treatments were applied once at an early stage of crop growth or in the elongated button stage. Moreover, cured leaf residues were only predicted to exceed the CORESTA GRL of 14 mg/kg in one of our statistical analyses. Our results suggest that strategic application of chlorantraniliprole should be utilized by farmers in order to minimize cured leaf residues.

Notes

AP 04

Crop protection agent container disposal / recycling project

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Turkey is an important tobacco leaf sourcing country as Oriental leaf is a key component of a wide variety of tobacco products. The major Turkish tobacco suppliers, represented by Aegean Tobacco Exporters Association (ATEA), continuously collaborate in non-competitive efforts to progressively engage in essential activities with growers, suppliers, local authorities, and stakeholders as part of a supply chain due diligence (SCDD) process. In 2021, three SCDD risk challenges (child labour, working conditions, and occupational health & safety) were identified by Twenty-Fifth Ltd consultancy and confirmed by ATEA's Sustainability Committee as priorities for broader industry-level collaboration. Proactively driven by JTI, the occupational health & safety workstream has prioritized key initiatives already initiated back in 2020, such as the crop protection agent (CPA) disposal / recycling project in the Denizli region (Acipayam, Beyagaç, Kale) where totally 22 CPA waste collection points were built following a successful partnership with the Municipality, Chamber of Agriculture, and CPA Companies (Syngenta, FMC, Agrobest). As we strive to positively impact our growing communities by driving behavioural change and improving social and environmental impacts across our industry, this paper will provide insight and describe this key ATEA / JTI initiative, including an overview of key performance indicators. A perfect example of a positive collaboration between all stakeholders initiated by the tobacco industry.

Notes

AP 06

Individual meeting with farmers: a tool for good agricultural practices training

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The training of farmers is a key factor in any good agricultural practices (GAP) program. At the end of the crop season, Trasformatori Tabacco Italia (TTI) discusses with each grower the field management and the quality of the tobacco harvested.

This study compares the different results achieved by two farmers A and B, who in 2022 cultivated the same variety.

All the field operations carried out by the growers are recorded on TTI's traceability platform and linked to the grading done by expert leaf buyers of TTI to understand which of them could have influenced the field's performance. Then, a report is made to highlight yield, quality index (QI), tobacco defects, and the presence of organic non-tobacco related material (NTRM).

Yield is expressed in kg/ha, QI is the result of the weighted average between the QI of each carton, percentage of defects and presence of NTRM are calculated on the base of the grading.

Farmer A had a yield of 3150 kg/ha, a QI of 68.5, and a strong presence of green tobacco and suckers on the 44.7 % and the 4 % of the cartons, respectively. Farmer B achieved a yield of 3340 kg/ha, a QI of 78.6, 4.4 % of green tobacco and no presence of NTRM.

A wrong fertilization influenced the results. Farmer A distributed 111 units of nitrogen and 152 of potassium against the 80 and the 216 estimated respectively from the soil analyses. This excessive nitrogen also promoted an extreme vegetative vigour and thus a weak control of the suckers. Farmer B, on the contrary, followed TTI’s fertilization advice by distributing 74 units of nitrogen and 234 of potassium.

The seasonal data collected allow TTI to support its farmers by offering objective data aimed to improve GAP and the consequent farmer’s profitability.

Notes

AP 08

Addressing livelihoods within small-scale tobacco grower base

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Farmer viability, decreasing net incomes and the associated lack of economic resilience is becoming increasingly significant throughout the small-scale grower base in Africa. This poses a direct threat to long term sustainability.

The objective of this initiative was to identify and implement a grower livelihood program which would contribute positively to farmers net income while strengthening understanding of financial and business literacy at a household level.

A cross section of farmers, who are by proxy contracted to Premium Active Tanzania were selected from Primary Societies in the Chunya growing region. Surveys to establish basic 'living income' data were conducted, and the selected farmers were trained using specific field tools from the Gender Action Learning System (GALS). Growers participated in focus group discussions which were guided by "living income" surveys and using the Challenge Action Tree (CAT) tool, a production challenge was identified.

One root cause was identified as a poor GAP practice (late topping), resulting in a loss of potential yield and therefore income. The time of topping is traditionally delayed in Tanzania beyond full bud emergence (CORESTA Guide No. 7) Stage 55/59 as opposed to Stage 50/51. The advantages of 'bud-topping' are well documented and have a significant impact on final yield and quality.

The implementation of a bud-topping program was identified as a simple, cost-effective method of improving farmer returns.

The study was laid out as a randomized complete block design involving three blocks (regions) and two treatments (bud topped and grower standard topping practices). There were a total of ten replications per region comprising 30 growers. Data collection involved leaf length x width measurements from ten plants per replication; 3 weeks after topping and 6 weeks after topping and yield data was collected at the end of the season.

Initial results are promising. Bud-topping trials will continue for three years to determine conclusively positive response. Once farmers are shown the positive impact, rollout of bud-topping practice will be included in Farmer Training Seminars and handbooks and coupled with improved financial management contributing towards an economically viable grower base.

Notes

AP 09

Use of mixed model for evaluation and selection of Burley tobacco

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Several traits must be considered simultaneously in selection of tobacco cultivars for meeting the requirements of farmers, industries and consumers. In this context, it is important to obtain information to improve the efficiency of the tobacco breeding programs. Experiments with tobacco hybrids are usually developed in several locations and years, a strategy that aims to increase selection efficiency, considering the effects of genotype x environment interaction. The complexity of identifying superior genotypes is simplified with the use of methodologies based on Mixed Linear Models, which allow modeling fixed and random effects simultaneously. In this research, the objective was to evaluate the performance of 24 Burley tobacco hybrids, generated by the Alliance One International, regarding the yield, nicotine and leaf quality Index characters, in seven environments in the southern region of Brazil, using a Mixed Linear Model approach. The tests were carried out in a randomized complete blocks design, with three replications, together with five commercial controls. Statistical analyses were performed in the JMP environment, using Mixed Linear Models. Based on the models selected by character, the average predicted values of the hybrids were obtained in the average of the environments and within each environment, making it possible to select the most adapted hybrids for each location. The use of the statistical approach of Mixed Linear Models proved to be effective with regard to the selection of superior genotypes, given the nature of the data set, which had imbalance in terms of the evaluated genotypes, in addition to a large number of environments where the tests were conducted. There were significant differences for the effect of genotype for the evaluated characters, which indicates that for these characters there is genetic variability. The behavior of the hybrids within the environments was verified, allowing the selection of hybrids according to the studied environments.

Notes

AP 10

Development of an alternative system for seed-based tobacco haploid plant generation

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Haploid plants can be extremely useful for expediting the production of new inbred lines, and also for rapidly converting female parental lines of new hybrids to cytoplasmic male sterility. Use of doubled haploid breeding can increase gain from selection per unit of time and reduce the number of years required for commercialization of new cultivars. One method of isolating haploid plants is the use of the *N. tabacum* × *N. africana* interspecific cross. This is a semi-lethal cross due to activity of a genetic interspecific lethality mechanism, and gynogenic haploid plants can be identified from seed due to parthenogenesis or chromosome elimination. *Nicotiana africana* is slow growing, however, and produces little viable pollen under hot conditions. In addition, it does not allow for efficient production of androgenic haploid plants from seed, rare events that are necessary for one-step transfer of the nuclear genome of a pollen parent to the cytoplasm of a female parental line. We have generated a modified method to identify haploid plants from seed utilizing hybrid lethality whereby (1) a *N. tabacum* gene controlling lethality in progeny derived from crosses with species of section *Sauveolentes* was identified and inactivated via gene editing, and (2) a genomic region affecting interspecific hybrid lethality was subsequently introgressed from *N. umbratica* into *N. tabacum*. A derived genetic stock of *N. tabacum* is being evaluated for its potential to assist in the identification of maternal and paternal haploid tobacco plants from seed.

Notes

AP 11

Use of principal component analysis to cluster flue-cured-Virginia tobacco genotypes

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Tobacco research have been intensively carried out with a high number of variables, genotypes and environments for the evaluation and discrimination of genotypes, as well as the identification of optimal cultivation conditions and limiting factors for the agronomic traits. The use of statistical tools such as multivariate data analysis techniques can be more appropriate for the purpose of discriminating genotypes. The objectives were to apply unusual statistical approach to discriminate and grouping flue-cured Virginia tobacco genotypes. The performance of 14 genotypes, developed by Alliance One International. Yield, nicotine, sugar and quality index grade were evaluated across 11 environments in the southern region of Brazil. The trials were conducted in a randomized complete blocks design, with three replications and four commercial controls. Statistical analyses were performed in the JMP software program (SAS Institute Inc.). The estimates of genetic parameters were estimated by the restricted maximum likelihood (REML) method. Multivariate and principal components analysis (PCA) using the breeding values of all traits were performed. Out of four, two of the principal components reached more than 1.00 Eigenvalue and explained about 71.95 % of the variability. The first principal component recorded the highest variation 42.61 % followed by the second PCA 29.34 %. The PCA1 was found to be mostly related with traits such as yield, nicotine, sugar and a small part of quality index. In the second PCA, the traits quality index and nicotine were more related. In conclusion, the main component analysis methodology, considering all the traits together, allowed a more precise grouping of the genotypes when compared to a conventional approach and allowed understanding agronomic traits based on their correlations considering their interaction with the genotypes.

Notes

AP 12

Genome-wide association analysis combined with quantitative trait loci mapping reveal the genetic loci of leaf traits in cigar tobacco

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Cigar is a special tobacco product made entirely from a roll of leaf tobacco. In China, the cigar market continues to grow, while the serious shortage of high-quality cigar wrapper, the cigar´s outermost leaf, limits the development of the domestic cigar industry. It is therefore necessary to develop high-quality varieties of cigar wrapper to meet industrial needs. The leaf traits of cigar wrapper, including leaf flatness, leaf thickness, lateral vein thickness and leaf vein angle, play a crucial role in cigar wrapper quality. Thus, it is of importance to study the genetic basis of leaf traits in cigar. In this study, a natural population comprising 185 accessions, and F_2 populations from Xiawanna × Segedinska Ruca cross were collected for genome-wide association analysis (GWAS) and quantitative trait loci (QTL) mapping, respectively. Here, we completed the whole genome resequencing for the two populations, and obtained 2119142 high-quality SNPs in GWAS population. A high-density genetic map containing 1682 SNP markers was constructed using F_2 population, with a total genetic distance of 3107.13 cM and the average genetic distance 1.85 cM. A total of 26 QTLs related to leaf traits were mapped in the F_2 population at three different developmental stages, and some QTL intervals were repeatedly detected in terms of different leaf traits and developmental stages. Furthermore, some significant SNPs identified by GWAS analysis co-located with the QTL intervals, indicating that the genetic regulation of the relevant leaf traits was stable, and the identified SNPs and QTLs were important genetic loci. At the end, we combined the two mapping results and predicted some candidate genes associated with leaf traits in cigar.

Notes

AP 13

Improving the efficiency of tobacco breeding using multivariate statistical methods

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At present, selection based on individual traits and isolated environments is the standard method for genetic selection in tobacco. However, when genetic selection is performed based on two or more genetically correlated traits and these are analysed individually, selection bias may arise. The present study was thus developed to examine the efficiency and applicability of Pearson's Correlation by the Residual Maximum Likelihood (REML/BLUP) and approaches in the genetic selection of flue-cured Virginia hybrids. The performance of 14 flue-cured Virginia tobacco hybrids, developed by Alliance One International, were evaluated regarding the yield, nicotine, sugar and quality grade characters, in 11 environments in the southern region of Brazil. The trials were conducted in a randomized complete block design, with three replications and four commercial controls. Statistical analyses were performed in the JMP software program (SAS Institute Inc.), using the REML method and Pearson's correlation coefficient. Genetic parameters were estimated via REML, with genotypic means adjusted and estimated using the BLUP (Best Linear Unbiased Predictor) procedure. The Likelihood Ratio Test (LRT) was performed and the significance was verified by the Chi-Square test. The heritability was considered from medium to low for all traits, as well as the residual variance was always higher when compared to the genotypic variance or that referring to the G×E interaction. In view of this, it is important that trials be conducted in different locations and agricultural years and with a greater number of repetitions. Pearson's correlation was significant only between yield and nicotine. Although, in this study, most traits did not show significant correlations, it is important to emphasize the importance of evaluating the data jointly, using appropriate statistical approaches in order to obtain more accurate results. The statistical analysis carried out was efficient. Some characteristics must be carefully evaluated when used at the selection level.

Notes

AP 14

Genetic control of enantiomeric specificity of nicotine biosynthesis in tobacco

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Living things frequently produce metabolites that can exist in optically pure form or as enantiomeric mixtures. Interestingly, enantiomers often confer different biological activities. Nicotine can exist naturally in the tobacco plant as either (*R*)- or (*S*)-enantiomeric isoforms, with the (*S*)-enantiomers predominating. (*R*)-nicotine typically accounts for < 0.5 % of the total nicotine pool on a dry leaf weight basis. (*S*)-nicotine has been reported to have increased physiological potency in humans relative to the *R* isoform. The mechanism of formation and accumulation of nicotine enantiomers is largely unknown. We have researched the relative impact of different isoforms of an enzyme involved in nicotine biosynthesis. Evaluation of lines carrying induced mutations in genes coding for these enzymes suggests that some isoforms are preferentially involved in the biosynthesis of (*S*)-nicotine, and one isoform is preferentially associated with the biosynthesis of (*R*)-nicotine. Certain tobacco lines with specific mutant combinations were found to preferentially accumulate (*R*)-nicotine. Our results shed light on the genetic control of nicotine enantiomer accumulation in *N. tabacum*.

Notes

AP 16

Isolation, identification of a lytic bacteriophage PQ43 for *Ralstonia pseudosolanacearum* and its application in biocontrol of bacterial wilt

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Bacterial wilt (BW) is a devastating soil-borne bacterial disease caused by *Ralstonia pseudosolanacearum*. Many efforts have been made to control BW, however there is still a lack of effective, green and safe measures. Bacteriophages are expected to become an important biocontrol resource for prevention and control of the latest generation of wilt diseases. Many bacteriophages infecting *R. pseudosolanacearum* have been isolated at present, however, the bacteriophages are still insufficient to bio-control the *R. pseudosolanacearum* which presents much diversity in the environment. In this study, a lytic bacteriophage PQ43 infecting *R. pseudosolanacearum* was isolated from the rhizosphere soil of tobacco in Ningxiang, Changsha, China. Transmission electron microscopy and genomics analysis indicated that PQ43 belonged to the family *Siphoviridae*, order *caudoviridaes*. The genomics with a full length of 47,156 bp and a mean G+C content of 64.27 %, contains 74 open reading frames (ORFs), and does not contain tRNA, resistance or virulence genes. Bacteriophage PQ43 also exhibited high temperature stability (< 60 °C, 2 h), strong pH tolerance (pH 3-10), and strong UV tolerance (< 110 min). A pot experiment showed that the addition of bacteriophage could effectively control tobacco BW, especially the bio-control effect of PQ43 application in 108 PFU/ml and 107 PFU/ml treatments by root irrigation, and could reduce the incidence of tobacco BW significantly (to 92.80 % and 58.84 %, respectively), within 30 days. So, bacteriophage PQ43 is considered as an effective candidate biocontrol agent to control tobacco BW disease caused by *R. pseudosolanacearum*.

Notes

AP 17

Evaluation of sunhemp treated with *Trichoderma* in short tobacco rotations for control of root-knot nematode (*Meloidogyne javanica*) and disease complexes

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The use of non-host crops in rotations with tobacco is a component of an IPM strategy used to suppress root-knot nematode (RKN) populations. Nematode damage usually predisposes infected plants to other soil-borne pathogens, such as *Fusarium* spp. and *Rhizoctonia* spp. resulting in disease complexes that are more damaging than either on its own. In Zimbabwe, low hectarages have constrained the ability for growers to practise the recommended rotations and the monocropping has led to the re-emergence of root-knot nematode disease complexes on tobacco. Rotation crops notably sunhemp and Katambora grass have been recommended for the control of plant parasitic nematodes but there is need to control soilborne pathogens as well. The objective of this trial was to evaluate the efficacy of Sunhemp and *Trichoderma harzianum 77* on root-knot nematodes and soreshin (*Fusarium* spp. and *Rhizoctonia* spp.) control in tobacco fields and to investigate whether *Trichoderma* modulates the hormone signalling network in the host to induce nematode resistance. *Trichoderma harzianum* (T77) was applied as a seed-treatment to the relay sunhemp crop, then tobacco was transplanted and evaluated in the field over three successive seasons. The experimental design was factorial replicated three times using an RKN-susceptible and RKN-resistant tobacco variety in microplots with high RKN populations of at least 50 RKNs per 200 g of soil. RKN population assays, disease and end-of-season root galling assessments were done. The results showed that tobacco seed-treated with T77 had markedly lower soreshin damage than those from disease control plots with merit noted when T77 was applied at transplanting. Additionally, susceptible tobacco plants grown in plots initially planted with sunhemp seed-treated with T77 had markedly lower galling (< 3 on a scale of 0-8) than plants from the disease controls. This observation was even more apparent in plots planted with resistant tobacco, which had root damage scores of 1 or less on a scale of 0-8. Used in combination with resistant varieties and recommended cultural control practices, T77 provides a promising sustainable control option for RKNs on tobacco lands in Zimbabwe.

Notes

AP 18

Use of symbiotic nitrogen fixation endophytes in tobacco seedling production

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Inadequate nutrition leads to poor plant growth and subsequently poor yields. This is a challenge especially in tobacco seedling production where planting of vigorous and healthy seedlings is the main pre-requisite for successful plant establishment in the field. To remedy this, farmers usually resort to increasing the usage of chemical fertilisers. This, however, places a burden on the environment such as groundwater pollution and greenhouse gas emissions. It is against this backdrop that there are concerted calls worldwide to reduce chemical fertilisers usage with the aim of achieving profitable production capacity under sustainable agriculture. One such way is the use of nitrogen-fixing microorganisms that increase plant nutrient supply, thus, reduce chemical fertiliser usage. In this experiment, *Gluconacetobacter diazotrophicus* (Gd) a nitrogen-fixing endophyte capable of also producing plant growth hormones, was evaluated. *Gluconacetobacter diazotrophicus* is mainly associated with sugarcane and has also been known to colonize many other sugar and non-sugar plants like *Pennisetum purpureum* and *Ipomea batatas*. However, to the best of our knowledge its effect on tobacco has not been evaluated. Thus, a trial was conducted at Kutsaga Research Station to determine the effect of inoculating tobacco seed with Gd bacteria on seedling growth and quality. The seed was sown both in the float system and conventional systems in a randomised complete block design. Inoculation was done prior to seeding and at two weeks after germination. Subsequent reductions in nitrogen rates were as follows 100, 75, 50, 25 and 0 %. Results show some increase in seedling height, diameter and biomass under the 25 and 0 % N treatments, suggesting nitrogen fixation in extreme nitrogen deficient environments. This paper discusses the results obtained and gives recommendations on further investigations.

Notes

AP 19

Improvement of the output value, chemical and processing quality of flue-cured tobacco by application of humic acid solution

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The overuse of N fertilizer in vegetable growing season leads to the high amount of N remained in soil and the decrease of resistance of tobacco plants in vegetable-tobacco continuous cultivation system, which will seriously threaten the sustainable development of flue-cured tobacco production. Humic acid (HA) rich in bioactive substances and microelements may enhance leaf growing rate and improve the quality of flue-cured tobacco. A two-factor randomized block experiment was carried out over two years to evaluate the effects of additional applying HA (0, 150 $L.ha^{-1}$) combining with three N fertilization rate (0, 60 and 90 kg $N.ha^{-1}$) on tobacco growth, output value, chemical and processing quality. (1) Addition of HA significantly increased the tobacco output value by 5 % - 28 %. The yield of superior tobacco positively correlated with the total output value (R^2=0.88), which was the key factor determining the increase of the total output value. (2) Applying HA decreased the disease index and significantly increased the microelement uptake and the daily leaf growth rate in prosperous growing stage which positively correlated with the yield of superior flue-cured tobacco. (3) Application of HA significantly improved the internal chemical quality and reduced the leaf midrib proportion. (4) At the N rate of 60 kg $N.ha^{-1}$, HA showed the best effect of improving quality and increasing income. In conclusion, our results demonstrated that addition of humic acid can significantly improve the total output value, the chemical and processing quality of flue-cured tobacco. The best effect can be achieved by adjusting N application rate appropriately according to the amount of soil residual N before the transplanting of tobacco seedling.

Notes

AP 20

Identification and manipulation of trichome specific controlling enhanced flavor and aroma in tobacco

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Glandular trichomes are epidermal outgrowths found on vascular plant species, including members of the genus *Nicotiana* capable of synthesizing and secreting large amounts of specialized secondary metabolites (e.g., terpenoids, flavonoids, phenylpropanoids, and acyl sugars) that contribute to plant defense, chemoattraction, as well as flavor and aroma. We have identified a set of genes whose promoters are expressed in a glandular-trichome-specific manner and have characterized the cis-regulatory elements conferring their trichome specific expression in genetically engineered K326 (commercial flue-cured varieties), TN90 (Burley type), Izmir Ego (an Oriental type) and *Nicotiana benthamiana* (a model species) plants. We have used these promoters to examine the regulatory factors and metabolic activities involved in the formation of key terpene metabolites that contribute to flavor and aroma, including biosynthetic pathways controlling the formation of the diterpenoid cis-abienol. We have also examined the effects of altering trichome density and trichome-specific gene expression on trichome and whole leaf gene expression, terpene profiles, and trichome densities of genetically engineered K326, Izmir Ego, and *N. benthamiana* plants. We have shown that it is possible to selectively alter leaf trichome numbers and terpene composition, and thereby flavor and aroma characteristics. Progress toward the understanding of the broader sets of regulatory factors that are involved in trichome expression and metabolism will be discussed.

Notes

AP 21

Computational-based genomic, transcriptomic, and metabolic analyses of biosynthetic gene clusters in tobacco

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Terpenes and terpenoids contribute to the aroma and flavor that influence consumer preferences in selecting plant-based products. Computational identification of biosynthetic gene clusters (BGCs) in plants can pave the way for future biosynthetic genetic engineering. Using integrative genomic, transcriptomic, and metabolic pathway annotation analyses, 35 BGCs in tobacco were identified with high confidence. Out of the 35 BGCs belonging to seven biosynthetic classes, seven BGCs were terpene-related. Two BGCs found in C13 and C14 chromosomes belonged to terpene and saccharide-terpene biosynthetic classes that were only 93 Mb and 189 Kb apart, respectively. Other clusters have the length ranging from 120 Kb (Cluster 9) to 1.6 Mb (Cluster 18). Each cluster contained five (Cluster 21) to 20 genes (Cluster 32) and the number of terpene synthase genes present in the clusters also varied from one (Clusters 18 and 21) to eight (Cluster 32). Gene expression profiling using diurnal and topping transcriptome datasets identified co-expressing genes within modules and varying levels of expression among modules as represented by the normalized enrichment score measured in each module. The positions pinpointed from these computational analyses will allow for more efficient modifications of specific genes and BGCs for development of tobacco-based products with improved aroma and flavor.

Notes

AP 22

Colchicine treated tobacco – changing biochemical composition of the leaves

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Colchicine has been used in agriculture since the 1930s to induce polyploidy, or the duplication of chromosome sets, in plants. In addition to its use in inducing polyploidy, colchicine has also been studied for its potential effects on secondary metabolite production increase in certain plants.

Several experiments have been conducted to determine the optimal conditions for treating tobacco with colchicine, such as the concentrations and durations of treatment. Seeds of two tobacco lines were treated with 0.5 and 1 percent aqueous solutions of colchicine for eight hours.

The treated seedlings and controls were grown to maturity and leaves of the resulting mature tobacco plants were harvested for analysis. The colchicine treatment affected the growth and development of the tobacco plants, leading to biochemical composition changes of the leaves. Our experiments have shown that colchicine treatment increases the production of secondary metabolites in tobacco plants. Preliminary internal panel observed different aerosol profiles between controls and treated lines. Compared to the control, the treated lines gave higher physiological impact and more complex sensorial notes (such as woody, phenolic, aromatic, burning, etc.). The chemical analysis showed more and higher contents of flavor relevant compounds in the treated lines versus controls.

Notes

AP 23

Study on the volatile organic compounds of flue-cured tobacco with different sweet aroma intensity by flavoromics approach

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Volatile organic compounds (VOCs) are mainly the chemical basis of sweet aroma, which is one of the most important flavour characteristics for high-quality flue-cured tobacco. In order to reveal differences of VOCs between the higher (MTQ) and lower (MTR) intensity of sweet aroma, the VOCs of flue-cured tobacco were analyzed by the headspace solid phase microextraction (HS-SPME) combined with comprehensive two-dimensional chromatography-time-of-flight mass spectrometry (GC×GC-TOFMS). The results showed that there were 1109 kinds of VOCs in both tobacco samples with different intensity of sweet aroma, 569 kinds of the unique VOCs in MTQ, and 559 kinds were only found in MTR. The relative contents of ketones, hydrocarbons, heterocyclic compounds, aldehydes, esters, alcohols and carboxylic acids in MTR accounted for 38.71 % of total volatiles, which was 25.15 % lower than that in MTQ. Fifty-nine kinds of VOCs had a significant difference ($p < 0.05$, VIP > 1) between MTR and MTQ, including 13 VOCs that were down-regulated and 46 VOCs that were up-regulated. The down-regulated VOCs were closely related to sweet aroma and fruity aroma, while the up-regulated were not only related to sweet aroma and fruity aroma, but also associated with unpleasant odours, such as pungent odour, sour odour and rancid odour. There were 10 kinds of key aroma compounds with relative odour activity value (ROAV)=1, including five kinds in MTQ and 10 kinds in MTR. 2,3-butaneone and vanillin mainly give the tobacco leaves sweet and milky aroma, while a-pinene, glutaraldehyde, 2-methylnaphthalene and isoamyl alcohol also contribute to turpentine odour, pungent, camphor odour, scorched odour for MTR. In this study, flavoromics approach was used to reveal the volatile organic components of flue-cured tobacco with different sweet aroma intensity. This work provided a method for the study of aroma characteristics of tobacco.

Notes

AP 24

Map-based cloning of the gene responsible for solavetivone accumulation in *Nicotiana sylvestris*

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Solavetivone, which is a sesquiterpenoid phytoalexin in *Nicotiana*, has a citrus-like aroma. In the present study, we identified a gene involved in solavetivone accumulation in *Nicotiana* by means of forward genetics. Due to the difficulties of forward phenotypic screen of mutants in allotetraploid tobacco (*N. tabacum*), we used a chemically-induced mutant library in diploid *N. sylvestris* and screened for mutants with increased solavetivone content. Through GC-MS analysis in 3289 mutant lines, we obtained a mutant with 30 times increase in solavetivone content compared to wild-type *N. sylvestris*. Intriguingly, this mutant developed hypersensitive response (HR)-like lesions in leaves, implying that deregulated immune response promotes the solavetivone accumulation. An F_2 population derived from a cross between the mutant and a wild-type *N. sylvestris* showed a one to three ratio for the mutant-type to wild-type phenotypes, suggesting that the mutant phenotype was caused by a single recessive mutation. MutMap analysis showed the causal mutation within a 3 Mb genomic region, and one missense mutation was found in a gene annotated as P-type ATPase. RNAi-mediated knockdown of the gene in *N. sylvestris* resulted in increased solavetivone content with HR-like lesion formation similar to those in the mutant. Thus, it is likely that the missense mutation impeded the gene function and is responsible for the increased solavetivone phenotype.

Notes

AP 25

Developing and deploying climate-resilient tobacco (*Nicotiana tabacum*) varieties in marginal areas of Zimbabwe: stability and adaptability analysis in new tobacco varieties

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To ensure farmers continue to reap the best in tobacco production, breeding and development of novel genetics is pivotal in the tobacco value chain. Recently, some new varieties were released to address climate change-associated shocks and new pathogen variants. Moreover, farmers in Zimbabwe are now growing tobacco outside of the traditional growing regions hence the need to develop new varieties for the new growers. The objective was to evaluate the agronomic performance of promising experimental tobacco hybrids (ETHS) under marginal conditions. Four sites: Karoi Tengwe, Lower Gweru, Insiza, and Masvingo that are marginal for tobacco production were used for the study in two seasons (2021-22, 2022-23) and compared to an optimum site (Kutsaga). Trials were laid out in a randomized complete block (RCBD), with three replications. Agronomic variables measured were speed to topping, leaf expansion metrics (largest, penultimate), yield (mass at untying, saleable), grade index and leaf chemistry (nicotine, reducing sugars, total nitrogen) were subjected to analysis of variance (single, across site) using Genstat 21st edition. The AMMI model and GGE biplots were used to analyze stability. The results revealed significant differences among genotypes across sites and seasons. The experimental hybrids ETH07/17 and ETH11/17 had the highest yields, while ETH12/11 had the fastest speed to topping. Partitioning of the genotype by environment effects revealed that the most stable and best genotypes were ETH07/17, ETH 11/17 and ETH12/17. The implication of this work in identifying varieties with broad and specific adaptation and its impact on tobacco production in Zimbabwe will be discussed.

Notes

AP 26

An investigation into the influence of climate smart agriculture on the soil properties, yield and quality of Burley tobacco in maize and groundnut rotation in Malawi - a comparison of different cultivation techniques in a small-scale model

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Malawi is impacted by climate change, population increase, low pH, soil degradation, loss of soil organic matter and erosion due to poor soil management. These factors impact food security. In tobacco, this has resulted in smallholder yield stagnation at less than 800 kg/ha against the potential of 3000 kg/ha. Climate Smart Agriculture (CSA) improves physical, chemical and biological soil properties through constant water flow, infiltration and holding capacity. Residues retention reduces emission of greenhouse gasses hence retention of carbon in the soil. CSA promotes zero/minimum tillage, yearly movement of the soil, compacts, reduce water infiltration, reduce soil aeration and increase farming costs hence declining productivity. The objective of this study was to evaluate and understand the trends of the effect of CSA practices on soil tillage, on reducing soil degradation, on rotation, and on mulching, in order to increase in yield and return. The CSA study was conducted at Mpale Farm from 2017 to 2023. Three tillage systems: conventional, minimum and no-till. Beds were only tilled in CY17 in the min-till and no-till. In conventional, tractor tillage was carried out annually. The trial had three crops in rotation: tobacco, maize and groundnuts. The trial was laid out in a split design with tillage systems as the main plot and the crop types as sub-plots.

No-till produced high pH, organic carbon and high organic matter. In seven years, no-till tobacco outperformed conventional by 20 % while on minimum-till by 15 %. For maize no-till outperformed conventional by 25 % while on minimum-till by 22 %. For groundnut no-till outperformed the conventional by 20 % while on minimum-till by 15 %. With no-till there was 40 % savings in labour compared to conventional. This performance is due to increased organic matter that maintains soil moisture hence increasing microbial activity, allowing for better nutrient availability. With no-till, there is soil structure improvement due to the reduction in tillage.

Notes

AP 28

Use of *Chrysopogon zizanioides* (L.) Roberty (Vetiver) to deliver ESG projects throughout the tobacco leaf supply chain

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Vetiver grass (*Chrysopogon zizanioides*) has been extensively studied in Asian countries due to its multiple uses in soil conservation, infrastructure protection, ecological sanitation, biological pest control, ecological restoration, and other agronomic and bioengineering applications. The establishment of closed hedgerows in contour lines or linear plantations is known as The Vetiver System (TVS) (Greenfield, 1993) and is considered a low-cost, easily adoptable, and replicable technology in different contexts. This study aims to demonstrate and validate the use of the Vetiver System in the development of Environmental and Social Governance (ESG) projects throughout the tobacco leaf supply chain. Based on documentary research (Greenfield, 2008; Truong *et al.*, 2015), and personal experience, Keyline Vetiver Hedges were established in strategic tobacco supply farms of Nueva Matacapan Tobaccos since 2019 to stop extreme erosion issues and crop loss due to severe precipitation events, as well as to be compliant with STP supplier improvement cycle. The association of TVS with tobacco production is a cost-efficient nature-based solution that produces short-term results and provides farmers with an important tool for climate risk management. The standardization of an adequate land planning and project management framework was delivered by Estampa Verde to scale up this solution throughout the industry. The incorporation of *Chrysopogon zizanioides* in tobacco farms following Estampa Verde's standard provides a starting point for climate risk reduction and further development of ESG related projects throughout the leaf supply chain.

Notes

AP 29

Effect of intercropping on crop productivity and quality of flue-cured tobacco in Malawi

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Limited land amidst growing population is one of the major constraints to producing more food and cash crops in Malawi. Intercropping has huge potential to utilize land more efficiently. A study was conducted to assess effects of intercropping legumes (common bean, cowpea, groundnut, pigeon pea and soybean) on cured leaf yield of flue-cured tobacco, crop productivity and compatibility of the tobacco-legume system. Eleven treatments were laid out in a randomized complete block design with three replications. Each of the legumes was rain-planted in mixed stand with tobacco and pure stands. A plot with pure tobacco was also included. In mixed stands, all legumes were grown between tobacco ridges spaced at 1.2 metres. Ridges in all legume pure stands were spaced at 0.75 metres. Tobacco-legume productivity and compatibility was determined using the land equivalent ratio (LER). Preliminary results showed that intercropped tobacco with common bean, groundnut and pigeon pea produced similar cured leaf yield to pure tobacco while intercropped tobacco with cowpea and soybean significantly reduced cured leaf yield. The vigorous vegetative growth of the two intercrops triggered competition for resources hence stressing tobacco growth. On the other hand, intercropped tobacco with legumes except for common bean had positive LERs of 1.79, 1.16, 1.11 and 1.09 for pigeon pea, groundnut, soybean and cowpea respectively. The results showed that pigeon pea and groundnut were compatible in a tobacco-legume system. These could serve as alternatives for growers to produce both food and cash crops under dwindling land holding sizes.

Notes

AP 30

NNN detection using monoclonal antibody

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N-Nitrosonornicotine (NNN) is a major carcinogen in tobacco products and listed as a HPHC chemical to be regulated by FDA. Quick, robust, and cost-effective NNN detection methods are highly desired for consistent tobacco product quality control, regulation compliance, and waste/cost reductions. Liquid chromatography-mass spectroscopy (LC-MS) has been the gold-standard for NNN quantification in tobacco products. However, LC-MS analysis requires intensive personnel training and expensive instruments purchase/maintenance. Enzyme-linked immunosorbent assays (ELISA) have been utilized as an alternative to LC-MS for the analysis of small organic compounds such as dioxin, mycotoxin, etc. These antibody-based screening methods have been adopted as industrial standards for some chemical contaminants. In this study, we attempted to develop a competitive ELISA assay based upon NNN specific rabbit monoclonal antibodies. We prepared NNN-KLH (keyhole limpet haemocyanin) conjugates which were used to immunize rabbits. The rabbit sera were collected and analyzed by ELISA using BSA-NNN conjugates as the probe and free NNN as the analyte competitor. For clone selection, we selected the rabbit, of which the anti-serum binding to BSA-NNN probes was most inhibited by free NNN in the ELISAs. Its spleenocytes were isolated and analyzed using flowcytometry with a fluorescent labelled BSA-NNN probe. Clones with the strongest fluorescent labelling were sorted out and their respective antibody coding sequences were cloned. This enabled the subsequent recombinant expression of these mono-clonal antibodies (mAb). Those mAb were further tested for their sensitivity and specificity to NNN. We successfully identified a clone with the IC50 of 1.5 ppm for free NNN when 1 ug/ml BSA-NNN probe was used. This clone showed high specificity to NNN and its binding to BSA-NNN probe was not inhibited by other TSNAs, nicotine, or nornicotine at relevant tested concentrations. We are investigating ELISA methods using this mAb for different tobacco products/matrixes.

Notes

AP 32

Diamine oxidase enzyme with respect to tobacco alkaloids and tobacco specific nitrosamines formation

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Nornicotine is known to be the major source of N-nitrosonornicotine (NNN) that is included on the Food and Drug Administration (FDA)'s list of harmful and potentially harmful constituents (HPHCs). It is typically produced during the curing and burning of tobacco and biosynthesized by the direct conversion from nicotine-by-nicotine N-demethylase (NND) enzyme. Aside this biosynthetic pathway, there are no other pathways that exist for nornicotine biosynthesis. In 2014, Hashimoto, T. *et al.*, reported that methylputrescine oxidase 2 (MPO2), was constitutively expressed at low basal levels without responding to methyl jasmonate (MeJA) induction and nic1nic2 regulatory signaling pathway. More importantly, they showed that MPO2 had a higher affinity to non-N-methylated amines such as putrescine than to N-methylputrescine, which made it distinct from traditional MPO, therefore named it diamine oxidase (DAO). In a previous field study, we detected higher levels of nornicotine than nicotine in very low nicotine (VLN) Burley tobacco. The excess amount of nornicotine suggested either another route of nornicotine biosynthesis, besides the formerly known NND-mediated conversion from nicotine, or abnormally enhanced expression of NND enzyme which led to more conversions. In this study, we initially verified that the NND expression level remains unchanged, as a measure to rule out the second possibility. Then we investigated the function of DAO as a new route of nornicotine biosynthesis by generating transgenic Burley and dark tobacco with modified DAO expression and measuring their alkaloids and tobacco specific nitrosamines (TSNA) concentrations. The overexpression of DAO in NLM-P dark tobacco increased nornicotine content more than 3-fold in both nursery and field test. The NNN content after curing in barn also increased proportionally. However, DAO modification showed minimal effect in Burley tobacco.

Notes

AP 33

Tobacco leaf transcriptomic analysis unveils differential effects of potassium chloride and potassium sulphate on formation of tobacco specific nitrosamines

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Tobacco specific nitrosamines (TSNA), such as N'-nitrosonornicotine (NNN), are carcinogenic metabolites accumulated in tobacco leaves during curing. Nornicotine is the major precursor of NNN. A recent study has shown that applying potassium chloride (KCl), a less expensive potassium alternative to potassium sulfate (K_2SO_4), as a potassium fertilizer significantly reduces TSNA levels of Burley and dark tobacco. However, the influences of KCl and K_2SO_4 on TSNA formation at the molecular levels were completely unknown. To gain mechanistic insights into the effects of KCl and K_2SO_4 on TSNA formation, we analyzed tobacco seedlings treated for three weeks with 100mM KCl or 50mM K_2SO_4. Metabolic analysis revealed that although KCl-treated samples accumulated more nicotine, nicotine-to-nornicotine conversion was much higher in K_2SO_4-treated plants than KCl-treated plants. To elucidate the underlying molecular mechanisms, we used RNA-sequencing to capture the transcriptomic landscapes of tobacco leaves under KCl or K_2SO_4 treatments. Differential gene expression (DEG) analysis showed that K_2SO_4 had profound impacts on gene expression compared to KCl. Upregulated DEGs in the K_2SO_4-treated sample are largely related to the tricarboxylic acid (TCA) cycle, amino acid metabolism, plant-pathogen interaction, stress-response, and protein-degradation, whereas downregulated DEGs are mostly associated with photosynthesis, nitrogen metabolism, and sulfur metabolism. Senescence plays an important role in nicotine-to-nornicotine conversion, and the nicotine demethylase gene *CYP82E4* (*E4*) is responsible for up to 98 % nicotine conversion in senescing leaves. The expression of senescence associated genes, including *E4*, is elevated in K_2SO_4-treated leaves, but repressed in KCl-treated leaves, suggesting that K_2SO_4 boosts nicotine conversion possibly by upregulating key conversion genes. We identified several transcriptional networks in both KCl and K_2SO_4 transcriptomes that may play roles in primary and secondary metabolic processes, opening the door for continuing research.

Notes

AP 34

Effect of the carbon nanoparticles on the tobacco growth by modulating the rhizosphere microbiome

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Carbon nanoparticles (CNPs) can potentially promote plant biomass and root growth by modulating rhizosphere microbial community. However, the mechanism between CNPs and rhizosphere microorganism remains largely elusive. The purpose of this study was to systematically explore the effects of CNPs on the diversity and structure of rhizosphere soil bacterial and fungal communities, as well as soil enzyme activities and nutrients. In this study, we applied 16S and ITS sequencing techniques to analyze the changes of bacterial and fungal communities for tobacco under CNPs (0.6 and 1.2 $g.L^{-1}$ by concentration) treatment at three different developmental stages. Our results demonstrated there were significant reductions in pH and available phosphorus contents after the treatment of CNPs, while the total nitrogen and total phosphorus were increased by CNPs application. Also, less sucrase, ß-1,4-glucosidase, polyphenoloxidase and acid phosphatase were observed in CNPs treatment soils. This study indicated that CNPs affected the diversity, composition and structure of the rhizosphere bacterial community. Fungal and bacterial communities had different response pattern for CNPs treatment, with phased and dose-effect effects. Compared with control, CNPs significantly increased the relative abundances of some potential beneficial bacteria, including *Burkholderia genus*, *Sphingomonas genus*, *Lactobacillus genus*. Furthermore, 278 culturable bacteria and 25 culturable fungi were isolated from soil and roots, and the strains enriched under CNPs treatment were tested for their ability to promote plant growth. Finally, five of them were validated for their microbial-mediated growth effects on tobacco. Metagenomic analysis revealed that CNPs increased functional diversity of root endosphere, with genes related to nutrient metabolism and plant hormone biosynthesis enriched after CNPs treatment. In general, our results have demonstrated the key role of rhizosphere microorganism in the interaction between CNPs and plants, and provide evidence and strategies for promoting tobacco growth with CNPs.

Notes

AP 35

Evaluation of the performance of shisha tobacco under Zimbabwean growing conditions

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Flue-cured, Burley, and Oriental tobacco varieties have historically been the most extensively produced in Zimbabwe. However, lately there has been interest among merchants in the development of other tobacco types, such as Shisha. Shisha is sweetened hookah tobacco that has Middle Eastern and some Asian origins. This type of tobacco use is on the rise all over the world and has the ability to give farmers access to additional sources of income and accelerate Zimbabwe's economic development. Shisha needs to be supported by deliberate variety selection and agronomic practices in the specified regions of production in order to be a profitable commodity. Thus, the main objective of this study was to evaluate the suitability of growing imported Shisha tobacco varieties under Zimbabwean environmental conditions. Two test Shisha varieties sourced from Europe, and another three locally developed varieties (exhibiting a Shisha profile), were used in the study. Three population densities were evaluated namely: 32000 plants.ha^{-1}, 25000 plants.ha^{-1} and 15000 plants.ha^{-1}. Two fertilizer regimes were applied i.e., Rate 1: 37.5 units of nitrogen (N) and Rate 2 (control treatment): was the standard quantity for flue-cured tobacco. A spilt split plot design with three factors was used i.e., main plot (fertilizer rate), sub-plot (population density), and the sub-sub-plot being the variety. Each factor was established in two-row plots over three blocks. In this study, imposing treatment effects (fertiliser, plant population densities and varieties) on the tobacco resulted in declines of the cured leaf yield and nicotine content. The higher number of leaves per plant (untopped) typically lead to relatively lower nicotine concentrations even at 15000 plants.ha^{-1}. The reducing sugar content of the test varieties were observed to be strictly constant even with increase in population densities. This paper will detail and analyze the results of each variety's performance in terms of leaf expansion metrics (largest and penultimate), yield (mass at untying, saleable), grade indices, and leaf chemistry (nicotine, reducing sugars, and total nitrogen).

Notes

AP 37

Capturing data electronically: modern problems require modern solutions

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Physical means of data collection and note taking can be laborious and costly. A modern approach to fieldnotes utilizes electronic data capturing technology. With a few simple tools and software, pre- and post-harvest measurements can be automatically entered into digital files. Electronic data capturing improves data quality and accuracy throughout the collection process, while increasing efficiency and reducing the number of personnel required per task. Recently, the NC State University Tobacco Agronomy and Breeding programs implemented digital platforms for large scale postharvest data collection. Through the integration of Microsoft Excel, WinWedge Software, basic laptops, barcoding systems, a multi-use printer, and Google Drive the labor requirements to handle ~21,000 hands of cured tobacco have been reduced by ~1,200 man hours. This accounts for an estimated cost savings that range from $USD 8,700 to 17,400 per growing season. In addition, the use of a tablet, Field Book software, and a wireless hotspot connection can further reduce the redundancy of data collection, input, organization, and processing. We estimate the time savings to range from a few hours to > 30 hours for each field trial, depending upon the parameters of interest, data collection frequency and methodology, as well as the number of field sites. The estimated payback time on this equipment ranges from one to three growing seasons. This presentation outlines the process we implemented in 2019, the material cost and training, and changes that continue to be implemented through 2023. These systems are adaptable to other research programs and may be of use to private industry for collecting GAP/STP information from growers.

Notes

AP 38

IPM and pest epidemics: *p*R = pr_Ie^{prI} and all that

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Commercial agriculture has removed many constraints to the success that pests and diseases would normally incur in a natural environment by providing large expanses of genetically identical plants in very close proximity. Man has been forced to develop a system of protecting these artificial plant communities with various forms of disease containment which can be improved with greater understanding of the biology of the plant and the pest, and the interaction between them and the effect of the environment on this interaction. The basic premise of an integrated pest management (IPM) program is to use a multi-faceted approach to break, or at least disrupt, the life cycle of a pest, or alter the microenvironment so that the pest population is unable to increase to a level at which it will cause an economic loss. The mechanics of IPM can be better appreciated by understanding plant fungal disease epidemics. An epidemic is comprised of the lag, the exponential and the decline phases. These can be mathematically modelled using parameters analogous to those of monetary saving account: an increase (interest) rate, the initial inoculum (principal) and the time period (term of investment). The value of each of these parameters varies widely depending on the lifecycle of the disease and the host plant environment. By understanding the biology of a disease and determining the most vulnerable stage of the life cycle, the most practical agronomic methods can be implemented by growers to slow the progress of an epidemic, and consequently its effect on yield and quality of the crop. These principles can also be loosely applied to the management of nematodes, insect pests and weeds.

Notes

AP 39

Diversity of *Fusarium* species infecting tobacco in Zimbabwe

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Tobacco (*Nicotiana tabacum*) production is severely hampered by many diseases including those caused by the ascomycete fungus *Fusarium*. A study was carried out to investigate the diversity of the *Fusarium* species infecting tobacco in Zimbabwe. Tobacco plants that displayed yellowing, wilting and root rot symptoms were collected during nationwide disease surveys in 2021-22 farming season. Symptomatic plant parts were surface-sterilized, blot-dried on filter paper and plated on PDA at 24 °C for five days. The colony colours, mycelia and spores that developed on PDA were viewed under the microscope. In greenhouse experiments, three-week-old tobacco seedlings were inoculated with fungal inoculum suspension (10^6 conidia/ml) and disease progress was measured by counting and recording the numbers of yellowing leaves, wilting and dead plants. DNA was extracted from each fungal isolate and sequenced targeting the internal transcribed spacer region. The consensus sequences that were generated for each isolate using BioEdit software were blasted into MEGA6.0 programme. The evolutionary history of the isolates was inferred using the maximum likelihood method. Morphological characterization revealed the presence of fusaria that produced white, creamy, grey and purple coloured colonies on PDA, and some rice-shaped spores. Greenhouse experiments confirmed the ability of the isolates to induce yellowing, wilting and root rot in the tobacco seedlings, though there were no significant differences ($p > 0.05$) in the amount of disease among the isolates. Four fusaria species namely *Fusarium falciforme*, *F. foetens*, *F. fujikuroi*, and *F. nygamai* were identified by molecular characterization. *Fusarium falciforme*, an emerging pathogen of legumes, cucurbits and solanaceous crops, was the most prevalent species with 80 % incidence. This study increases our knowledge on the diversity of *Fusarium* species infecting tobacco in Zimbabwe and provides a base for developing disease control tactics.

Notes

AP 40

Correlation of management practices and environmental conditions on incidence of angular leaf spot in dark fire-cured tobacco

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An observational study was conducted in 2020, 2021, and 2022 to evaluate possible correlations between grower management practices, environmental factors, and angular leaf spot (*Pseudomonas syringae* pv. *tabaci* tox-) incidence in dark tobacco fields in Kentucky and Tennessee, USA. Ninety fields were monitored in this three-year study (30 fields per year). Fields were located in seven counties in western Kentucky and northwestern Tennessee and ranged in size from three hectares to 32 hectares. All fields evaluated had some previous history of angular leaf spot incidence. Growers were not asked to implement new practices or change any current practices in their management program. Four fields were confirmed to have angular leaf spot in 2020, eight fields were confirmed in 2021, and four fields were confirmed in 2022. Correlations occurred between angular leaf spot incidence and levels of three nutrients found in plant tissue: phosphorus, boron, and copper; and levels of three nutrients found in soil: sulfur, manganese, and copper. An increased probability of angular leaf spot infection was also found to occur if the average temperature increases by only 1 °C. With one degree C increase in average air temperature, the chance of angular leaf spot being detected increased by approximately 47 %. There were also significant correlations between dark tobacco variety and angular leaf spot incidence. There were no significant correlations detected between transplant production practices, time of transplanting, tillage practices, rotation interval, or previous crop and angular leaf spot incidence.

Notes

AP 41

Transplant water application: investigations of in-furrow & sidedress systemic insecticide placement

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In the last decade, many commercial tobacco farmers in North Carolina have transitioned away from greenhouse tray drench applications of imidacloprid, opting instead to include the systemic insecticide in transplant water applications for early-season flea beetle (*Epitrix hiritipennis*) control and reduced stand losses associated with Tomato Spotted Wilt Virus (TSWV). More recently, producers have expressed interest in sidedress applications of transplant water solutions that would place imidacloprid approximately 5 cm away from seedlings. Research was initiated at two field sites in 2022 in order to compare the efficacy of traditional in-furrow placement versus that of the sidedress placement. At each site, four treatments were tested: 0.14 kg imidacloprid + 935 L water/ha (in-furrow), 0.29 kg imidacloprid + 935 L water/ha (in-furrow), 0.14 kg imidacloprid + 187 L water/ha (sidedress) + 935 L water/ha (in-furrow), and 0.29 kg imidacloprid + 187 L water/ha (sidedress) + 935 L water/ha (in-furrow). A non-treated control (935 L water/ha) was included for comparison. Flea beetle herbivory was consistently reduced by in-furrow treatments of imidacloprid (89 to 94 %), regardless of application rate. In contrast, the sidedress placement of imidacloprid did not reduce herbivory relative to the non-treated control, nor did any treatment reduce stand losses associated with TSWV. Imidacloprid residues in green tissue collected from two to four weeks after transplanting were consistently highest following in-furrow placement of the 0.29 kg ai/ha application rate. The lowest residue measurements were recorded in sidedress treatments. Preliminary results indicate that the sidedress placement of imidacloprid is unlikely to be as effective as in-furrow placement relative to flea beetle herbivory, and that neither placement strategy may help reduce the stand losses associated with TSWV. Field trail results from 2023 will be included in this presentation.

Notes

AP 42

Flue-cured tobacco response to sub-lethal doses of HPPD-inhibiting herbicides

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Herbicide-tolerant (HT) crops that are genetically engineered to withstand applications of multiple herbicidal modes of action are commonly produced in fields adjacent to flue-cured tobacco. The next generation of HT crops will express tolerance to a class of herbicides known as 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors. The impact of HPPD-inhibiting herbicide exposure to flue-cured tobacco foliage has not been reported. Research was initiated at two field sites in 2022 to measure the effects of isoxaflutole and mesotrione to cured leaf yield, quality, and value. Within each active ingredient, five sub-lethal concentrations (25, 5, 1, 0.2, and 0.1 % v/v of the full rate) were applied at two different times (five and 10 weeks after transplanting). A non-treated control was included for comparison. Visual injury was greatest when exposure occurred five weeks after transplanting, and was not apparent at the later exposure time. Within the early exposure timing, visual injury declined with sub-lethal dose, although mesotrione was generally more injurious than isoxaflutole when pairwise comparisons were made between common doses. Moreover, visual injury was difficult to identify when sub-lethal dose concentrations were less than one percent. Finally, cured leaf yield was impacted by the interaction of sub-lethal dose concentration and exposure timing, with the 5 and 25 % v/v concentrations at five weeks after transplanting yielding significantly lower than the 0.1 and 0.2 % v/v concentrations at 10 weeks after transplanting. The decline in yield translated into a decline in crop value that followed an identical trend. Cured leaf visual quality was not impacted. Our results suggest that HPPD-inhibiting herbicides pose a threat to flue-cured tobacco when exposure occurs early in the growth cycle and when the concentration exceeds 5 % v/v.

Notes

AP 43

Assessment of Romex 22.15 SC (Lambda-cyhalothrin 9.5 % and Thiamethoxam 12.6 %) efficacy for pest control in tobacco crop

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Tobacco (*Nicotiana tabacum* L.) is a major cash crop in Sub-Saharan Africa that contributes to the growing countries' economies. However, insect pests affect the quality and production of tobacco leaf yield. Confidor 70WG (Imidacloprid) is a systemic insecticide that has been used for over a decade for controlling pests in Tanzania and is widely known to have ecotoxicological effects on non-target species. Some farmers apply imidacloprid excessively, which may result in excessive residues in soils and tobacco leaves. The objective of this study was to assess the efficacy of the new product Romex 22.15 SC (Lambda-cyhalothrin 9.5 % and Thiamethoxam 12.6 %), as well as determining their residual levels both in tobacco leaf and soils at Tumbi, Tabora; Mtanila, Chunya, Ulowa, and Kahama. The randomized completely block design (RCBD) was used in the study with three replications and four treatments, the absolute control (no agrochemical-T1), the T2 treated seedlings at the nursery with 15ml Romex 22.15 SC/10 L of water before transplanting in plots, (T3) treated seedlings with Romex 15ml 22.15 SC/10 L of water in field three weeks after seedlings transplanting and compared with the (T4) control treatment Confidor, 10 g/10 L of water. Results showed that Romex 22.15 SC at 15 ml in 10 litres of water applied in the field significantly decreased ($P < 0.001$) levels of pests compared to the control treatment (Confidor 70WG) at 10g in 10 L of water. Furthermore, it was revealed that the combination of two active ingredients, Lambda-cyhalothrin and Thiamethoxam for Romex 22.15 SC contributed significantly to the control of bollworms (*Helicoverpa armigera*) compared to the standard Confidor. Therefore, the preliminary study showed promising efficacy results for the Romex 22.15 SC on pest control in the tobacco crop.

Notes

AP 44

Field evaluation of fungicides for management of frogeye leaf spot caused by *Cercospora nicotianae* on Burley tobacco

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Frogeye leaf spot (FELS) caused by *Cercospora nicotianae* has been increasing in severity in recent years in tobacco grown in Kentucky. Current management practices include the use of fungicides such as azoxystrobin since 2005, but within the last ten years there have been several reports of moderate to high resistance to this fungicide. In order to minimize the spread of fungicide resistance, it is recommended that growers manage these kinds of fungal diseases with alternating sprays of fungicides that have different modes of action. The objective of this field trial was to evaluate experimental chemicals and mixes, and compare their control of FELS with fungicides currently registered for tobacco such as azoxystrobin and mancozeb. Foliar sprays were done every other week and three disease evaluations were completed in a grower's field planted with tobacco KT215 in 2022 with a history of FELS. All chemical sprays significantly reduced the average percentage of FELS (4-6 %) on lower leaves, when compared to the average of FELS in the non-treated disease check (19.4 %). None of the treatments were significantly different from each other in terms of weight of cured leaves for yield. The experimental chemicals and mixes reduced disease severity at very similar levels to the two already recommended fungicides, and with their different mode of action have the potential to be registered and included for control of FELS in tobacco. These experimental chemicals will be tested again during the 2023 season in the same field.

Notes

AP 45

Fungicide evaluations for foliar leaf spot disease management

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Foliar leaf spot diseases such as *Rhizoctonia solani* and *Cercospora nicotianae* have increased in severity over the last decade in North Carolina. A recent survey of County Agents suggests that yield losses from *R. solani* may exceed six percent across the state and 30 % in isolated situations. Alternatives to azoxystrobin fungicides are needed to address this growing issue. In 2022, field trials were established to screen seven foliar fungicide products that are new or not currently labelled for commercial tobacco production. *Pseudomonas chlororaphis*, *bacillius subtillis* strain AFS032321, inpyrfluxam, pydiflumetofen + difenoconazole, picoxystrobin + cyproconazole, azoxystrobin + flutriafol, azoxystrobin + fluindapyr + flutriafol were evaluated. Azoxystrobin and mancozeb were included for comparison. Treatments were applied in sequential applications four, six, and eight weeks after transplanting. Foliar leaf spot pressure was low in 2022; however, preliminary results indicate that products such as difenoconazole + pydiflumetofen, mancozeb, inpyrfluxam, and azoxystrobin + flutriafol may help manage foliar leaf spot disease. However, visual chemical injury (flecking) was commonly observed within treatments comprised of strobilurin products (FRAC Group 11) but it did not exceed 11 % and declined with tobacco growth. Relative to the non-treated control, difenoconazole + pydiflumetofen, azoxystrobin + flutriafol, and azoxystrobin + flutriafol + fluindapyr improved cured leaf yield by 275 to 447 kg/ha at one research site. Results from the 2023 field season will be included in this presentation.

Notes

AP 46

Supply chain due diligence for compliance and sustainability of the tobacco industry in Malawi

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The tobacco sector in Malawi, prior to 2012, had one system of producing and selling, where growers produced the crop on their own and sold it on auction. However, over the years there have been numerous concerns in the tobacco supply chain ranging from human rights violations to environmental degradation. This necessitated the need for a mandatory human rights and environmental due diligence for market players. The need for Supply Chain Due Diligence (SCDD) led to the introduction of the Integrated Tobacco Production System (IPS) to ensure traceability, compliance and sustainability of the tobacco.

In view of the foregoing, about 80 % of the tobacco crop regarded as compliant is produced under IPS while the remaining 20 %, regarded as non-compliant, is produced independent of any agreement and supervision. To achieve total compliance, in 2020, Agricultural Research and Extension Trust (ARET) developed a SCDD framework for ensuring that independent growers are complying to Agricultural Labour Practices (ALP) and Sustainable Tobacco Production (STP) practices.

The key pillars of the framework are: grower traceability, training, data management, monitoring and evaluation (M&E) and reporting. To achieve this, ARET has identified 4,374 independent growers, collected their profiles, mobilised them into 246 contact groups and geo-tagged the groups. In addition, certified tobacco seed sales have been digitized to ensure seed traceability. ARET is providing trainings on STP and ALP through the groups. Data on groups, grower profile and participation in trainings is collected digitally and uploaded into an ARET server ready to be made available to any industry stakeholder. The results from the first season of the initiative evince that independent growers are complying to GAP and STP practices. This initiative, if well harnessed, will assist in making Malawi tobacco compliant for the sustainability of the sector.

Notes

AP 47

Farmer Advantage Card for improving growers' livelihood in Turkey

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Turkey is an important tobacco leaf sourcing country of Oriental leaf. In Turkey, the major Turkish tobacco suppliers, represented by Aegean Tobacco Exporters Association (ATEA), continuously collaborate in non-competitive efforts. In 2021, three supply chain due diligence (SCDD) risk challenges (child labour, working conditions, and occupational health & safety) were identified by Twenty-Fifth Ltd consultancy and confirmed by ATEA's Sustainability Committee as priorities for broader industry-level collaboration.

The Farmer Advantage Card, which consists in setting up an online platform / physical card distribution by a third-party company, includes exclusive discounts for improving growers' livelihoods is one of the projects identified under the working conditions topic.

The objective of the project is to decrease grower's production and living costs and to increase their profitability with the ultimate goal of ensuring a sustainable tobacco production.

Thanks to the SCDD collaborative approach at our country level, six leaf companies dedicated themselves to this project and an online platform was hired from a third party company and all exclusive discounts displayed. In addition, physical discount cards were distributed to the growers and many partner stores agreed to provide additional discounts to the growers who show their Farmer Advantage Card.

For effective communication, ATs were trained regarding the details of this project and, in order to produce a better understanding in the field, brochures were distributed to all growers and posters were hung in crowded places such as village cafes, governmental institutions, etc.

Our objective is to deploy this project to 100 % of our grower base (47.000 growers) with an involvement expectation of 10-15 %.

Growers will have exclusive discounts from 5 %-20 % for their production and living expenses such as fuel oil, fertilizer, education, medical, CPA, grocery, white-goods and home appliances, tyres, etc.

As a result, thanks to the Farmer Advantage Card, growers will receive an approx. 10 % discount on their farming and living expenses as their expenses will be reduced by approx. 10 %. This will have a positive impact on growers' household savings and on their livelihood. They will then increase their profitability and gain an economic advantage that will help to tackle poverty and ensure a more sustainable tobacco production.

Notes

AP 49

Evaluation of the potential of greenhouse gas emissions in tobacco producing properties in the southern region of Brazil

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The agricultural life cycle assessment is a tool for quantitatively analyzing and evaluating materials production and operations in different stages of the agricultural process. Agriculture's environmental impact is comprehensively summarized using the life cycle concept. The work aimed to evaluate the potential for emission of greenhouse gases in three properties in the South region of different locations and profiles. To this end, the particularities of each property were evaluated *in loco* concerning the area occupation and management. The area occupation at the properties was quantitatively measured (Google Earth Pro) and the assessment of greenhouse gas emissions was carried out using the SimaPro Life Cycle Analysis software, using the Recipe Midpoint 1.03 method. Rating the variation in south Brazilian tobacco growing properties, the three study sites present values of tobacco planting of 12 %, 12 % and 55 %, native forest of 22 %, 44 % and 74 %, and corn crops of 1 %, 6 % and 18 % concomitantly with the tobacco crop or in the off-season. As well as the profile of properties, the potential emission of equivalent greenhouse gases differed, showing values (input) of 9.000, 14.900 and 17.600 Kg $CO_{2\text{-eq}}$. The hotspots were represented by corn cultivation in two properties, with average values of 4,600 Kg $CO_{2\text{-eq}}$ /ha, and the planting and drying of tobacco with average emission of 1.189 Kg $CO_{2\text{-eq}}$ /ha and 1050 Kg $CO_{2\text{-eq}}$ /ha, respectively. The reduction (output) by capture of CO_2 by the tobacco plants (leaf with stems) reached amounts of 26.57 %, 12.75 %, and 52 % of kg $CO_{2\text{-eq}}$ in each property, respectively. Through the identification of hotspots based on the data obtained by the life cycle assessment, it will be possible to create strategies to mitigate emissions, increasing the environmental and economic sustainability of tobacco-producing properties in the southern region of Brazil.

Notes

AP 50

Resistance risk and resistance mechanism of *Ralstonia solanacearum* to SYP-14288

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Tobacco bacterial wilt is a destructive soil-borne vascular disease in tobacco production, and it cannot be effectively controlled by bactericides. SYP-14288 is a typical uncoupler with a broad spectrum, but the action site of SYP-14288 is unknown. The objective of this study was to assess resistance risk and clarify resistance mechanism of *Ralstonia solanacearum* to SYP-14288. In this study, the inhibition spectrum of SYP-14288 showed that it had a good inhibitory effect on 10 plant pathogenic bacteria. The sensitive baseline of tobacco bacterial wilt to SYP-14288 was established, and the average EC_{50} values were 0.0378 μg/ml. Sixteen resistant mutants were obtained using SYP-14288-amended media method, which can be divided into low resistance, medium resistance and high resistance levels. Based on the survival suitability of sensitive isolates and resistance mutants, the resistance risk of tobacco bacterial wilt to SYP-14288 could be moderate. Through the joint analysis of transcriptome and proteome, the key metabolic pathways and main differential genes were cleared. The drug efflux mediated by ABC transporter was one of the main reasons for the resistance of tobacco bacterial wilt to SYP-14288. This study could provide a technical support for the rational use of SYP-14288 and delaying the development of resistance in fields.

Notes

AP 51

Eukaryotic elongation factor (*eIF4*) targeted sequence analysis of PVY tolerant Kutsaga accession to determine nucleotide responsible for PVY resistance and CRISPR/Cas9 mutagenesis of a Kutsaga line for PVY resistance

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Potato virus Y (PVY) is a destructive pathogen in tobacco with the potential to cause 100 % loss of affected plants if the disease sets in early in the crop's growth. To prevent virus spread in tobacco, affected plants are usually rouged off as no control methods are available once the virus infection occurs. Genetic resistance has been successfully used for the management of this problem in a wide range of crops. Studies have shown that the VPg protein sub-unit of PVY genome interacts with the host's protein synthesis complex (translation initiation factor *eIF4E*), which works as a susceptibility factor to facilitate infection. In this study, we aimed at inducing PVY resistance by disrupting the interaction between VPg and the *eIF4E* gene using CRISPR-Cas9 in tobacco. The *eIF4E* gene in the tobacco parental named *ONC* was mutated by introducing a knockout on the gene using CRISPR-Cas9. Plants harbouring the desired mutation were tracked using Sanger sequencing using specific primers targeting the *eIF4E* gene. A comparison of the wild-type and the mutated *ONC* was carried out using CLUSTALW pairwise alignment. Results indicated significant nucleotide differences of about 45 % between the wild type and the mutated plants. Furthermore, the mutated plants exhibited increased resistance to PVY after disease assessments. The T_0 seeds were grown further to T_1 and T_2 seeds to observe the presence of the Cas9 enzyme and segregation of the transgene. In conclusion, we observed that CRISPR-Cas9 gene mutation of the *eIF4E* could be a cost and time effective strategy of introducing PVY resistance in tobacco, and thus, it can aid in the breeding of PVY-resistant tobacco.

Notes

AP 52

PIF1, a phytochrome-interacting factor, negatively regulates drought tolerance and carotenoids biosynthesis in tobacco

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The phytochrome-interacting factors (PIFs) function crucially in multiple physiological processes, but the biological functions of some PIFs remain elusive in some species. Here, a tobacco (*Nicotiana tabacum* L.) PIF transcription factor *NtPIF1* was cloned and characterized. The transcript of *NtPIF1* was significantly induced by drought stress treatments, and it localized in the nuclear. Knockout of *NtPIF1* by CRISPR/Cas9 system led to the improved drought tolerance of tobacco with increased osmotic adjustment, antioxidant activity, photosynthetic efficiency and decreased water loss rate. On the contrary, *NtPIF1*-overexpression plants display drought-sensitive phenotypes. Moreover, *NtPIF1* reduced carotenoids biosynthesis and regulated the expression of carotenogenic genes upon drought stress. Electrophoretic mobility shift and dual-luciferase assays illustrated that *NtPIF1* directly binds to the promoter of the carotenogenic gene *Ntß-LCY* to repress its transcription. Overall, these data suggested that *NtPIF1* negatively regulates the tobacco adaptive response to drought stress and carotenoids biosynthesis; moreover, *NtPIF1* has the potential to develop drought-tolerant tobacco plants using CRISPR/Cas9 system.

Notes

AP 55

Developing nitrogen and potassium fertilizer rate recommendations for cigar wrapper tobacco in North Carolina

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Cigar wrapper tobacco is a new crop to North Carolina. In the United States, it is traditionally grown in Pennsylvania and the Connecticut River Valley area; therefore, local nutrient application recommendations are not available to producers in the mid-Atlantic region. This project aims to provide research-based nitrogen and K_2O fertilizer rate recommendations that maximize yield and quality potential without being environmentally or economically detrimental. From 2021 to 2022, increasing nitrogen rates (0 to 448 kg N/ha) and potassium rates (0 to 336 kg K_2O /ha) were applied in two separate but co-located trials at seven locations. Growth and nutrient data were collected throughout the growing season, and yield and quality data were collected after the tobacco was harvested and cured. Across all growing environments, a linear plateau model indicated that leaf yield was maximized at 198 kg N/ha. Cured leaf from plots that did not receive supplemental N up to 90 kg/ha were not usable, as they tended to scald during wilting or developed mold infestations while curing. These observations were specific to the two lowest N treatments and were consistent across sites. A slight linear increase in yield was documented as K_2O application increased to 336 kg/ha. The yield gain was small and generally had little effect on grade distribution, although wrapper grades and K_2O rate were positively correlated in a sandy loam soil type in 2021. Based on preliminary results, it is plausible that appropriate N application rates may range between 175 and 200 kg N/ha and that K_2O rates may range between 75 and 100 kg/ha, which are essentially maintenance application rates. Additional testing will be completed in 2023 for confirmation.

Notes

AP 56

Agrochemical programs for cigar wrapper tobacco: impacts on cured leaf residues

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In order to support the creation of Cigar-Guidance Residue Limits for the CORESTA Guide No. 21, field research was conducted in North Carolina from 2019-2021. Across the three growing environments, PA-41 (Grower's Choice or Welk's Pride) was produced using best local agronomic practices and treated with one of two agrochemical spray programs, which varied slightly across seasons. Research plots were harvested by stalk-cutting and priming. The primary goal of this research was to generate cured leaf samples for agrochemical residue quantification. In total, 21 different agrochemical active ingredients were applied at various crop stages and in accordance with federal guidelines outlined on each product label and with increasing carrier volume to account for plant size. After curing, leaf samples were separated into lower, middle, and upper stalk positions for residue analysis. Across all growing seasons residues of acephate, bifenthrin, pendimethalin, sulfentrazone, and thiamethoxam were not detected, most likely due to pre-transplanting and/or early-season applications. Of the remaining active ingredients, only clomazone and maleic hydrazide residues would have exceeded C-GRLs. Importantly, acetamiprid, azoxystrobin, chlorantraniliprole cyantraniliprole, dimethomorph, dithiocarbamates (mancozeb), flumetralin, fluopicolide, imidacloprid, indoxacarb, mandipropamide, metalaxyl, oxathiapiprolin, and spinosad residues were well below the C-GRLs found in Guide No. 21. Our results suggest that strategic application and rotation of agrochemicals can mitigate CPA residues in cigar tobacco production. In addition, adjustments in treatment protocols or adjustment of the application pattern relative to clomazone and maleic hydrazide should alleviate residues concerns for those products.

Notes

AP 57

Effects of cooking rice water on chemical composition and aroma quality of fermented cigar tobacco leaves

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Cooking rice fermentation is a unique and traditional method of tobacco leaf fermentation in Shifang, Sichuan. However, the characteristic of cooking rice fermentation is based solely on experience, and the mechanism is still unclear. Field trials were conducted to explore the influence of the characteristic cooking rice fermentation on the quality of cigar tobacco leaves. The cigar varieties Shiyan No.1 and Dexue No.1 were used as fermentation materials. Based on the different cooking time (7, 10 and 13 min), three different rice cooking degrees were set up for experiment. With the water fermentation as the control group, the changes of conventional chemical components, neutral aroma components and sensory quality of cigar tobacco leaves during fermentation were analyzed. The results showed that the content of total sugar, reducing sugar and starch in tobacco leaves increased significantly after being treated with cooking rice water, and the contents of total sugar, reducing sugar, starch, protein, total nitrogen and nicotine gradually decreased during the fermentation process. The content of total sugar, reducing sugar, starch, total nitrogen and nicotine in the tobacco leaves of the control group decreased slowly during the fermentation process. Compared with the pre-fermentation and control groups, most of the aroma components increased significantly after cooking rice fermentation, while the neophytadiene content significantly decreased; the sensory quality of tobacco leaves after cooking rice fermentation was significantly improved, mainly reflected in the improvement of aroma quality, the increase of sweetness, purity and mildness degree, the reduction of irritation, the weakening of strength. These findings suggest that the more suitable degree of cooking rice for Shiyan No. 1 and Dexue No. 1 is to cook the rice into a black mass (10 min), until the core of the hand-twisted rice is still white.

Notes

AP 58

Effects of aging with characteristic media on the sensory quality, chemical composition and microbial community of cigar

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Aging is an important process to improve the quality of cigar, but the effect of aging with characteristic media on cigar has not been reported. Therefore, this study aimed to prepare and obtain the aging media that could improve the aroma and quality of cigar, and to clarify the influences of media on cigar. Effective media were firstly screened by sensory quality evaluation, then the effects of aging media on chemical compositions and microbial communities of cigars were investigated. The results showed that: (1) As aging media, coffee formula and cocoa formula could improve the smoke plumpness, as well as increase the burnt-sweet aroma, roasted aroma and nut aroma of cigar. Thus, the relatively richer and softer aroma and higher sensory quality could be found in cigars aged with media. Besides, thirty days was the optimal time for cigar aging. (2) Aging with coffee and cocoa media had no significant effect on the starch content of the cigar, but increased the content of amino acids, non-volatile organic acids (49.60 % and 15.84 %, respectively), malic acid and aromatic components. Particularly, the aromatic components were increased from 2.48 $mg.g^{-1}$ (W-30) to 3.21 $mg.g^{-1}$ (C-30) and 3.70 $mg.g^{-1}$ (K-30), respectively. The content of saturated fatty acids was significantly increased with the addition of coffee medium, and starch content showed a decreasing trend with aging proceed. (3) Aging with coffee can improve the diversity of bacteria and fungi on the cigar surface and change the succession rule of the bacterial community. In contrast, aging with cocoa had no significant effect on microbial diversity of cigars. In this study, the influence of aging medium on cigar quality was analyzed multidimensionally for the first time, which provided a reference for the development of new aging media and technologies of enhancing the quality of cigars.

Notes

AP 59

Identification and application evaluation of functional microorganisms in cigar fermentation

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Fermentation is an important process for improving the quality of cigar tobacco leaves, with the main goal of enhancing the flavour richness while reducing irritancy. The purpose of this study was to explore the functional microorganisms in cigar fermentation and evaluate their fermentation performance, thereby improving the quality of cigar tobacco leaves. First, functional microorganisms were predicted by correlation analysis, and pure-culture was adopted to directionally isolate the functional microorganisms. Then, the isolated strains were evaluated and screened based on fermentation indexes. Finally, the fermentation performance of preferred functional microorganisms was verified by bioaugmentation. Test data show: 1) Correlation analysis revealed that *Candida* had the functional potential to degrade nitrogen-containing substances and synthesize flavour substances. 2) By directional isolation, five strains of *C. parapsilosis* and four strains of *C. metapsilosis* were obtained. 3) By comparing the fermentation performance of nine strains of *Candida* , it was found that *C. parapsilosis* P1 and *C. metapsilosis* M4 not only reduced the alkaloid content (by 25.3 % and 32.6 %, respectively) but also increased the content of flavour components (by 25.2 % and 20.6 %, respectively). 4) On the 21st day of fermentation, group P1 and M4 presented the highest content of flavour components and lower alkaloid content, which could enhance the flavour richness and reduce the irritancy. Among them, P1 could elevate the content of chlorophyll and carotenoid degradation products, enhance the bean and nutty flavour. M4 could increase the content of chlorophyll, carotenoid and cembranoids degradation products, and Maillard reaction products, improve the baking, nutty, cocoa and honey flavour. In this study, the functions of *Candida* strains in cigar fermentation were predicted and verified, and two functional strains suitable for cigar fermentation were screened, which would help guide the development of microbial starters and the directed regulation of cigar quality.

Notes

AP 60

Low nicotine flue-cured tobacco: influences of cultivar selection and agronomic management

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The reintroduction of proposed standards by the US-FDA warrants continued investigations of selected tobacco cultivars and management strategies that may reduce concentrations of nicotine and other metabolites in cured leaves. To quantify these effects in flue-cured tobacco, field research trials were conducted in 2021 and 2022 in North Carolina. The cultivars were selected based on conventional nicotine concentration (K326 and NC196) and low nicotine concentration (NCLA926 and ITB697). Each cultivar was produced using local best management (LBM) practices (14,820 plants/ha, 84 kg N/ha, and topped at the early flower stage) and reduced nicotine management (RNM) practices (20,748 plants/ha, 62 kg N/ha, and not topped) in accordance with the LNTP Task Force research protocol. The main effect of cultivar was significant for yield and value, with K326 and NC196 outperforming NCLA926 and ITB697. The main effect of management program was likewise significant for the same parameters. The LBM program produced a higher yield (+1,432 kg/ha) and value (+$US 5,871/ha) than the RNM program. In contrast, cured leaf visual quality and nicotine concentration were influenced by the interaction of cultivar and management program. Visual quality was lowest in NCLA926 in the RNM program (55) but was improved when NCLA926 was grown using LBM practices (72). Visual quality was significantly better in leaves sourced from other cultivars in both management programs. Not surprisingly, nicotine concentration was highest in NC196 (27.1 mg/g) and K326 (24.9 mg/g) produced using LBM practices. RNM practices reduced nicotine in NC196 (18.1 mg/g) and K326 (18.6 mg/g), though not enough to meet the proposed FDA standards. Similar trends in nicotine concentration were documented from ITB697. The lowest recorded nicotine concentration was in NCLA926, which was similar between management practice (2.11 and 2.62 mg/g) and is approximately 10-fold greater than the proposed FDA nicotine standards. Ultimately, agronomic performance and cured leaf factors continue to be negatively affected by proposed low nicotine management practices and low nicotine cultivars compared to conventional cultivars and production practices. Moreover, the outlined RNM practices appear to have no effect on nicotine accumulation when paired with low nicotine cultivars.

Notes

AP 61

Effect of low-nicotine tobacco / eggplant grafting on nicotine reduction and origin of low nicotine in grafted tobacco leaves

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Reducing nicotine content of tobacco leaves to a level below the threshold of addiction is a strategy proposed by the World Health Organization to reduce smoker's dependence on tobacco. However, the current low-nicotine varieties developed alone are not sufficient to meet the required standard. In this experiment, the combination of low-nicotine varieties and grafting method was employed to investigate the effect of low-nicotine tobacco / eggplant grafting on nicotine content of tobacco leaves, and the origin of small amount of nicotine produced and accumulated in grafted tobacco was explored by PCR analysis on materials from the stem close to grafting surface. The results showed that the nicotine content in the middle leaves of flue-cured tobacco of low-nicotine variety NC926 and domestic low-nicotine variety CD-01 was 0.19 % and 0.17 %, respectively. While the nicotine contents of tobacco from NC926 / eggplant grafting and from CD-01 / eggplant grafting were further reduced to 0.046 % and 0.036 %, respectively, which were close to or below the proposed nicotine limit. Compared with normal high nicotine tobacco, low nicotine tobacco had similar sugar and nitrogen contents and aroma quality, while the strength was significantly decreased. It was assumed that the trace amount nicotine in grafted tobacco was produced from the stems above the grafting surface, which was verified in this study. PCR analysis results from stems close to grafting surface showed that the expression of key nicotine biosynthesis genes of *PMT*, *BBLs*, *A622* were significantly up-regulated in grafted tobacco stems compared with non-grafted tobacco stems, indicating that grafting was able to stimulate nicotine biosynthesis in stems close to grafting surface. We also found that the expression levels of these genes varied with varieties, with the expression levels being much higher in normal high nicotine variety than that in low nicotine varieties. The conclusion is that the combination of low nicotine variety and grafting with eggplant was an effective approach to further reduce nicotine content in flue-cured tobacco. The stems close to the grafting surface were able to compensate to some extent for nicotine biosynthesis.

Notes

AP 62

Understanding the molecular mechanism underlying the poor leaf quality of the low alkaloid tobacco varieties

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The low nicotine (LA) phenotypes of Burley tobacco are associated with lower yields, delayed ripening, and senescence resulting in poor cured leaf quality and higher susceptibility to insect herbivory compared to the high alkaloid (HA) variety. Nicotine comprises a pyridine and a pyrrolidine ring, each of which is generated via two independent primary metabolic pathways, the NAD and polyamine metabolic pathways, respectively. The homologous ETHYLENE RESPONSE FACTOR (ERF) transcription factors ERF199 and ERF189 belong to two independent loci, NIC1, and NIC2, which influence the nicotine accumulation in the plant by orchestrating the transcription of multiple nicotine biosynthesis-related genes. Primary metabolites are essential for the growth and development of plants, whereas specialized metabolites mediate plant–environment interactions. In tobacco, it is reported that the part of the NAD pathway is co-ordinately regulated with nicotine biosynthesis. NAD is an ubiquitous coenzyme in oxidation-reduction reactions essential for the growth and development of plants. Putrescine is one of the main polyamines (PAs) produced in plants. They are ubiquitous DNA- and RNA-binding organic cations and are involved in the regulation of diverse physiological processes such as flowering, embryogenesis, organogenesis, senescence, and fruit maturation and development. Plants maintain a precise balance in the metabolite pool for normal growth and development. Thus, it is assumed that the disturbance in NAD or PA homeostasis in low-nicotine tobacco plants resulted in pleiotropic effects in the cellular developmental aspects. We employed the RNA-sequencing technique to compare the global expression pattern of the genes in HA and LA leaves. Our data suggest that cell expansion-associated gene/regulators are downregulated, possibly resulting in high cell density and small cell size in the LA leaf. Also, the downregulation of auxin-responsive factors may contribute to the delayed senescence of LA leaves.

Notes

AP 63

Development and evaluation of newly-generated ultra-low nicotine tobacco lines and hybrids

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The potential for mandated lowering of cigarette nicotine levels by various regulatory agencies has caused increased interest in development of tobacco cultivars with lower potential for accumulation of this alkaloid. Suggested threshold levels of tolerance have been in the vicinity of 0.4 mg/g, or below. Few, if any, tobacco cultivars currently exist that would *routinely* meet this ultra-low nicotine content when grown under conventional agronomic management, however. We have produced and evaluated a series of new non-GMO and non-gene edited genetic combinations for alkaloid accumulation in different field experiments. A number of these materials accumulate nicotine at levels far lower than previously described genotypes. Data suggest that some new genotypes could routinely produce cured leaf with nicotine levels (averaged over all stalk positions) below 0.4 mg/g. Corresponding changes in yield and quality characteristics will be reported.

Notes

CORESTA AP2023 CONFERENCE

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

ABSTRACTS

POSTER PRESENTATIONS

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

IGPOST 01

CORESTA strategy, cooperation and achievements

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The mission of CORESTA is to promote and facilitate international cooperation and best practices in scientific research relative to tobacco and its derived products. Solidly based governance rules are defined and published to optimally fulfil this mission, and responsibilities are delegated by a General Assembly to a Board and a Scientific Commission.

The vision of CORESTA is to be recognised as an authoritative source of publicly available, credible science and best practices. In 2023, hundreds of scientists from 168 organisations and 43 countries are cooperating in working groups to develop consensual methods, tools and guidelines, and to conduct collaborative studies.

A Strategy House composed of four strategic areas, eight strategic subjects and 16 workstreams has been implemented with the objective to efficiently identify and drive key activities. A circular process of cooperation has also been deployed to prioritize, monitor and report progress on projects, to debate and align contributors' views, and to inform stakeholders. Such a framework leads to the production and publication of an impressive number of quality materials on a wide range of topics.

This poster will describe and illustrate the Strategy House, the collaborative framework, the 5-year plan and 2-year expected deliverables, and the main achievements over the last three years.

Notes

APPOST 02

Mancozeb application in tobacco: a revival of an older chemistry?

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In North Carolina, foliar leaf spot diseases, such as *Rhizoctonia solani* and *Alternaria alternata*, have increased in severity in recent growing seasons. Historically, azoxystrobin has provided sufficient control of *R. solani*; however, commercial farmers have reported failures since 2018. Moreover, nothing at present is federally approved for commercial application that would offer suppression of *A. alternata*. Research was initiated in 2022 to evaluate the efficacy of mancozeb for the suppression of these diseases and to quantify cured leaf dithiocarbamate residues. Across research sites, mancozeb was applied three times (four, six, and eight weeks after transplanting), each at 2.24 kg/ha (1.68 kg ai/ha). A comparison treatment of azoxystrobin was applied at the same intervals with each application delivering 0.58, 0.66, and 0.66 L/ha (0.14, 0.16, and 0.16 kg ai/ha, respectively). Foliar leaf spot pressure was low in 2022 due to above average temperature and below average precipitation. However, in reference to *A. alternata*, the number of leaf spot lesions was reduced by applications of azoxystrobin and mancozeb in lower stalk leaves at one research site. At the same research site, mancozeb significantly reduced *A. alternata* severity (3.3 %) relative to the non-treated control (5.4 %). Yield was not affected by treatments. Cured leaf samples obtained from separate evaluations with identical treatment protocols suggest the mancozeb residues are likely to fall below the limit of quantification (1.0 mg/kg) and should not exceed the CORESTA GRL (5.0 mg/kg). Preliminary data suggests that mancozeb could be a useful fungicide to use in rotation with azoxystrobin and that cured leaf residues can be managed with early to mid-season product applications. Additional research will be conducted in 2023 and results will be included in this presentation.

Notes

APPOST 03

Flutriafol residues in non-traditional Burley tobacco

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Flutriafol is a fungicide that is not currently labelled for tobacco production, which may offer suppression of common foliar leaf spot diseases such as target spot (*Rhizoctonia solani*) and frogeye leaf spot (*Cercospora nicotianae*). In spite of such promise for disease suppression, the cured leaf residues resulting from flutriafol application in Burley tobacco have not been reported. Field trials that serve to quantify agrochemical residues for two experimental fungicides applied to field grown Burley tobacco were conducted in North Carolina from 2019 through 2021. Across these growing seasons, Burley tobacco treated with flutriafol (Topguard® at 1.02 L/ha/application; 128 g ai/ha/application) or a premix combination of azoxystrobin + flutriafol (Topguard® EQ at 0.58 L/ha; 173 g azoxystobin + 128 g flutriafol/ha/application) was evaluated in one growing environment within each season. Both agrochemicals were applied twice, once at layby (36-49 days after transplanting) and again 30 days before harvest. Residue results varied across growing seasons. In 2019, flutriafol residues from Topguard® were highest in lower stalk samples (9.73 mg/kg) but declined in middle and upper stalk samples (5.75 and 4.85 mg/kg, respectively). In contrast, flutriafol residues were similar between lower and middle stalk samples in 2020 (1.63 and 1.50 mg/kg, respectively) and 2021 (1.40 and 1.17 mg/kg, respectively). Within the same two seasons, flutriafol residues were lowest in upper stalk samples (1.13 and 0.53 mg/kg, respectively). When Topguard® EQ was applied, flutriafol residues were similar across all stalk positions in 2019 but generally demonstrated a reduction from lower to upper stalk positions in 2020 and 2021. Azoxystrobin residues were similar across stalk positions and were consistently less than 2.5 mg/kg. Our results provide cured leaf residue guidance relative to these fungicides, should they be approved for application in the commercial production of Burley tobacco.

Notes

APPOST 04

Estimation and prediction of genetic parameters and breeding values through REML/BLUP approach

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The Mixed Linear Models provides advantages compared to ordinary linear models. One is the ability to consider variables as random and other variables as fixed. The objective of this analysis is for the selection of superior genotypes. As the genotypes are a random sample of the hybrids within the trial, the genotypic effects are assumed as random and the consequent use of best linear unbiased predictor (BLUP) is justified. The objective was to predict the genetic value of tobacco hybrids through their BLUP, using Mixed Linear Models and parameters estimation by the Restricted Maximum Likelihood method (REML), for purposes of selection. The performance of 14 flue-cured Virginia tobacco hybrids, developed by Alliance One International, was studied. Yield, nicotine, sugar and quality index grade were evaluated across 11 environments in the southern region of Brazil. The trials were conducted in a randomized complete blocks design, with three replications and four commercial controls. Statistical analyses were performed in the JMP software program (SAS Institute Inc.), using Mixed Linear Models. Genetic parameters were estimated via REML, with genotypic means adjusted and estimated using the BLUP procedure. The likelihood ratio test (LRT) was performed for the variables evaluated in the experiment, and the significance was verified by the Chi-Square test. The use of the statistical approach of Mixed Linear Models showed to be effective in the selection of superior genotypes. The G × E (genotype x environment) interaction had a statistically significant impact on the genotypic and phenotypic parameters associated with tobacco yield, nicotine and quality index. It was possible to identify genotypes adapted to certain regions of the study, as well as widely adapted. This statistical analysis methodology allowed a better estimate of the genotypic values, making the selection and decision-making process more efficient on the part of the breeders.

Notes

APPOST 05

Estimating genetic variability and correlation of agronomic traits in Burley tobacco for selection purposes

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The breeding values, genotypic and phenotypic variances, heritability and the correlation coefficients of the agronomic traits are some of the key parameters, which determine the efficiency of a breeding program. The phenotypic correlation is important because it shows how selection for one trait influences the expression of other traits. Therefore, the objective of this study was to obtain the genetic parameters estimated among the traits including the genotypic variances, phenotypic variances, genotype by environment variances, heritability and correlation coefficients, aiming to improve selection for important agronomic traits. The performance of 24 Burley tobacco hybrids, developed by Alliance One International, was studied. Yield, nicotine and quality index grade were evaluated across seven environments in the southern region of Brazil. The trials were conducted in a randomized complete blocks design, with three replications with five commercial controls. Statistical analyses were performed in the JMP software program (SAS Institute Inc.) using the restricted maximum likelihood method (REML) and Pearson's correlation coefficient. Genetic parameters were estimated via REML, with genotypic means adjusted and estimated using the best linear unbiased predictor (BLUP) procedure. The likelihood ratio test (LRT) was performed and the significance was verified by the Chi-Square test. Based on the predicted mean values, Pearson's correlation between traits were estimated. There were statistically significant differences for the effect of genotype, indicating there is genetic variability for this effect. The behaviour of the hybrids within the environments was verified, allowing the selection of hybrids according to the studied environments. The heritability estimates decreased due to the complexity of the character evaluated. The characters leaf quality index and nicotine have a positive association. The statistical method used showed to be efficient for this type of data set, allowing greater efficiency with regard to the selection of superior genotypes.

Notes

APPOST 06

Functional identification of tonoplast membrane potassium channel gene *NtTPK* in tobacco

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The presence of K^+ is crucial for maintaining the quality of tobacco, and hence the study related to understanding the molecular regulation of the concentration of K^+ in tobacco could be of great significance to improve the quality of tobacco. In this study, a two-pore K (TPK) channel gene *NtTPKa* was cloned from tobacco. The gene was found to encode a protein containing the unique K^+ selection motif GYGD and the transmembrane region of TPK1. The expression of *NtTPKa* gene was substantially increased by low potassium stress. In addition, analysis of subcellular localization showed that *NtTPKa* was primarily localized in the tonoplast membrane. The concentration of K^+ in tobacco was significantly increased by application of different materials that inhibited the expression of *NtTPKa* gene by RNAi. We used CRISPR/Cas9 technology to create *NtTPKa* gene knockout materials, and the concentration of K^+ in tobacco was observed to be significantly increased. Therefore, *NtTPKa* could serve as a negative regulatory gene for concentration of K^+ in tobacco. We also examined the K^+ transport function of *NtTPKa* channel protein through patch clamp technique. The results showed that the *NtTPKa* protein only selectively transported K^+, but possessed no transport function for Na^+, Mg^{2+} and Ca^{2+}, and its transport activity for K^+ exhibited specific concentration of K^+ dependence. The clone identification of *NtTPKa* can potentially provide a new gene resource for increasing the concentration of K^+ in tobacco.

Notes

APPOST 07

Chromosome-level genomes of the *Nicotiana tabacum* progenitors, *Nicotiana sylvestris* and *Nicotiana tomentosiformis*

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As descendants of the *Nicotiana tabacum* progenitors, *Nicotiana sylvestris* and *Nicotiana tomentosiformis* are important diploid *Nicotiana* species. In addition, ancestors of *N. sylvestris* were involved in the polyploidization events that gave rise to the *Suaveolentes* and *Repandae clades*, reinforcing its importance within the *Nicotiana* species.

The genomes of these two *Nicotiana* species were published almost 10 years ago based on second-generation short-read sequencing, which resulted in fragmented scaffold-level assemblies. However, the fragmented nature of these assemblies and the lack of chromosome-anchoring limits their usability for synteny-based cross-species research.

Here we present *de novo* chromosome-level genomes for *N. sylvestris* and *N. tomentosiformis* built using primarily third-generation long-read sequencing, thereby bringing them to the completion level of other reference diploid *Solanaceae* genomes.

Philip Morris International is the sole source of funding and sponsor of this research.

Notes

APPOST 08

Exploring the microbial diversity and composition of three cigar product categories

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Cigars and cigarillos are emerging as popular tobacco alternatives to cigarettes. However, these products may be associated with similar adverse health effects. We used environmental DNA (eDNA) sequencing to extensively characterize the microbial diversity of cigar and cigarillo products and investigate differences in microbial composition across 23 different product types. Our findings showed the three categories of cigars (large, filtered, and cigarillos) differed significantly in observed richness and Shannon diversity, with filtered cigars exhibiting lower diversity measures compared to large cigars and cigarillos. We also found a shared and unique microbiota among different product types. *Firmicute* was the most abundant phyla in all product categories, followed by *Actinobacteria*. Nine genera were exclusively shared by large cigars and cigarillos and an additional thirteen genera were exclusive to filtered cigars. Analysis of individual cigar products showed consistent microbial composition across replicates for most large cigars and cigarillos while filtered cigars showed more inter-product variability. These findings provide important insights into the microbial diversity of cigar and cigarillo products, which can inform the development of more effective tobacco control policies and public health interventions.

Notes

APPOST 09

Deep tillage enhances the spatial homogenization of bacterial communities by reducing deep soil compaction

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Pressure from machinery and continuous agricultural production exacerbate soil compaction. Deep tillage (DT) can reduce subsoil compaction, but the effects of DT on soil bacterial communities at various spatial and temporal scales and the sustainability of tillage effects remain unclear. Here, we collected soil profile samples from 18 farmlands in a tobacco-rice multiple cropping area in southern China to investigate the current status of soil compaction. DT effects on soil physicochemical properties and bacterial communities at different soil depths were investigated by establishing three long-term experimental sites, and the sustainability of the effects of DT were preliminarily explored. Our results showed that soil compaction occurred at soil depths greater than 20 cm, as evidenced by higher soil bulk density, and sharp decreases in water and nutrient contents and bacterial community diversity. Although the ameliorative effect of DT on deep soil compaction diminished in the fourth year, the water and organic matter content and bacterial α diversity remained high. DT resulted in a more homogeneous bacterial community across the soil profile in terms of community similarity and compositional stability, along with increased alpha diversity, all of which were associated with reduced heterogeneity in soil variables, increased soil organic matter content, and an increase in the importance of homogeneous selection in the community assembly mechanism. Additionally, the homogenization of bacterial communities under DT promoted an enhancement in bacterial network complexity and stability. Collectively, our findings reveal the importance of deep soil improvement on the spatial homogenization of bacterial communities, which has far-reaching implications for comprehensively understanding the spatial and temporal patterns of microbial communities in agricultural ecosystems and their response to tillage.

Notes

APPOST 10

A multi-county survey of plant-parasitic nematodes in North Carolina tobacco fields

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The genera of plant-parasitic nematodes (PPN) known to infect tobacco include root-knot nematodes (*Meloidogyne* spp.), lesion nematodes (*Pratylenchus* spp.), the tobacco cyst nematode complex (*Globodera tabacum*), and stunt nematodes (*Tylenchorhynchus* spp.). Their prevalence in commercial fields planted to flue-cured tobacco have not been quantified in North Carolina. In August 2021 and September 2022 a total of 188 soil samples were collected from tobacco fields in 24 counties in order to identify nematode genera and quantify density. Where *Meloidogyne* spp. was identified, PCR was performed to determine the presence of *M. enterolobii*, or Guava root-knot nematode, which is a newly identified invasive species. Stunt nematodes were identified in 2021 and 2022 in 67.8 and 80 % of the soil samples, respectively. Slightly less prevalent were root-knot nematodes at 31.4 and 50 % in 2021 and 2022, respectively. *Meloidogyne enterolobii* were only identified in 2022 in Pitt and Granville Counties. This was the first report of *M. enterolobii* in Granville County. Lesion nematodes were generally as common as root-knot, accounting for 33.1 and 50 % in 2021 and 2022, respectively. Tobacco cyst nematodes were only identified in 2021 in Stokes County, which is in the Old Belt production region of the state. Tobacco cyst nematode was present in 100 % of the Stokes County samples (n=8) but only accounted for 6.8 % of all nematode genera identified in 2021. Our results suggest that PPN remain a serious pest for tobacco producers and that the spread of the invasive Guava root-knot nematode should continue to be monitored and managed. In contrast, the presence of tobacco cyst nematode appears to remain isolated to the Old Belt production region; therefore, sanitation measures and management strategies have proved to be effective tools to reduce its spread.

Notes

APPOST 11

Screening of insecticides for controlling *Myzus persicae* in cigar leaf fields and safety evaluation of *Rhynocoris fuscipes*

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In order to identify effective and *Rhynocoris fuscipes*-friendly aphid control agents in tobacco fields, the toxicity of nine common insecticides to *Myzus persicae* and *R. fuscipes* was assessed using the leaf-dip and residual film methods in glass tubes, with a subsequent evaluation of the safety of *R. fuscipes*. Plot experiments were then conducted in tobacco fields. The results showed the following LC_{50} values of the nine agents for *M. persicae*: acetamidine (44.311 mg/L), fludizonitrile (46.696 mg/L), rotenone (71.499 mg/L), eucalyptol (77.538 mg/L), dipropirenol (139.697 mg/L) and pyrazidone (175.142) mg/L), pyrethrin (427.589 mg/L), veratrine (322.856 mg/L), *Metarhizium anisopliae* CQMa421 (205.921 cfu/mL). The highest and lowest LC_{50} values for second stage nymphs of *R. fuscipes* were rotenone (139.596 mg/L) and *Metarhizium anisopliae* CQMa421 (> 1500 cfu/mL). Combined with the safety coefficient and the toxicity ratio, eucalyptol, *Metarhizium anisopliae*, acetamiprid, pyrzidone and *Metarhizium anisopliae* CQMa421 demonstrated effective control on *M. persicae* with less toxicity to second stage nymphs of *R. fuscipes*. Field experiments showed that the combination of eucalyptol, pyrzidone and *Metarhizium anisopliae* CQMa421 with *R. fuscipes* achieved effective control percentages of 93.14 %, 91.22 % and 91.00 %, respectively, surpassing the efficacy of using eucalyptol or *R. fuscipes* alone. This combined approach exhibited a favourable control effect on tobacco aphids, thus recommending its practical implementation. The results of this study provided valuable guidance for the control of *M. persicae* in tobacco fields through the alternating use of *R. fuscipes* and chemical agents, while establishing a basis for large-scale control of aphid giannis and *Noctuidae* pests in tobacco fields.

Notes

APPOST 12

A new index "root-knot density" was created which can objectively evaluate the damage of root nematodes on tobacco

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Root-knot nematode disease occurs frequently due to continuous mono-cropping and excessive water and nitrogen input. The disease degree and gall index are often used to evaluate the damage of root-knot disease. However, the weak correlation between these two indicators to tobacco leaf dry weight has often been reported. The objective of this study is to verify whether root-knot density (RKD) as a new indicator, the root-knot number per unit root weight or volume, can describe the damage of root-knot disease on tobacco growth and yield quantitatively. A total of 3000 tobacco plants from 60 independent plots were classified according to the damage symptoms of leaves *in situ*. A total of six plants in each plot were selected and sampled to represent 6 damage levels with a total of 360 plants. The responding roots were taken out with a root auger. Dry weights of leaf, stem, root and root-knot as well as root volume, root-knot number, disease degree and gall index were determined for all 360 plants separately. Our results showed that (1) the disease degree and gall index of root-knot nematodes had a weak negative correlation with tobacco leaf dry weight. Meanwhile, leaf dry weight and the dry weight, volume and number of root-knots were not correlated. (2) The root dry weight, volume and length of diameter ≥ 2 mm roots were significantly positively correlated with leaf dry weight. (3) The RKD of root diameter ≥ 2 mm roots was significantly negatively correlated with the leaf dry weight. (4) The dry weight of leaves, stems and roots decreased significantly with the increase in the average RKD of diameter ≥ 2 mm roots in reclassified groups, which was significantly positively correlated with the average reclassified disease degree and gall index. Our results highlighted that the proposed RKD in this paper can be used to evaluate the damage degree of root-knot disease quantitatively, as a new indicator for future research and practical diagnosis of root-knot nematodes.

Notes

APPOST 13

Photosynthetic performance as a tool in detecting infection in tobacco (*Nicotiana tabacum*)

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Photosynthesis is the key process for plant growth and development. By calculating the rates of chlorophyll fluorescence kinetics, it is possible to quantify the impacts of environmental stresses like phytopathogen infection on photosynthetic processes. Tobacco mosaic virus (TMV) is one of the most economically damaging plant viruses because of its wide host range and transmission mode. TMV is a positive-sense single-stranded RNA virus in the family *Tobamovirus* that attacks a variety of plants, particularly tobacco. The infection produces distinctive patterns on the foliage, such as "mosaic"-like mottling and discoloration, which can decrease photosynthetic efficiency and, as a result, yield and quality. Unfortunately, little is known about how viruses, particularly TMV, influences complex mechanistic processes like photosynthesis. The objective of the present study was to understand how the commonly available isolates of TMV found in Zimbabwe affect the mechanistic processes of photosynthesis and to generate data for future research on other commercially significant plant viruses. Ten plants of the tobacco variety ONC with established susceptibility to TMV and an additional ten plants of its isogenic variant ONCR with TMV resistance imparted by the N gene were grown in rainout shelter over the course of two seasons (2019-20 and 2022-23), at the Kutsaga Research Center in Harare, Zimbabwe. Results showed that infection decreased the maximum quantum yield of PSII (F_V/F_M), the effective quantum yield of PSII (ΦPSII), the CO_2 assimilation rate (A) and the stomatal conductance (g_s) in the studied TMV-susceptible plants, while non-photochemical quenching (NPQ) increased. This article will discuss in detail and demonstrate the usefulness of research on plant-virus interactions using modulated chlorophyll fluorescence measurements. In future, this will be especially important in circumstances where there are no visible signs of infection. Additionally, this research contributes to the body of information for future studies on other economically important plant viruses.

Notes

APPOST 16

Fungicide and starter fertilizer placement at transplanting

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Starter fertilizer and fungicide products are commonly included in transplant water solutions. It has been hypothesized that a different placement strategy is needed in order to minimize chemical and soluble salts injury. Research was initiated in 2022 to compare the efficacy of traditional in-furrow fungicide and fertilizer placement versus that of a sidedress placement. Two placement techniques were evaluated, a traditional in-furrow application that places solution in the planting furrow and a sidedress application that places the solution 5 cm away from seedlings. Within each placement technique three treatments were subsequently evaluated: oxathiapiprolin (0.07 kg ai/ha) + mefenoxam (0.21 kg ai/ha), 9-45-15 starter fertilizer (12.4 kg/ha), and oxathiapiprolin + mefenoxam + 9-45-15. A non-treated control was included for comparison (935 L water/ ha only). In-furrow placement was delivered in 935 L water/ha, while sidedress placement was delivered with 187 L water/ha. Two weeks after transplanting, visual injury (45-48 %) and chemical residues were highest in treatments receiving oxathiapiprolin + mefenoxam in-furrow. Sidedress placement of this fungicide combination reduced injury to 16 and 5 % and residues of mefenoxam to < 20 mg/kg. Oxathiapiprolin residues were below the Limit of Quantification. In the same measurement interval, the non-treated plots contained the largest plants based on dry mass and stem height measurements. Plant recovery was observed four weeks after transplanting, as chemical injury was not visible and in-furrow placement of starter fertilizer resulted in plants that were taller with a higher dry weight mass than the fungicide only treatment or any treatments that were applied sidedress. By six weeks after transplanting there were no differences in growth or biomass accumulation recorded across treatments. Preliminary results indicate that sidedress applications of fungicides and starter fertilizers may reduce injury; however, plant assimilation of those inputs may be delayed relative to in-furrow placement. Moreover, the addition of 9-45-15 in-furrow may promote plant recovery from fungicide injury. Results from 2023 field trials will be included in this presentation.

Notes

APPOST 18

Study on hyperspectral multivariate linear prediction model of tobacco leaf nitrogen content

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The nitrogen content of tobacco leaves has a direct bearing on the gene expressions of nitrogen metabolism-related enzymes and on the amount of nitrogen metabolites (Alinat *et al.*, 2015). Hyperspectral remote sensing, originating in the 1920s, offers an important tool for experimental science, and this technique can be used to identify molecular and atomic structures (Fan *et al.*, 2022). Since different biochemical components of crops have distinct absorption bands (El-Naggar *et al.*, 2021), it is feasible to monitor crop quality parameters based on optical remote sensing data. The objective of this study was to analyse the quantitative relationship between the nitrogen content of tobacco leaves and hyperspectral variables in three developmental stages and establish the hyperspectral prediction model for nitrogen content of tobacco leaves in order to obtain the nitrogen content of tobacco leaves accurately and effectively during the whole growth period. This study used the field canopy spectrum of the three critical periods of tobacco rosette stage, vigorous growth stage and topping stage. The correlation analysis of field canopy spectrum, first derivative spectrum, hyperspectral parameters and vegetation index with the nitrogen content of tobacco leaves was carried out one by one, and the prediction model was established by multiple linear regression using the variables with the best correlation coefficient. The results show that the first derivative spectrum, EVI II and green peak position show strong correlation, which is suitable for introducing multivariate equations as independent variables. Finally, the modeling determination coefficient (R^2) is 0.66, RMSE is 0.40, and MAPE is 11 %. The validation results showed that R^2 was 0.73, RMSE was 0.38, and MAPE was 8.33 %. It proved that this model could accurately predict the nitrogen content of tobacco leaves and could meet the requirements of large-scale statistical monitoring of tobacco quality indicators in the field.

Notes

APPOST 19

Automatic discrimination planting areas of flue-cured tobacco based on near-infrared spectroscopy technology and support vector machine improved by whale optimization algorithm

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A study was carried out to accurately and rapidly identify planting areas of flue-cured tobacco. A total of 201 flue-cured tobacco samples from three different areas in Kunming, Honghe and Qujing, Yunnan Province were selected for the study. After collecting the near-infrared spectra of different areas and reducing the interference factors through the spectral preprocessing method, followed by principal component analysis (PCA) for dimensionality reduction, a whale algorithm (WOA) was established to optimize support vector machine (SVM) parameters to establish an automatic identification method. In the wavenumber range of 8000 to 4000 cm^{-1}, the standard normal variable transformation (SNV) combined with the second derivative method (2D) was used for near-infrared spectroscopy preprocessing, and the data after the PCA dimensionality reduction was used as the input variable, after which the WOA-optimized support vector parameters could achieve a better recognition effect. The classification accuracy rate of the training set is 97.18 %, and the classification accuracy rate of the test set is 98.31 %. This shows that using near-infrared spectroscopy technology combined with WOA algorithm to optimize SVM can achieve accurate identification of area of flue-cured tobacco.

Notes

APPOST 20

Design and testing of sinusoidal curve variable frequency control system for circulating fan in bulk curing barn

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Scientific control of wind speed between tobacco leaves can improve quality of tobacco after cured in bulk curing barn. Regarding the light colour and high power consumption caused by inaccurate control of the current circulating fan, and different requirements for wind speed during the tobacco curing, this paper designed a variable frequency drive (VFD) to control the rotation speed of the circulating fan by a sinusoidal curve way, which includes speed value, amplitude, and frequency. With the design of anti-interference components, the VFD is integrated with the tobacco curing controller. The curing adoption of anti-interference for the VFD system will not affect the normal operation of the tobacco curing controller during tobacco curing. Compared with the traditional circulating fan with high speed and low speed options, the VFD system can accurately control the air speed during the tobacco curing process. This paper also presents an alternative method in terms of ventilation and humidity removal for industrial and agricultural production.

Notes

APPOST 21

"Di@gnoPlant Tabaco" for Brazilian tobacco stakeholders

MARIGNAC E.

CORESTA, 11 rue du Quatre Septembre, 75002 Paris, France

In 2013 INRA published Di@gnoPlant® Tobacco translated from French into English by CORESTA thus allowing access to this tool by English speaking farmers and technicians. With the ability to identify tobacco diseases, the use of crop protection agents could be reduced. The early and reliable identification of diseases and the detection of emergent pests have proven to be crucial for effective plant protection. An early diagnosis enables implementation of the most appropriate protection method(s). What disease causes the symptoms? And what control methods can be used?

This identification tool was found to be useful by tobacco farmers and technicians, but the problem was its existence in only French and English. During the AP2017 Conference in Santa Cruz do Sul it emerged that the Brazilian farmers did not always use this tool and therefore CORESTA decided to translate the website and the application into Portuguese. A successful cooperation project with the help of native Brazilian Portuguese speakers was undertaken. Brazilian farmers can now gain immediate access via smartphone or tablet to research knowledge and expertise in plant protection with the Portuguese version of Di@gnoPlant® Tabaco. The user is able to identify diseases by means of an image database. Fact sheets detail the symptoms and biology of the incriminated pest or disease and the optimised protection methods adapted to the context.

The application will be demonstrated during the poster session.

Notes

APPOST 23

Multiresidue method for the determination of pesticides in tobacco *(Nicotiana tabacum)*

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The development and validation of an analytical Multiresidue Method for the determination of 155 pesticides in the *Nicotiana tabacum*, or cultivated tobacco, using QuEChERS extraction followed by different techniques: (1) HPLC-MS/MS (55 compounds), (2) GC-MS/MS (98 compounds) and (3) GC-ECD (one compound) and (4) GC-MS (one compound) to fulfil the requirements of the CORESTA and ISO/IEC 17025 accreditations.

At the beginning of the tests, one of the problems faced was obtaining pesticide-free tobacco samples that could be used as a blank in validation tests. After testing several commercial samples, the best alternative was to utilize plants used in the experiments of residue studies carried out by our Agroscience team, guaranteeing the absence of target compounds.

All necessary parameters, such as linearity of analytical curves, matrix effect and accuracy (veracity and precision), robustness, limits of the instrument and the method of detection and quantification that meet the Guidance Residual Levels (GRLs) of CORESTA were evaluated.

Blank samples were spiked with different concentrations considering in six replicates for each concentration level plus the blank sample. From the 157 studied compounds, 156 showed recoveries within the acceptable range of 70-120 % for all evaluated concentrations and all compounds showed precision < 20 %. The validated method limits of quantification were below Guidance Residual Levels (GRLs).

In our routine analysis, the most frequent compound above the GRL was acetamiprid. In 3.75 % of the analyzed samples the concentrations found of this compound were above the permitted level. 100 % of the samples analyzed were positive for some compound.

Notes

APPOST 24

Complete sequencing of the tobacco *(Nicotiana tabacum)* genome and transcriptome as a tool for discovering new resistance markers and gene functionalities

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With advances in next-generation sequencing technologies (NGS), it becomes increasingly accessible to use this technique in relation to traditional molecular biology methodologies. Nowadays, most experiments for tobacco genetic improvement are carried out using a few markers through conventional PCR. This methodology, despite being effective and inexpensive, limits the number of markers used and becomes costly on a large scale, thus limiting the technique.

Another factor is that the technique only gives results in markers already known, making it impossible to discover new markers or resistance genes.

Reproduction of pest-resistant species is important in any crop, especially tobacco. In this way, understanding more broadly how to produce more resistant and long-lasting plants becomes essential. Thus, we present the NGS as a solution and tool for the discovery of new genetic markers and resistance genes in tobacco. For this, the complete sequencing of the tobacco genome was carried out on the Illumina NovaSeq 6000 platform, generating about 5 GB of data, as well as the transcriptome was sequenced to cross gene expression data with the genome.

This makes it possible to align gene expression data from resistant plants and under different cultivation conditions to arrive at the ideal cultivation. Our results show that it is still necessary to apply the technique to more plants and under different growing conditions to obtain a more specific test, but it is extremely innovative and counts as an initial step in precision genetics for tobacco cultivation.

In the field, complete genome sequencing allowed a better-quality control of the seedlings used in our Field Stations and later used in various experiments both in the field and in the laboratory such as efficacy and residues studies and tests guiding good practices and sustainable use of resources. In addition, quality control was essential in the development and validation of an analytical Multiresidue Method (MRM) for the Determination of Pesticides in *Nicotiana tabacum* (tobacco) used in the analysis of residues in commercial products.

Notes

APPOST 25

Physiological and molecular analysis on colour mutant lines reveals relationship between chlorophyll and nitrate and TSNA content in flue-cured tobacco

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College of Tobacco Science / Tobacco Harm Reduction Research Center, Henan Agricultural University, Zhengzhou 450002, China

Burley tobacco is particularly susceptible to TSNA formation because the cultivars exhibit a chloroplast-deficient phenotype. Previous studies showed that the lack of chlorophyll had negative effect on photosynthesis and nitrogen use efficiency (NUE), resulting in higher levels of nitrate and TSNA. In this study, a stable albino leaf mutant (*Al*) and light-green leaf mutant (*LG*) obtained from the common flue-cured tobacco (*Nicotiana tabacum* L.) cultivar 'Zhongyan 100' (ZY100) by mutagenesis with ethyl methanesulfonate (EMS) were used as materials. The differences between mutants and the wild-type (*WT*) were analyzed for biomass, photosynthetic fluorescence parameters, carbon- and nitrogen-related physiological parameters, and NO_3-N and TSNAs content. The results showed that lower total pigment content and leaf biomass in mutant plants were observed compared with the *WT* (15.51 and 6.44 times for *Al*, 2.77 and 1.29 times for *LG*) while a significantly higher NO_3-N content was observed (18.85 times for *Al* and 4.09 times for *LG*) under the same nitrogen application. The net photosynthetic rate, photosynthetic fluorescence parameters, carbohydrate, soluble protein, and carbon- and nitrogen-related enzyme activities all decreased in leaves of mutants and the development of chloroplasts was abnormal. Similar biomass, higher photosynthesis, and enzyme activities were achieved when the slight-green mutant was given three-fold more nitrogen fertilizer; however, a much higher NO_3-N accumulation was observed. Notably, the NO_3-N content in *LG* was increased by 16.96 times compared with the *WT* when they had similar leaf biomass. Moreover, the NO_3-N and TSNA content in cured leaves of the mutants were also significantly increased. RNA-sequencing demonstrated the down-regulated genes in mutants were enriched in plant hormone signal transduction and nitrogen metabolism, which are involved in pigment biosynthesis and the carbon fixation pathway. The conclusion is that lower expression level of genes involved in these pathways leads to reduced pigment, which in turn affects photosynthetic carbon fixation and nitrogen assimilation, resulting in accumulation of nitrate.

Pot and field experiments showed that salicylic acid (SA) spraying was able to improve chlorophyll synthesis and photosynthesis, as a result, NO_3-N and TSNA contents in cured tobacco leaves were significantly decreased.

Notes

CORESTA AP2023 CONFERENCE

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

SUSTAINABILITY WORKSHOP

WS 01

Use of Sentek soil probes in tobacco research

REED T.D.

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Good agricultural practices (GAP) have been a formal industry requirement for most U.S. tobacco growers for a decade. Environmental stewardship is one of the three cornerstone principles of GAP and promoting soil conservation and minimizing the run-off of fertilizer nutrients are just two practices that growers address. Sentek soil probes (Sentek Technologies, Stepney SA, Australia) have been utilized as a research tool at the Virginia Tech Southern Piedmont Center to study multiple soil parameters and develop sustainable production practices. Soil temperature and moisture as well as volumetric ion content (VIC) are measured at 10 cm intervals to depths of 0.5 or 1.0 m in the soil profile. Supporting software (IrriMAX Live) allows for the examination of the fate of rainfall and irrigation events with quantification of the amount of infiltrating the soil versus running off the soil surface. Water infiltrating the soil can be described as remaining in the active crop root zone, passing below this zone, or leaching beyond this depth. Each of these have been quantified following rainfall events of varying intensities and duration. Grower profitability is paramount to the sustainable production of tobacco and producing a high yield of high quality, marketable tobacco is essential to profitability. The capacity to estimate the active crop root depth and examine rooting activity of a tobacco crop as well as examine the movement of fertilizer ions are potentially useful research tools. One interesting research topic where this has been used is the comparison of root size and development between different tobacco varieties.

Notes

WS 02

Enhancing sustainable tobacco production practices in Zimbabwe

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Zimbabwe is known for producing flavour style flue-cured tobacco leaf that is widely sought after. To ensure that this tobacco is produced in a sustainable manner, the Tobacco Research Board is mandated with ensuring that growers have up-to-date information on current production practices to enable science-based crop management and that they adhere to set regulations and industry requirements. A number of activities are therefore, undertaken to ensure growers have information on recommended cultural and production techniques, access and use suitable tobacco varieties and greener crop protection agents. In this paper we elucidate on grower interaction sessions being undertaken for advisory purposes. We also discuss new climate-smart varieties developed for marginal growing areas that were put out on limited release in the 2023 season for grower evaluation and the status on the evaluation of green crop protection and production products including biofertilizers and biocontrol agents which are at various stages of testing and those that have been registered and already in use by growers.

Notes

WS 03

A decade of the Agrochemical Advisory Committee's (ACAC) achievements and their impact on sustainability

SCOTT L.

Universal Leaf Tobacco, Inc., 9201 Forest Hill Ave, Richmond, VA 23235, U.S.A.

The mission of the Agrochemical Advisory Committee (ACAC) is to address matters relating to agrochemicals and topics associated with product stewardship and integrity in tobacco by gathering relevant information and disseminating guidance to stakeholders. During the last 10 years, ACAC has intensified its work to collect, assess, and distribute science driven information relative to agrochemical selection, application, and residues across numerous tobacco types. This has resulted in multiple CORESTA Guides, such as No. 1, No. 3, No. 19, No. 21, and No. 27, which are publicly available to a global audience. In addition, ACAC monitors agrochemical residue trends and has noted specific agrochemicals that are being phased out and typically replaced by other CPAs that have a lower risk profile. Specific examples of these agrochemicals will be shared during this presentation.

Notes

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ESG WORKSHOP

WS 04

STP overview - accelerating positive impacts on social and environmental footprints in the tobacco industry

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(2) Sustainable Tobacco Program (STP), Secretariat Coordinator, Peterson Projects S.A., Rue Blavignac 10, 2nd Floor, 1227, Carouge, Switzerland

Programs that incorporate environmental, social, and governance (ESG) factors are increasingly prevalent in the business world, as they are drivers of long-term value especially in industries with labor-intensive supply chains such as tobacco. The expectations of companies to meet standards related to their environmental footprint (net zero or reducing greenhouse gases) and impact on society (forced labor and child labor) have steadily moved beyond the boundaries of direct operations. Industries are subsequently collaborating in pre-competitive ways to identify common issues of industry suppliers and develop more effective and impactful solutions.

Currently, seven tobacco manufacturer members are collaborating through an industry ESG initiative called the Sustainable Tobacco Program (STP). This will enhance agricultural supply chain due diligence and accelerate positive impacts on social and environmental footprints. Participants report continuous improvement efforts across eight sustainability themes (water, human and labor rights, crop, soil health, climate change, natural habitats, livelihoods, and governance). STP helps members and participants identify risks and permits them to prioritize efforts with the largest positive impacts for suppliers, growers, and their workers, as well as the environment.

As the 2023 chair of STP, Altria will provide an overview of the framework; highlighting how STP modifies tools and processes, ensures data integrity and relevance, engages with stakeholders, and stays aligned with evolving ESG laws and regulations that impact the industry.

Notes

WS 05

Exploring different ways to enhance the livelihoods of tobacco growing communities and further help contracted growers and their families achieve and sustain a living income

QUATKE C.

JT International SA, 8 rue Kazem Radjavi, Geneva 1202, Switzerland

The concept of living income is referenced in the UN declaration of human rights and widely recognized as an enabler for reducing other human rights risks in supply chains such as that of tobacco. There are also clear demonstrable benefits that can impact such supply chains.

While labour practices have predominantly been the focus in the tobacco supply chains, additional focus is now shifting towards enabling and ensuring that tobacco growers get a decent standard of living from their farming enterprise. While being driven by changing legislative environments, competitive landscapes and increasing investor demands, attainment of a decent standard of living for growers is set to become a basic standard and license to operate in the agricultural and tobacco supply chains.

From 2023, Japan Tobacco International (JTI) has commissioned studies aimed at understanding how its growers fare in the attainment of a living income. Using a globally accepted benchmark, JTI is in the process of determining each of its (Vertically Integrated, VI) supply origin's living income using a Living Income Calculator. This will consequently lead to addressing and closing out gaps and impediments to attainment of country-specific targets.

Notes

WS 06

A farmers' perspective on ESG and future opportunities

RENN J.; GRIFFIN S.; BOYD G.

Tobacco Growers Association of North Carolina, 3901 Barrett Drive #202, Raleigh, NC 27609, U.S.A.

Environmental, Social, and Governance (ESG) initiatives across the global tobacco industry will undoubtedly impact commercial farming entities of all sizes. The Tobacco Growers Association of North Carolina (TGANC) believes that industry representatives throughout the entire supply chain must work together to create equitable and sustainable ESG programs. To meet this objective, these programs must foster open dialogue among farmers, buyers, and manufacturers. In this presentation, TGANC will outline the manner in which impactful ESG programs might be developed. We will also discuss the current Labour, Environmental, and Crop Management programs that are already being utilized in the United States through the training and certification modules offered by the Good Agricultural Practices program.

Notes

CORESTA AP2023 CONFERENCE

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

CIGAR TOBACCO WORKSHOP

WS 07

Challenges for cigar tobacco production

RAMOS R.

ITG Brands LLC, 420 N. English Street, Greensboro, NC 27405, U.S.A.

The cigar industry has been growing massively, demanding more cigar tobacco production. To produce marketable tobacco, the farmers are required to produce perfect leaves, with restricted or zero tolerances to physical damages, especially in the wrappers. This unique situation plus weather conditions (high humidity and temperatures) in the cigar tobacco growing origins, increase the dependency on use of crop protection agents. Analyzing the current situation across all origins in America and Asia especially, there are various challenges that cigar tobacco growers/suppliers are facing as, using more crop protectant agents, molecules availability, low number of resistant varieties, inconsistent measurement by industry and lack of research on these tobacco types.

As an Industry it is imperative to look for solutions to address these challenges and bring it to an open discussion. CORESTA Guide No. 21 is a starting point establishing C-GRLS as guidelines for the usage of crop protection agents. There are other opportunities to reduce dependence on the use of crop protection agents, such as improving the sharing of information with all actors through better communication channels and promoting more research to support cigar tobacco farmers and suppliers.

Notes

WS 08

Cigar tobacco production in Pennsylvania

GREEN B.; HARTLEY M.

Lancaster Leaf Tobacco Company, PO Box 25099, Richmond, VA 23260, U.S.A.

Tobacco production in Pennsylvania occurs on more than 2,000 hectares. Historically, PA-41 and Mayland were the dominant types produced in the state. After 2004, additional types, such as Burley, and Green River One Sucker, gained prominence. More recently, another transition has occurred, whereby Connecticut Broadleaf is now produced on commercial farms. The introduction of Connecticut Broadleaf has been widely accepted by producers because of their experience with PA-41 and other air-cured styles. In addition, the intensive management required for production fits well with the local Amish culture. Our presentation will highlight Connecticut Broadleaf and PA-41 production in Pennsylvania as well as the unique management practices and equipment used by local farmers.

Notes

WS 09

Cigar tobacco leaf: a critical agricultural raw material for EU manufacturing of cigar and cigarillo products

VARAKAS P.

European Cigar Manufacturers Association (ECMA), Rue Véronèse 73, 1000 Brussels, Belgium

EU manufacturing of cigars and cigarillos mainly consists of medium-size family-owned companies located throughout Europe. With 27 manufacturing plants, Europe remains a world-leading exporter of cigars and cigarillos, exporting more than 1372 tons worth over 257 million euros (FOB) in 2022.

As such, a substantial part of European manufacturers' cigar and cigarillo production is typically made from mainly fermented, dark air-cured tobacco, blended from different countries of origin, different harvests and different tobacco varieties grown in a limited number of countries, such as Indonesia, Brazil, Dominican Republic, Ecuador, Italy and the United States of America. The objective of this study was to quantify the amount of imported raw tobacco into Europe and analyse the upcoming European legislation impeding the free flow of imports. The method of calculation involved the collection and aggregation of data of tobacco purchased by ECMA members meant for manufacturing.

Already buying the largest share of European dark fire and fire-cured tobacco leaf, the lack of sufficient supply of high-quality tobacco leaf for cigar filler and cigar wrapper grown in Europe makes it compulsory for European manufacturers to import between 70 to 80 % of their supply in raw tobacco.

It is in this context that European manufacturers attribute a capital importance to having appropriate rule of origin and import legislation. With legislative measures to avoid or minimise the placing of products associated with deforestation, due diligence requirements, import tolerances and mirror clauses incorporated in free trade agreements, the European legislative framework has seriously increased the risk of disruption in the supply of cigar leaves meant for European manufacturing of cigars and cigarillos.

Becoming a critical raw material, special emphasis should be placed on how information from the sustainable and responsible cigar tobacco supply chain can be used to allow for a smooth international flow of cigar tobacco leaves.

Notes

WS 10

Perspectives on crop protection agent analysis in cigars

ANSPACH T.

Eurofins Dr. Specht International GmbH, Am Neuländer Gewerbepark 2, 21079 Hamburg, Germany

Cigars represent a highly diverse global category of tobacco products. As other commercial high-value agricultural crops, cigar raw materials shall be sampled and tested to validate recommended good agricultural practices and quality standards. However, there is limited scientific data available for cigars, in particular crop protection agent (CPA) residues.

Since cigars come in different shapes and sizes, contrasting products were identified and sampled in key markets to obtain a general understanding of potential dynamics of CPA residue profiles. Analytical state-of-the-art multi-residue methods were applied to analyse CPAs in whole cigar samples and main product components such as wrapper, binder and filler.

The testing results showed high variability in CPA residue levels in products as well as in their main components. Contrasting CPA residue profiles will be discussed and assessed against the CORESTA Guide No. 21 listing cigar specific guidance residue levels.

Overall, presented analytical residue data may further support local production teams, including those dedicated to the design of cigar crop protection programs, and establish recommendations towards industry-wide stewardship practices.

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CORESTA AP2023 CONFERENCE

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

PROGRAMME SUMMARY

Abbreviations

GENERAL
SG ..... Sub-Group
TF ..... Task Force

AGRONOMY & LEAF INTEGRITY STUDY GROUP
AA ..... SG Agrochemical Analyses
GMO ..... SG Proficiency Testing for Detection of Transgenic Tobacco
GTS ..... TF Green Tobacco Sickness
LNTP ..... TF Collaborative Study of Low Nicotine Tobacco Agronomic Production Practices
PSMST ..... SG Pest and Sanitation Management in Stored Tobacco
RFT ..... SG Agrochemical Residue Field Trials
TSNA ..... SG TSNA in Air-cured and Fire-cured Tobacco

PHYTOPATHOLOGY & GENETICS STUDY GROUP
BIO ..... SG Efficacy of Biological and Eco-Friendly CPAs
BKS ..... SG Collaborative Study Black Shank
IPM ..... SG Integrated Pest Management
NGPC ..... TF *Nicotiana* Germplasm Collection
XDES ..... SG Diagnosis Expert System Translation

OTHER
ACAC ..... Agrochemical Advisory Committee

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

SUB-GROUP / TASK FORCE MEETINGS

	Saturday, 14 October		Sunday, 15 October		
Meeting Rooms	*Mérida*		*Querétaro*		*Meeting Rooms*
8:00-11:00	**SG GTS** 9:00-11:00		**ACAC** 8:00-12:00		8:00-11:00
11:00-12:00	**SG BIO** 11:00-12:00	REGIST-RATION		REGISTRATION	11:00-12:00
12:00 - 14:00			*Mérida*		**12:00 - 14:00**
14:00-15:00	**SG BKS** 14:00-15:00		**SG IPM** 14:00-15:00		14:00-15:00
15:00-16:00	**TF LNTP** 15:00-16:00		**TF TSNA** 15:00-16:00		15:00-16:00
16:00-17:00			**SG GMO** 16:00-17:00		16:00-17:00
17:00-18:00			**SG RFT** 17:00-18:00		17:00-18:00
			19:00-22:00		
			Welcome Reception		

AGRONOMY & LEAF INTEGRITY and PHYTOPATHOLOGY & GENETICS

Mtng Room	Sat 14 Oct	Sun 15 Oct	Monday 16 October	Tuesday 17 October		Wednesday 18 October	Thursday 19 October	Mtng Room
	Guadalajara		Yucatán	Yucatán		Yucatán	Yucatán	
			Chair: XU Dongmei	**Chair: GOEPFERT**		**Chair: ZHANG Limeng**	**Chair: GOEPFERT**	
			OPENING SESSION	BREEDING & GENETICS 1		PEST & DISEASE CONTROL	CIGAR TOBACCO PRODUCTION	
8:30 - 8:50			**Welcome** - XU Dongmei	**AP09** - BATISTA - *AOI*		**AP38** - FISHER CR - *Uni. Kentucky*	**AP55** - JAMES - *NCSU*	8:30 - 8:50
8:50 - 9:10			**Invited Speaker:**	**AP10** - MOORE - *NCSU*		**AP39** - KARVINA - *TRB*	**AP56** - VANN M - *NCSU*	8:50 - 9:10
9:10 - 9:30			**TURRENT A.** - *Casa Turrent*	**AP11** - BATISTA - *AOI*		**AP40** - BAILEY - *Uni. Kentucky*	**AP57** - SHI Hongzhi - *Henan Ag. Uni.*	9:10 - 9:30
9:30 - 9:50			**IG01** - STEVENS / FLORA - *RAIS / Altria*	**AP12** - WANG Jun - *CNTC Deyang*		**IPM SG Report** - FISHER A	**AP58** - HU Wanrong - *CNTC Sichuan*	9:30 - 9:50
9:50 - 10:10			**CORESTA Prize:**	**AP13** - BATISTA - *AOI*		**PSMST SG Report** - WINEGARDNER	AP59 - JIA Yun - *CNTC Sichuan*	9:50 - 10:10
10:10 - 10:30			**Presentation - PRAT M.**	**AP14** - ALLEN - *NCSU*		COFFEE	COFFEE	**10:10 - 10:30**
10:30 - 10:50			COFFEE	COFFEE		Yucatán	Yucatán	
			Yucatán	Querétaro	Yucatán	**Chair: LALANDE**	**Chair: CARUSO**	
			Chair: LALANDE	**Chair: VANN**	**Chair: KUDITHIPUDI**	CPA MANAGEMENT 2	CIGAR TOBACCO WORKSHOP	
(10:30 - 10:50)			CPA MANAGEMENT 1	BIOLOGICAL PROCESSES	FLAVOUR & AROMA	**AP41** - STAINBACK Cody - *NCSU*	**WS07** - RAMOS - *ITG Brands*	10:30 - 10:50
10:50 - 11:10	REGISTRATION	REGISTRATION	**AP01** - POCHUCHA - *JTI Germany*	**AP16** - YI Ke - *CNTC Hunan*	**AP20** - TIMKO - *Uni. Virginia*	**AP42** - VANN M - *NCSU*	**WS08** - GREEN - *Lancaster Leaf*	10:50 - 11:10
11:10 - 11:30			**AP03** - VANN M - *NCSU*	**AP17** - MAHERE - *TRB*	**AP21** - TIMKO - *Uni. Virginia*	**AP43** - LISUMA - *TORITA*	**WS09** - VARAKAS - *ECMA*	11:10 - 11:30
11:30 - 11:50			**AP44** - MARTINEZ OCHOA - *Uni. Kentucky*	**AP18** - CHINAMO - *TRB*	**AP22** - DELLA VECCHIA - *Altria*	**AP45** - STAINBACK Chris - *NCSU*	**WS10** - ANSPACH - *Eurofins*	11:30 - 11:50
11:50 - 12:10			**AA SG Report** - POCHUCHA	**AP19** - PU Yi - *China Agric. Uni.*	**AP23** - LIN Yechun - *CNTC Guizhou*	**RFT SG Report** - SEBATA	**Q&A**	11:50 - 12:10
12:10 - 12:30			**ACAC Report** - SCOTT	**GMO / BIO SG Reports** - FISHER C / MAVUKA	**AP24** - TAKEUCHI - *JT*	LUNCH	LUNCH	**12:10 - 13:40**
12:30 - 14:00			LUNCH	LUNCH			Yucatán	
			Yucatán	Mérida		Yucatán	**Chair: CHAMANGO**	
			Chair: LIVESAY			**Chair: LIVESAY**	BREEDING & GENETICS 2	
			SUSTAINABILITY: GAP TRAINING			ESG	**AP50** - MU Wenjun - *CNTC ZTRI*	13:40-14:00
14:00 - 14:20			**AP04** - BADEMCI - *JTI Turkey*			**AP46** - CHIWAYA - *ARET*	**AP51** - ZVOBGO - *TRB*	14:00 - 14:20
14:20 - 14:40			**AP06** - FRANCESCHETTI - *TTI*	POSTER SESSION		**AP47** - CETINTAS - *Sunel*	**AP52** - LIU Shaohua - *CNTC CAAS*	14:20 - 14:40
14:40 - 15:00			**AP08** - ROUSSOS - *Premium*			**AP49** - FRANTZ - *Premium*	**AP25** - MUKOYI - *TRB*	14:40 - 15:00
15:00 - 15:20			**AP26** - INSAURRALDE - *AOI*			**AP28** - CARRILLO - *Nueva Matacapan Tabacos*	**BKS SG Report** - FISHER C	15:00 - 15:20
15:20 - 15:40			**AP29** - CHIPETA - *ARET*			**GTS TF Report** - SCOTT	TEA	15:20 - 15:40
15:40 - 16:00			TEA	TEA		TEA		**15:40 - 16:00**
			Yucatán	Querétaro	Yucatán	Yucatán	Yucatán	
			Chair: VANN	**Chair: XU Dongmei**	**Chair: CARUSO**	**Chair: XU / LALANDE**	**Chair: FISHER CR**	
			SUSTAINABILITY WORKSHOP	TSNA	TECHNOLOGY	ESG WORKSHOP	LOW NICOTINE	
16:00 - 16:20			**WS01** - REED - *Virginia Tech*	**AP30** - QI Dong - *Altria*	**AP34** - MU Wenjun - *CNTC ZTRI*	**WS04** - IRVING - *Altria*	**IG02** - FISHER AM - *Uni. Kentucky*	16:00 - 16:20
16:20 - 16:40			**WS02** - DIMBI - *TRB*	**AP32** - KIM Taejin - *Altria*	**AP35** - RUZANE - *TRB*	**WS05** - QUATKE - *JTI*	**AP60** - CHEEK - *NCSU*	16:20 - 16:40
16:40 - 17:00			**WS03** - SCOTT - *ULT*	**AP33** - SINGH Sanjay - *Uni. Kentucky*	**AP37** - MACHACEK - *NCSU*	**WS06** - GRIFFIN - *TGANC*	**LNTP TF Report** - KUDITHIPUDI	16:40 - 17:00
17:00 - 17:20			**NGPC TF Report** - LEWIS	**TSNA SG Report** - FISHER C	**XDES SG Report** - MARIGNAC	**Q&A**	**AP61** - SHI Hongzhi - *Henan Agric. Uni.*	17:00 - 17:20
17:20 - 17:40			**Q&A**				**AP62** - PATRA - *Uni. Kentucky*	17:20 - 17:40
17:40 - 18:00							**AP63** - LEWIS - *NCSU*	17:40 - 18:00
		19:00-22:00					20:00 - 23:00	
		Welcome Recep.	Free Evening	Free Evening		Free Evening	Closing Dinner	

Please note that due to the withdrawal of some papers, the programme has had to be rearranged and some of the presentation numbers may no longer be consecutive.