

**ABSTRACTS OF PRESENTATIONS MADE AT THE
2015 CORESTA JOINT MEETING OF THE
AGRONOMY & LEAF INTEGRITY AND PHYTOPATHOLOGY & GENETICS
STUDY GROUPS
IZMIR, TURKEY**

*(in alphabetical order of first authors)
(Presenter's name is underlined when the main author listed first did not present the paper)*

ARAI M.; TAJIMA T.; SATO S.; KOMATSU T.; FUJIMURA T

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 08

Molecular mechanism of powdery mildew resistance in tobacco

Powdery mildew caused by *Erysiphe cichoracearum* is a major disease of tobacco affecting a wide area including Asia. The Japanese domestic air-cured tobacco cultivar Kokubu (syn. Kou-fan) exhibits extremely high resistance to powdery mildew by recessive alleles at two loci. This resistance has been introduced into several cultivars. Although the genes responsible for this recessive resistance are not identified, the loss-of-function mutation in the *Mildew resistance locus O (MLO)* gene confers broad-spectrum resistance to powdery mildew in various plant species. To elucidate the molecular mechanism of powdery mildew resistance of Kokubu, transcripts and genomic sequences of tobacco *MLO* orthologues were compared between powdery mildew resistant and susceptible cultivars. The tobacco genome included two *MLO* orthologues (*NtMLO1* and *NtMLO2*) corresponding to powdery mildew resistance. However, transcripts of both *NtMLO* genes of powdery mildew resistant cultivars included partial deletions or insertions. In DNA sequence alignment analysis, the dinucleotide substitution at the 3' end of intron 7 in *NtMLO1* gene and the dinucleotide deletion at the 5' end of intron 6 in *NtMLO2* gene were found in powdery mildew resistant cultivars. These mutations triggered the splicing alterations in *NtMLO* transcripts in powdery mildew resistant cultivars. Transgenic Kokubu expressing wild-type *NtMLO1* or *NtMLO2* exhibited severe disease symptoms, as did susceptible cultivars. These results demonstrate that powdery mildew resistance in Kokubu results from the splicing mutations of two *NtMLO* genes and the subsequent inhibition of functional *MLO* protein synthesis. Using the differences of nucleotide sequence of *NtMLO* genes, we developed DNA markers to detect the powdery mildew resistant or susceptible genes. These markers are expected to simplify the breeding of powdery mildew resistant cultivars and to obviate the use of test crosses for genotype determination.

Japan Tobacco Inc., Leaf Tobacco Research Center, 1900, Idei, Oyama, Tochigi 323-0808, Japan

BERBEC A.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 28

***Nicotiana* hybridisation map – "blank spots" yet to be filled**

The genus *Nicotiana* comprises, depending on adopted knowledge and criteria, from 69 to as many as 80 taxa. Of the more "exotic" species, some (*N. ameghinoi*, *N. azambujae*, *N. rastruensis*) exist only as herbarium specimens or on paper, some (e.g. *N. paa*, *N. fatuhivensis*, *N. stenocarpa*, *N. burbridgeae*, *N. monoschizocarpa*) have a dubious taxonomic status and some (*N. longibracteata*, *N. cutleri*, *N. truncata*, *N. heterantha*) do not seem to be readily available for study. Scientific experiments on hybridising *Nicotiana* species using various methods and for various purposes have been an important part of tobacco science and tobacco improvement for more than a century. A total of ca. 1,100 reports of various types, mostly research papers, and pertaining, directly or indirectly, to diverse aspects of interspecific hybridisation in *Nicotiana* were identified using popular web search engines and conventional approaches to literature retrieval. The cultivated *N. tabacum* was by far the most frequently taxon cited in that context (891 records) followed by *N. debneyi*, *N. glauca*, *N. glutinosa* and *N. rustica* (161, 153, 143 and 140 records, respectively). On the other end there were species for which no hybridisation or introgression-related data were located (*N. acaulis*, *N. spagazzini*) or which are mentioned in that context in no more than a single record (*N. mutabilis*, *N. cordifolia*, *N. petunioides*, *N. corymbosa*, *N. linearis*, *N. thyrsoflora*, *N. wigandioides*). For none of those aforementioned nine species, plus *N. attenuata* and *N. miersii*, has an attempt to hybridise with *N. tabacum*, successful or otherwise, ever been reported. For another eight species (*N. forgetiana*, *N. bonariensis*, *N. solanifolia*, *N. pauciflora*, *N. clevelandii*, *N. umbratica*, *N. kawakamii*, *N. arentsii*) evidence to that effect is very scant and sometimes only circumstantial. Those 19 species represent "blank spots" on the map of the genus treated as a source of potentially usable germplasm for tobacco. Their potential usefulness and probable reasons for missing from documented hybridisation efforts are briefly discussed.

Institute of Soil Science and Plant Cultivation - State Research Institute, ul. Czartoryskich 8, 24 100 Pulawy, Poland

BLANCARD D.(1); ARMAND J.M.(1); MARIGNAC E.(2)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 26

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

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

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

This development has two aims:

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

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

The application will be demonstrated during the poster session.

(1) *INRA, UMR Santé et Agroécologie de la Vigne, ISVV - IFR103, 71 Rue Édouard Bourlaux, 33883 Villenave-d'Ornon, France*

(2) *CORESTA, 11 rue du Quatre Septembre, 75002 Paris, France*

BROOKER A.G.P.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 26

Tobacco curing tunnels in Zimbabwe: a solution for sustainability

With pressure on to reduce demand on energy resources be they bioenergy or fossil fuels, there is a need to take advantage of the most efficient types of tobacco curing systems.

The continuous curing system in the form of either the cascade or tunnel is by far the most efficient system in use in Zimbabwe. Tunnels are slightly more efficient than cascades. Preference is being shown for the tunnels. A tunnel system will use approximately 25-30% of the energy used in a conventional barn.

To further reduce the energy demand of tunnels, provision can be made to use some solar energy. The additional cost of using the solar energy is quickly paid for in fuel savings.

Tunnels have been in use for nearly 50 years. However in the last 18 years constant improvements have been made that now make the tunnel a very sought after system. Improvements have been in the form of better understanding and control of the units as well as physical improvements.

As the percentage of tobacco cured through tunnels increases so the total energy used in curing will reduce. As the energy demand drops it will become easier to supply the demand from sustainable sources.

Brown Engineering, P.O.Box ST 311, Southerton, Harare, Zimbabwe

BUDIMIR A.(1); ŠARCEVIC H.(2); KOZUMPLIK V.(2); PLEŠA Z.(1)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 14

Testcross performance of new breeding lines of flue-cured tobacco for morphological and agronomic traits

In 2014 a field trial with ten new breeding lines of flue-cured tobacco, their test-hybrids with two line testers, and two controls, standard varieties widespread in the commercial production, was set up at three locations (Virovitica, Kutjevo1 and Kutjevo2) in Croatia. The cultural practices at all three locations were as recommended for commercial flue-cured tobacco production. At the location Kutjevo2 irrigation was additionally included. During vegetation, plant height, number of days to flowering, number of leaves per plant, 9th leaf length (L) and width (W), L/W ratio and the leaf area were assessed. After harvesting and curing leaf yield, average price and value/ha were determined. Analysis of variance revealed significant differences among genotypes for all the analysed traits except for number of leaves per plant. At the irrigated location (Kutjevo2) the average yield and value were significantly higher as compared with two no irrigated locations. Genotype by location interaction was significant for all traits except for number of leaves per plant, L/W ratio and value. Several lines and hybrids had significantly higher yield, price and value as compared to the standards. General combining ability (GCA) of the experimental lines was significant for all the traits except for plant height, L/W ratio and price and, GCA of testers was significant for all the traits except for plant height, L, yield and value. Specific combining ability of test-hybrids was significant only for width and area of the ninth leaf.

(1) *Croatian Tobaccos Inc., 33000 Virovitica, Croatia*

(2) *University of Zagreb, Faculty of Agriculture, Svetošimunska 25, 10000 Zagreb, Croatia*

CHAMANGO A.M.Z.; GOMONDA R.W.J.; MAINJENI C.E.D.; MSANGOSOKO K.R.; KUMWENDA R.L.N.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 31

New locally-bred nematode resistant Burley tobacco hybrids for Malawi

Burley tobacco is produced by over 200 000 farmers throughout Malawi. However, productivity losses caused by soil borne diseases, particularly root-knot nematodes (*Meloidogyne spp.*) remain a potential concern due to use of low yielding susceptible cultivars, diminishing crop rotations, and stringent global environmental standards associated with pesticide use. The majority of small-holder farmers cannot afford currently recommended nematicides because of high costs hence the need to develop nematode resistant cultivars. A study to evaluate nine hybrids and five checks for root-knot nematode resistance was conducted at four locations in Malawi from 2011/12 to 2013/14 cropping seasons. The trial was laid out as a randomised complete block replicated three times at each site. Nematode assessments were done at disease nurseries maintained through nematode augmentation practices in order to increase inoculum and pathogenicity. Agronomic traits including yield and quality aspects were assessed in rotated lands, applying all cultural practices as recommended. Data on nematode root gall-ratings were recorded using a 0-10 scale. Variable responses were observed between test hybrids and checks with respect to nematode infection. Three out of nine test hybrids had 30-40% lower root gall-ratings and were identified as being superior to currently recommended Burley varieties. Compared to the resistant check, BRK 4, nematode gall-ratings of three hybrids (ABH 12, ABH 31, and ABH 43) remained favourably within the acceptable range of a nematode resistant cultivar. These results suggest that breeding can serve as a tool for generating locally-bred nematode resistant cultivars thereby controlling root-knot nematodes sustainably without use of nematicides by all farmers in Malawi.

Agricultural Research and Extension Trust (ARET), Private Bag 9, Lilongwe, Malawi

CHIDA H.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 13

Mulching for tobacco cultivation in Japan

Mulching covering the ridge by plastic film is widely used for tobacco cultivation in Japan. It was introduced in the 1960s and was used in 98% of the total tobacco cultivation area in the 2014 crop. There are two main advantages of mulching by plastic film, and that closely relates to Japanese climate. One is to prevent the erosion of soil and fertiliser in the rainy season. Another is to accelerate initial growth by maintaining soil moisture and temperature that allows early harvest before the typhoon season. Today, many kinds of mulch are utilised in Japan.

Polyethylene film is the most popular and there are four types of transparent, black, silver and bicolour. Each of them has different properties in maintenance of soil temperature and in control of weeds and/or aphids.

Biodegradable plastic film has been used since the 2000s. It is eco-friendly material because it is decomposed to carbon dioxide and water by soil microorganisms. Therefore, it is not necessary to remove and discard after end of harvest. The penetration rate of biodegradable plastic film in the 2014 crop was only 9% because of its price and quality in the rate of decomposition.

Living mulch between tobacco rows using wheat or barley plants has recently been introduced. The penetration rate of living mulch in the 2014 crop reached 16%. In addition to the prevention of soil erosion and control of weed, it is effective to control the soil-borne disease, black shank, by preventing an adhesion of soil to tobacco leaves at the time of rainfall.

Japan Tobacco Inc., Leaf Tobacco Research Center, 1900, Idei, Oyama, Tochigi 323-0808, Japan

COLARD S.(1); GUITTON P.M.(2)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APOST 38

The process of CORESTA cooperation

The vision of CORESTA is “to be recognised by our members and relevant external bodies as an authoritative source of publically available, credible science and best practices related to tobacco and its derived products.”

To pursue this vision, CORESTA develops analytical methods, produces technical, study and reference reports, and publishes guides and recommendations on good practice and usage. CORESTA documentation is available from its website at www.coresta.org and reflects the work done within the Association through cooperation.

The steps leading to this work and the related outputs needed to be formally structured and documented in order to ensure sustainable improvements. A CORESTA Standards Task Force was then launched in 2012 to streamline the cooperation process and ensure that all steps were properly marked and reported to ease the follow-up of the on-going work and further archiving.

The poster will present the flow diagram that describes this process, and the responsible entities, starting from a new work item proposal up to the documents produced and made publically available.

Further perspectives on the future work undertaken by the CORESTA Standards Task Force and the corresponding impacts on the way of working will be commented.

(1) SEITA, Imperial Tobacco Group, 48 rue Danton, 45404 Fleury-les-Aubrais, France

(2) CORESTA, 11 rue du Quatre Septembre, Paris, France

COSIC J.(1); VRANDECIC K.(1); JURKOVIC D.(1); BUDIMIR A.(2); PLEŠA Z.(2); ZDELICAN J.(2)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 25

Tobacco diseases in transplant production in Croatia

In Croatia tobacco transplants are produced in greenhouses using the float tray system. The high plant density and high moisture provide extended periods of leaf wetness and promote development and spread of seedling diseases such as *Rhizoctonia* stem rot, *Pythium* root rot, *Sclerotinia* collar rot, *Botrytis* grey mould and blue mould. On the other hand, transplant production using sterile substrates significantly reduce the occurrence of disease in the initial stages of development. Notwithstanding the above, every year in transplant production we find plants with disease symptoms. The aim of this study was to identify pathogens that cause transplant damping-off. With the aim of identifying pathogens in the transplant production, we collected the young plants with disease symptoms during three years (2012-2014). Plant parts with symptoms were washed in running water, disinfected in a 96% ethanol and rinsed in sterile distilled water. Prepared plant parts were placed in Petri dishes with PDA and incubated in thermostat. Identifications were based on the mycelial growth rate, colony pigmentation, density of mycelia, presence or absence of spores and sclerotia and their morphology. In 2013 year we determined the occurrence of *Sclerotinia sclerotiorum* Lib. (de Bary) (causing agent of *Sclerotinia* collar rot), and in 2014 and 2015 years *Botryotinia fuckeliana* (de Bary) Whetzel (anamorph *Botrytis cinerea* Pers ex Fr.) (causing agent of *Botrytis* grey mould). Both pathogens affect a large number of plant species and their appearance is expected in the production of tobacco transplants in areas with well-developed agricultural production of field crops, vegetables, fruits and grapevine. Their appearance in the greenhouses is a consequence of the airborne spread of *B. cinerea* conidia and *S. sclerotiorum* ascospores. Soil borne pathogens (e.g. *Pythium* sp., *Rhizoctonia* sp.) have not been detected and due to the use of sterile substrates we in fact do not expect their appearance.

(1) *J.J. Strossmayer University, Faculty of Agriculture in Osijek, Kralja P. Svacica 1d, 31000 Osijek, Croatia*

(2) *Hrvatski duhani d.d. Virovitica, Osječka 2, 33000 Virovitica, Croatia*

CZUBACKA A.; KORBECKA G.; OLSZAK-PRZYBYS H.; DOROSZEWSKA T.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 22

Effectiveness of combining potato virus Y tolerance and resistance determined by transgenes in tobacco

Growing resistant cultivars is the most effective method of plant protection against viral diseases. Different sources of resistance are used for breeding new tobacco cultivars resistant to potato virus Y (PVY). The best source of resistance is *Nicotiana africana* because it is resistant to all tested PVY isolates. Tobacco breeding line BPA, derived from a cross between *N. tabacum* and *N. africana*, shows PVY tolerance introduced from the wild species. Other sources of resistance are transgenic lines: MN 944 LMV (containing gene of lettuce mosaic virus coat protein) and AC Gayed ROKY (containing antisense gene of PVY replicase). These transgenic lines show PVY resistance at least to some PVY isolates. Expecting that merging the factors of tolerance and transgenes in one genome will have the combined effect in plant protection, we obtained hybrid plants by crossing BPA line with each of the two transgenic lines. Then F₂ generation was tested for resistance to three PVY isolates differing with their virulence. Different levels of resistance among tested plants confirmed expected segregation of this trait. Results of biological tests were confronted with ELISA and PCR tests for presence of transgenes and markers linked to PVY tolerance.

Institute of Soil Science and Plant Cultivation - State Research Institute, ul. Czartoryskich 8, 24 100 Pulawy, Poland

DIMBI S.; KHUDDU G.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 42

Kutsaga's Tobacco Improved Productivity Sites (TIPS): for improved productivity and leaf quality of the small-holder crop

The last decade has seen unprecedented changes on the Zimbabwe tobacco landscape. For example, the grower base has increased from about 7 000 growers in 2000 to over 88 000 growers in the 2012/2013 season and currently, it is estimated that 80% of growers produce tobacco on a hectare or less. The average hectareage per grower has thus reduced from 40 ha in the 1990s to the current 1,5 ha. This led to a major strain in the provision of adequate training, extension and advisory services to growers especially as there has not been an increase in the research staff complement. In response, the Kutsaga Farmer Field Schools concept was introduced in 2007 with the idea of increasing both productivity and quality of tobacco grown by small-scale farmers. In 2011, the Field Schools evolved into the Kutsaga Tobacco Improved Productivity Sites (TIPS), which are group-based learning hubs where tobacco extensionists, agrochemical representatives and growers are taught skills in sustainable and profitable tobacco production. These sites are dedicated to the advancement of the tobacco industry through the provision of focused, local, relevant and practical farmer skills development. Additionally, strong collaboration of the Tobacco Research Board (TRB) with tobacco contractors, grower associations and the government tobacco extensionists in a train-the-trainer approach has enabled TRB to reach out to more growers than it would have been capable of on its own. Annually, since 2011, some ~70 sites have been established in all the 15 tobacco growing regions of Zimbabwe. At these sites some ~400 extensionists interact with well over 20 000 growers in the season. This modus operandi has enabled the TRB to cope with an ever increasing number of tobacco growers in Zimbabwe and to ensure a supreme quality flue-cured tobacco crop continues to be produced despite the changes in the grower base.

Tobacco Research Board, P.O. Box 1909, Harare, Zimbabwe

DIMITRIESKI M.; MICESKA G.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 17

Resistance to TMV (tobacco mosaic virus) and black shank (*Phytophthora parasitica* var. *nicotianae*) in some Oriental tobacco varieties and lines

The common tobacco mosaic virus and black shank disease can cause severe economic loss to tobacco production in some microregions of the Republic of Macedonia and in other tobacco producing countries worldwide. Considering the actuality of the problem, by means of generative hybridisation, a number of Oriental lines with high resistance to the above mentioned diseases have been developed at the Tobacco Institute-Prilep. Five of these lines and varieties were included in investigations carried out in the experimental field and in the bio-laboratory of the Institute. Three of them showed high resistance to TMV and black shank, one had high resistance to TMV and medium resistance to black shank. The standard variety YV125/3 was susceptible to both diseases. The resistant lines can be used as a starting material in hybridisation, for creation of new tobacco genotypes resistant to the economically important diseases.

Scientific Tobacco Institute-Prilep, University St. "Kliment Ohridski"-Bitola, st. "Kicevski pat" bb, 7500 Prilep, Republic of Macedonia

EDWARDS K.D.; BROMLEY J.R.; EVANS A.D.; HUMPHRY M.E.; SANCHEZ-TAMBURRINO J.P.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APW 01

'Omics': Opening a window to the workings of the tobacco plant

From its biochemistry and the underlying genetic architecture that regulates it, the composition of the tobacco plant is highly complex and far from understood. Applied to tobacco, the field of 'Omics' aims to characterise, quantify, and translate biological molecules and phenotypic traits into information that can help to decode the structure, function, and dynamics of the plant.

Technological developments in areas such as sequencing and mass spectrometry, as well as data-storage and -analytical capabilities, have led us to an era where 'big data' plays a major role in biological research. This is pertinent to tobacco, where growing amounts of genomic and transcriptomic data are becoming available, including assemblies of the tobacco genome. This data is providing fresh insight into the workings of the tobacco plant, and the tens of thousands of genes that its genome contains.

In addition to providing the basis for design of molecular markers to enhance quantitative genetic studies and molecular breeding capabilities, 'Omics' data also provides vital clues as to the function of tobacco genes based on an understanding of when and where they are expressed through transcriptomic and proteomic approaches. Complementary to this, metabolomics provides a broad view of the biochemistry of the plant and its tissues, which together with other phenomics approaches, provide an important handle on the next generation of traits desired in the tobacco plant.

Generating the data only covers half of the story and making sense out of it is equally, if not more, important. Systems biology, which ties together biological understanding with bioinformatics, and statistical analysis of 'big data' will play an important role in this.

In this talk the areas of genomics, transcriptomics and metabolomics will be introduced and some examples given of how they can be applied in the tobacco plant.

British American Tobacco, R&D Cambridge, 210 Science Park, Cambridge CB4 0WA, U.K.

FISHER C.R.(1); JACK A.M.(2); JI H.(2)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 19

TSNA accumulation in controlled curing environments

A three-year study from 2012-2014 was done to test the effects of temperature and relative humidity (RH) on TSNA (tobacco specific nitrosamine) accumulation in air-cured Burley tobacco. Twenty-four curing chambers were constructed, each holding six sticks of six stalk-cut plants. The temperature and humidity in each unit could be controlled independently of each other at constant levels for the duration of the cure and were monitored with data loggers. The tobacco was cured in the units at all combinations of three temperatures (16, 24 and 30 °C) and three humidities (60, 75 and 90% in 2012, and 50, 65 and 80% in 2013 and 2014). Each year, an additional treatment was cured in a conventional air-cured barn. In 2012, the variety was TN 90H (high converter selection of TN 90); in 2013, TN 90H and TN 90LC (commercial low converter); and KT 210 in 2014. Samples for leaf chemistry analysis were taken after the end of the cure each year, and also after 14 and 35 days in the 2014 test. Data were analyzed as a multiple regression of leaf chemistry parameters against RH and temperature. Conversion of nicotine to nornicotine was not affected by either temperature or RH in any of the three years. The multiple regression for N'-nitrosonornicotine (NNN) was significant in all years and all varieties. The RH component of the multiple regression was highly significant in all cases, but the temperature component was only significant in KT 210 in 2014, although it was almost significant in both varieties in 2013.

(1) *University of Kentucky, Plant and Soil Science Dept., 1405 Veterans Drive, 315 PSB, Lexington, KY 40546, U.S.A.*

(2) *University of Kentucky, Kentucky Tobacco Research and Development Center, KTRDC Building, 1401 University Dr., Lexington, KY 40546, U.S.A.*

FUKAZAWA N.; TAKAHASHI R.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 24

Effect of time and concentration on mortality of the cigarette beetle, *Lasioderma serricornis* (F.), fumigated with phosphine

Fumigant efficacy is determined by its concentration (C) and exposure time (t). For typical fumigants such as methyl bromide, the product of C and t is constant ($Ct = k$) to produce a specific toxic effect for a given insect species. Under these circumstances, double C requires half t to achieve the same effect. However, this model empirically does not apply to phosphine. Instead, some insect pests are known to respond according to a modified equation, $C^n t = k$ ($n < 1$). Here, double C is insufficient to achieve the same effect at half t . This study was conducted to clarify whether phosphine fumigation for the cigarette beetle, *Lasioderma serricornis*, follows the modified model, or not. Fifty adults each of six cultures with different phosphine-resistance levels (Culture S, R1, R2, R3, R4, and R5) were exposed to a range of concentrations of phosphine (1–2,000 ppm) at 25 °C for 24, 72, 120, or 168 h. A day after exposure, their viability was checked. The phosphine concentration necessary to achieve 50% lethality (LC_{50}) was calculated. Then the relation between C and t to achieve 50% lethality was determined. Results show that all six cultures fit the model $C^n t = k$ ($n < 1$) well: $C^{0.70} t = 169.8$ ($r^2 = 0.86$) for culture S, $C^{0.81} t = 1908.4$ ($r^2 = 0.84$) for culture R1, $C^{0.78} t = 2859.4$ ($r^2 = 0.85$) for culture R2, $C^{0.80} t = 3258.1$ ($r^2 = 0.89$) for culture R3, $C^{0.79} t = 2623.4$ ($r^2 = 0.84$) for culture R4, and $C^{0.64} t = 2464.4$ ($r^2 = 0.83$) for culture R5. These results demonstrate that extending the exposure time is more important for intensification of phosphine fumigation against *L. serricornis* than increasing the concentration, irrespective of the resistance level.

Japan Tobacco Inc., Leaf Tobacco Research Center, 1900, Idei, Oyama-shi, Tochigi 323-0808, Japan

GAO M.; ZAITLIN D.; JACK A.M.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APOST 20

Engineering resistance to TSWV (tomato spotted wilt virus)

Tomato Spotted Wilt Virus (TSWV) is a devastating tospovirus that ranks among the top 10 most economically important plant viruses, accounting for >1 billion dollars in annual crop losses. TSWV routinely causes extensive damage to field crops in the south-eastern U.S. While genetic resistance is known in tomato and pepper, there is no resistance in commercial tobacco (*Nicotiana tabacum* L.). Therefore, the use of RNA interference (RNAi) to introduce TSWV resistance into tobacco and *Nicotiana benthamiana* was investigated. TSWV is a complex RNA virus with a tripartite RNA genome consisting of small (S; 2.9Kb), medium (M; 4.8Kb), and large (L; 8.9Kb) single-stranded RNA molecules that encode five proteins (S1, S2, M1, M2, and L1 genes). Hairpin RNAi constructs were produced for each of the five TSWV genes using the pHellsgate8 binary vector. Stable transgenic plants were obtained via Agrobacterium-mediated transformation. T0-generation plants were inoculated with TSWV to screen for virus resistance.

For flue-cured tobacco variety K326, seven S1 lines, six S2 lines, seven M1 lines, two M2 lines, and nine L1 resistant transgenic lines were obtained. Two of the M2 transgenic plants displayed only weak resistance to TSWV infection.

For the Burley variety KY14, we obtained seven S1, three S2, 11 M1, and six L1 resistant transgenic lines.

For *N. benthamiana*, five S1, four S2, seven M1, three M2, and two L1 resistant transgenic lines were obtained. Three of the M2 transgenic plants expressed weak resistance to TSWV. TSWV inoculation of T1-generation plants derived from the T0 lines showed that virus resistance was segregating in all T1 progeny sets.

PCR amplification from genomic DNA showed that all resistant plants contain both the forward and reverse fragments of the RNAi silencing constructs. Also, results of Das-ELISA showed that the resistant plants were TSWV-free or contained low levels of the virus.

University of Kentucky, Kentucky Tobacco Research and Development Center, KTRDC Building, 1401 University Dr., Lexington, KY 40546, U.S.A.

GÜREL N.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 01a

Tobacco production in Turkey

Tobacco was introduced into the Ottoman Empire around 1603. It was banned several times and eventually accepted as a means of collecting taxes in 1860. During the Crimean War the Ottoman Empire defaulted on its debts. In 1874 a Tobacco Monopoly was established by the French “Regie de Tabac” and a mechanism was set up to collect all the tobacco produced and used to pay back the debt to the West.

As the end of the Ottoman Empire approached and the New Turkish Republic was established the Regie was transformed into “TEKEL”. There were also several buying arms for American cigarette companies as well as many dealer organizations trading in tobacco. The number of companies involved in tobacco was as high as 100 at one time.

In 2002 a new Law abolished the Monopoly Status of TEKEL and this was the beginning of the withdrawal of the Government from all aspects of tobacco in Turkey. Private firms were allowed to produce cigarettes in Turkey. Once the government sold TEKEL it also ended tobacco production. Turkish tobacco production went from a totally supported subsidized producer base to a free market system based on contract farming under regulation of the TAPDK.

The number of cigarette producers increased in the Turkish market and the number of leaf tobacco companies dramatically reduced. As the whole market went through a transformation several world class establishments and systems were applied to the Turkish production base. In the old system, tobacco had been produced all over the country based more on political whims than supply and demand.

Turkey is the biggest producer of aromatic Oriental tobacco and together with its product production base is becoming a sizable regional production hub. The tobacco business contributes over a billion dollars in exports and over 11 billion dollars is contributed to the inland revenue. The Turkish farmer base is about 80,000.

Several challenges exist that are being addressed, such as ageing farmer base, rapidly changing supply and demand and a constant struggle to become more efficient and sustainable. Rapidly changing technology is deployed in leaf tobacco processing to achieve these goals.

Aegean Tobacco Exporters' Association, Ataturk Cad. No: 382, Alsancak, 35220 Izmir, Turkey

GVEROSKA B.; MICESKA G.; DIMITRIESKI M.; KORUBIN-ALEKSOSKA A.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 27

Application of probiotics in tobacco production - possibility for protection against damping-off disease on tobacco seedlings

A long-term usage of chemicals in crop protection causes various negative effects such as resistance to pathogens, residues, expensive and not always effective protection, etc. The consumer demand for pesticide-free food imposed the need to substitute these products in agricultural production. Effective Microorganisms (EM) technology develops the concept of probiotics and their use in farming. Its basic principle is to apply and increase the population of effective and useful microorganisms in soil, which will displace degenerative microorganisms, especially soil pathogens. The aim of this study was to determine the impact of probiotics on damping-off disease and the possibility for their application in protection of tobacco seedlings. Investigations were made with the enhanced probiotic - the biofungicide Ema 5 (300 ml/100 m²) and the preparation EmFarma Plus (1000 ml/100 m²) in different variants - as a stand-alone application of biofungicides or in combination. In both assessments of the percentage infected area, the highest intensity of attack was noted in the variant where both agents were applied 15 days after sowing. The lowest intensity was recorded with combined application of the biofungicide Ema 5 with preparation EmFarma Plus in the soil before sowing. These results were obtained in both cases: without the use of herbicides or with their application after sowing. A minimum of two treatments are needed to achieve the full effect. The tested preparations reduced the intensity of attack and can be used in the control of damping-off disease on tobacco seedlings. Accordingly, probiotics have a good perspective in sustainable tobacco production.

Scientific Tobacco Institute-Prilep, University St. "Kliment Ohridski"-Bitola, st. "Kicevski pat" bb, 7500 Prilep, Republic of Macedonia

INMAN M.D.; VANN M.C.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 38

Alternative primary tillage practices for flue-cured tobacco in North Carolina

With increasing farm size and the necessity for timely field preparation, flue-cured tobacco producers are likely to benefit from alternative primary tillage methods used during field preparation. Research was conducted in 2012 and 2013 to determine the effects of differing primary tillage methods on soil physical properties and the growth and development of flue-cured tobacco. Three primary tillage systems were compared in two production regions of North Carolina: the Piedmont (fall ridging, rotary tillage, and spring ridging) and Coastal Plain (fall ridging, strip-tillage, and spring ridging). Treatments were arranged in a randomized complete block design and replicated four times. Soil bulk density, porosity, water content, and penetration resistance were determined after transplanting and after final harvest. Crop yield was determined following harvest and cured leaves were assigned a USDA grade to assess quality. Crop value was determined using a combination of leaf yield and quality. In the Piedmont region, fall ridging reduced leaf yield and value when compared to rotary tillage and spring ridging systems. The reduction in leaf yield and value with fall ridging is most likely associated with increased soil penetration resistance observed in 2012. Alternatively, in the Coastal Plain, primary tillage treatments did not affect agronomic aspects of production or soil physical properties. Results indicate that fall ridging and strip tillage systems were acceptable primary tillage methods for the production of flue-cured tobacco in the Coastal Plain of North Carolina. Alternatively, fall ridging in the Piedmont reduced tobacco yield and value, indicating that it is likely not an acceptable primary tillage method within the region.

North Carolina State University, Department of Crop Science, Williams Hall, Campus Box 7620, Raleigh, NC 27695, U.S.A.

JACK A.M.; FISHER C.R.; JI H.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 18

The effect on TSNAs of cutting and housing wet tobacco

The objective of this three-year study was to test our recommendation to growers not to cut or house wet Burley tobacco. The design was a split-split plot, with two cutting treatments, (1) cut wet, (2) cut dry; four housing treatments, (1) protected indoors on a railwagon, housed dry (2) exposed outdoors but kept dry, housed dry (3) stuck out in the field; exposed to wetting, housed dry (4) stuck out in the field; exposed to wetting, housed wet; and two varieties, high and low converter selections of TN 90. In the first year, 2011, there were no significant differences between any of the treatments. There were no differences between cutting treatments in any of the years, but in both 2012 and 2013, there were differences between housing treatments for some of the variables. When the years were combined, there were highly significant differences between years for all variables, and between housing treatments for some variables. In the lamina, there were significant differences between housing treatments in one or both varieties for NNN, NAT, NNK and total alkaloids, but not for total TSNAs (tobacco specific nitrosamines), nitrite nitrogen or nitrate nitrogen. Where there were differences between housing treatments in the individual TSNAs, the lowest were in the railwagon treatment and the highest were in the stuck out treatments. Total alkaloids were highest in the two housing treatments protected from rain, and lowest in the stuck out treatments, probably because some of the surface alkaloids were washed off by rain. We conclude that contrary to expectation, cutting tobacco wet (while inadvisable from other aspects) does not affect TSNA accumulation, but that housing treatments can affect it. Leaving tobacco stuck out for too long, particularly if it is subject to rain, can significantly increase TSNAs.

University of Kentucky, 1401 University Drive, 202B KTRDC Bldg., Lexington, KY 40546, U.S.A.

JIN Y.F.(1); WANG S.S.(1); LI J.Y.(2); PANG T.(2); ZHANG J.B.(1); GONG M.(1)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 41

Effects of different growth temperatures on alkaloid content and nicotine metabolism in roots, stems and leaves of tobacco (*Nicotiana tabacum* L.) plants

Alkaloids, especially nicotine content, significantly influences quality and flavour types of tobacco leaves, which is differentially regulated by various environmental conditions. To investigate the effects of different growth temperature on alkaloids and nicotine content as well as gene expression of nicotine metabolism-related enzymes in roots, stems and leaves of 12-leaf-old tobacco (*Nicotiana tabacum* L.) plants, the tobacco cultivar Yunyan 87 was cultivated in artificial climate chambers setting at three different growth temperature (mean temperature at 30.5, 23.5, and 16.5 °C with daily fluctuation). The results showed that the different growth temperatures influence growth, alkaloids and nicotine content as well as gene expression of seven nicotine metabolism-related enzymes in tobacco plants. The higher growth temperature at 30.5 °C lowered nicotine, anatabine, anabasine and nornicotine content in roots, stems and leaves. On the other hand, the lower growth temperature at 16.5 °C raised the alkaloid content in root and stem, and the medium growth temperature at 23.5 °C made leaves maintain the highest alkaloid content as compared with the lower and higher temperature treatments. RNA-seq and qPCR results showed that the seven gene expression of nicotine metabolism-related key enzymes was consistent with the change of nicotine content. These results suggested that the higher growth temperature quickly stimulated the synthesis of nicotine and translocation to tobacco leaves, the lower temperature inhibited the nicotine translocation, and the medium growth temperature was helpful for the long lasting accumulation of nicotine, which led to the highest nicotine content in tobacco leaves.

(1) School of Life Sciences, Yunnan Normal University, Kunming 650500, P.R. China

(2) Yunnan Academy of Tobacco Agricultural Sciences, Kunming 650021, P.R. China

JULIO E.; COTUCHEAU J.; DECORPS C.; LAURENT T.; DORLHAC de BORNE F.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 29

Characterisation of the *Rk1* resistance to *Meloidogyne* in tobacco by RNA-Seq analysis

The root-knot nematode (*Meloidogyne spp.*) is a major pest causing severe damages in many tobacco growing areas such as Europe, Brazil or Africa. The use of nematicides to fumigate soils is not compatible with efforts to reduce agrochemical residues on tobacco. With crop rotation, the most efficient alternative is to develop resistant cultivars adapted to the repartition of the different species of *Meloidogyne*: *M. incognita*, *M. arenaria*, *M. javanica*.

The *Rk1* dominant gene in tobacco conditions resistance to races 1 and 3 of the root-knot nematode *Meloidogyne incognita*. This resistance coming from *Nicotiana tomentososa* has been widely used for controlling nematodes in modern commercial cultivars such as K326 or NC95, with the help of RAPD markers to pilot the transfer.

In order to identify the *Rk1* gene responsible for nematode resistance, RNA-Seq analysis of a panel of 30 tobaccos was used to compare differentially expressed genes between resistant and susceptible flue-cured cultivars. Candidate contigs have been identified, including sequences having high homology with late blight resistance homolog protein of tomato and potato.

Two contigs belonging to the same gene were validated, by confirming the *N. tomentososa* origin of these sequences, and also by testing on a F2 segregating population characterised for the presence of Rk with a SCAR marker developed from RAPD. Finally, this gene was also validated on 800 cultivars of the Imperial Tobacco collection.

SEITA, Imperial Tobacco Group, La Tour, 24100 Bergerac, France

JULIO E.(1); VERRON T.(2); CAHOURS X.(2); DORLHAC de BORNE F.(1); COLARD S.(2)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. IG 01

Association mapping in a collection of tobacco reference cultivars. Step One: Variability of smoke constituents

Recent developments of sequencing technologies and computational methods have given the opportunity to detect natural variation underlying complex traits in crops. For this purpose, association mapping can be used to identify the link between tobacco genes or molecular markers to smoke constituents. Such association can support the development of new tobacco varieties suitable for future potential regulatory constraints.

In our study, a panel of 161 tobacco varieties, composed of flue-cured, Burley, dark air-cured and Oriental types, was grown in open field. Because of its large genome, RNA-Seq based sequencing was chosen to capture differences of gene expression together with SNPs variation in the 161 varieties. Three different tissues at two growth stages were used to do a comprehensive analysis of the transcriptome. After curing, cigarettes were made with each variety and were mechanically smoked according to the Canadian Intense smoking conditions. The mainstream harmful or potentially harmful constituents (HPHCs) listed in the FDA abbreviated HPHC list^[1] were determined using in-house and internationally recommended methods.

Taking into account multiple factors linked to the growing environment and weight of tobacco actively burnt during puffing, our first investigations showed significant differences of several smoke constituents between varieties, thus demonstrating the potential of association mapping for the development of future varieties. For some constituents, differences were however not significant. A description of the approach and preliminary results obtained from the smoking of a sub-group of flue-cured varieties is reported here.

[1] U.S. Food and Drug Administration, 2012. Reporting Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke under Section 904(a)(3) of the Federal Food, Drug, and Cosmetic Act.
<http://www.fda.gov/downloads/TobaccoProducts/GuidanceComplianceRegulatoryInformation/UCM297828.pdf>

(1) SEITA, Imperial Tobacco Group, La Tour, 24100 Bergerac, France

(2) SEITA, Imperial Tobacco Group, 48 rue Danton, 45404 Fleury-les-Aubrais, France

KARAMANLIS K.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 36

Carbon footprint of Oriental variety Katerini 53

One of the human activities that aggravates global climate change is the agricultural sector, which produces 9% of carbon emissions. In this paper the carbon footprint of the Oriental variety Katerini 53 is calculated at the farmer's level based on the GreenHouse Gas Protocol. Both direct and indirect emissions are released in order to measure the impact of farming on the environment in a crop year. The data collection was done by method of questionnaires and interviews, including information from the beginning of cultivation until the farmer's final product (cradle-to-gate inventory), of 35 farmers from Pieria prefecture which is the largest production area and the more representative.

The total carbon footprint of cultivation is 1,6968 tCO₂eha⁻¹ in a crop year. Per production stage, transplanting has the biggest impact at a rate of 54.78% of total carbon emissions. Harvesting/curing and field cultivation stages produce 21% and 12% of carbon footprint respectively. The lowest impact was observed on seedbed and baling/purchase stages with 9% and 3% of carbon emissions respectively. Based on inputs, 67.12% of emissions (1,1389 tCO₂eha⁻¹) are produced by fuel consumption. Fertilisers produce 18.69% of the carbon emissions, other inputs 11.11% and finally crop protection agents (CPAs) 3.07%.

As shown, producers need to move to a more efficient use of energy-fuel and fertiliser by addressing the problem of fragmented land and applying the principles of integrated crop management respectively.

Postgraduate Quality Assurance Course, School of Science and Technology, Hellenic Open University, Patra, Greece

KORUBIN-ALEKSOSKA A.; GVEROSKA B.; DIMITRIESKI M.; ALEKSOSKI J.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 13

Breeding for drought tolerance in tobacco

Today we are witnessing continuous spreading of drought as a result of global warming. The need for irrigation increases for all crops, including tobacco. The Oriental sun-cured tobacco does not require large quantities of water, but certain amounts of precipitation during the growing season are still necessary for obtaining a good quality raw material. The shortage of rain and possibilities for irrigation can be overcome by selection of varieties tolerant to drought. Tobacco Institute-Prilep has developed programmes for investigation of the assortment and improvement of the existing varieties and creation of new genotypes. The investigation included nine varieties belonging to different types of tobacco, a part of which have been commercially used in the Republic of Macedonia and others are kept for further breeding. The trial was set up in the experimental field of the Institute during 2012 and 2013 in randomised blocks with three replications. Each variant was investigated in conditions with and without irrigation. The aim of the paper was to study the Oriental varieties in order to obtain data on their tolerance to drought and to supply material for further selection. Drought tolerance was determined by classical breeding methods based on phenotypic expression of morphological and production traits. For this purpose, modern breeding programmes are using molecular markers in different stages of the selection process. The highest degree of tolerance to drought was observed in genotypes P-84 (type Prilep) and P-2 (type Djebel). These genotypes can be included in the programmes for improvement of the investigated trait.

Scientific Tobacco Institute-Prilep, University St. "Kliment Ohridski"-Bitola, st. "Kicevski pat" bb, 7500 Prilep, Republic of Macedonia

KRSTESKA V.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 33

Fauna of hoverflies (Diptera: Syrphidae) in tobacco biocenosis

Larvae of the aphidophagous species of hoverflies are of major importance in regulation of the number of aphids. The main goal of the investigations was to perform faunistic analysis of the family Syrphidae.

The analyses were carried out in the tobacco biocenosis in the Prilep area. We used the following methods: examination of 20 tobacco stalks, the examination of 100 tobacco leaves, the yellow water vessels method, the insect-catcher method and faunistic analyses with the following parameters: active dominance, active abundance, consistency or frequency.

The quantitative analyses is based on final evaluation of the total number of collected individuals: 2900 of *Sphaerophoria scripta* L., 2147 of *Sphaerophoria rueppelli* Wied., 1833 of *Scaeva pyrastris* L., 647 of *Episyrphus balteatus* De Geer, 146 of *Eupeodes corollae* Fab., 2 of *Syrphus ribesii* L., 184 of *Paragus quadrifasciatus* Meig., 5 of *Paragus bicolor* Fab., 19 of *Paragus tibialis* Fallen, 101 of *Melanostoma mellinum* L., 21 of *Eristalis tenax* L., 7 of *Eristalis arbustorum* L. and 15 of *Syrpita pipiens* L.

S. scripta, *S. rueppelli*, *S. pyrastris* and *E. balteatus* are dominant species. *E. corollae*, *P. quadrifasciatus* and *M. mellinum* are sub-dominant species. Other species have very low dominance and they are sub-recedent species. *S. scripta* was the most numerous species in each year of study and by all study methods. *S. scripta* was euconstant species in 2004 and 2005 and was constant in 2003. *S. rueppelli* was a constant species during all years. *S. pyrastris*, *E. balteatus* and *E. corollae* were confirmed as less constant. Other species were accidentally present in tobacco entomocenosis in Prilep.

The increase of larval abundance of aphidophagous hoverflies approximates the period of mass reproduction of aphids in tobacco. Based on the results, *S. scripta*, *S. rueppelli*, *S. pyrastris* and *E. balteatus* can be used for biological control within the integral protection of tobacco against aphids.

UKLO Scientific Tobacco Institute-Prilep, Kicevska bb, 7500 Prilep, Republic of Macedonia

LI Wei(1); ZHANG Hailiang(1); LIU Cheng(1); DU Yongmei(1); GAO Xiaoming(1); WANG Guodong(2); GUO Yongfeng(1)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APW 03

Transcriptomic and metabolomic studies reveal spatiotemporal switches of primary and secondary metabolism during leaf senescence of tobacco

Several features of tobacco (*Nicotiana tabacum*) make it a unique system in studying the metabolome of leaf senescence: 1) as topping being a routine agricultural practice, sequential senescence of leaves at different positions (leaves at lower positions senesce first) makes tobacco useful in studying nutrient remobilisation from old leaves to younger leaves at the top; 2) tobacco has a rich secondary metabolism. In this study the metabolome of tobacco leaf senescence was analysed through profiling of ~80 major metabolites of leaf samples from three representative positions at eight different time points. The metabolomic analysis was supplemented with RNA-seq data from the same leaf samples. Comprehensive analyses on metabolite accumulation and expression changes of enzyme-encoding genes in corresponding metabolic pathways indicated a significant up-regulation of the tricarboxylic acid cycle and related metabolism of sugars, amino acids and fatty acids, suggesting the importance of energy metabolism during leaf senescence. Taken together with an overall up-regulation of the gene expression machinery including ribosomal proteins and RNA polymerases, results from this study support leaf senescence being an exceedingly active process - with senescing leaves having higher activities of gene expression and energy metabolism than young leaves. Other changes of the metabolome during tobacco leaf senescence include increased biosynthesis of nicotine and chlorogenic acid. Our results also suggest that a number of compounds seem to be transported from senescing leaves at lower positions to younger leaves at upper positions. This includes glutamine, sucrose, 2-furoic acid, 4-hydroxy-3-methoxybenzoic acid, caffeic acid, chlorogenic acid, phenylalanine, D-turanose, guanosine, hydroquinone, L-cysteine, L-mimosine, L-tyrosine, melibiose, m-hydroxybenzoic acid, nicotine, nicotinic acid, uracil, and 4-hydroxybenzoic acid, some of which could play a role in nutrient remobilisation.

(1) *Tobacco Research Institute, Chinese Academy of Agricultural Sciences, Qingdao 266101, P.R. China*

(2) *Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, P.R. China*

LI Yafei(1); SHI Hongzhi(1); SUN Junwei(2); YANG Huijuan(1); WANG Jing(1); HU Kun(1)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APOST 09

Effect of changing curing methods on TSNA formation for flue-cured and Burley tobacco

Curing is the most important stage of TSNA formation. Two curing methods, flue-curing and air-curing, are commonly used for flue-cured tobacco and Burley, respectively. Since different curing methods differ in curing environments, this might have significant impacts on TSNA formation during curing. The experiment was carried out to investigate the effect of changing curing methods for flue-cured and Burley tobaccos on TSNA contents in cured samples. Flue-cured tobacco variety Hongda and Burley variety TN90 were planted in the Yunnan Province of China. Matured leaves from both flue-cured and Burley tobacco were subjected to three curing methods, flue-curing (FC), air-curing (AC), first air-curing then flue-curing from the end of yellowing (F+A). The results showed that TSNA contents in Burley tobacco were generally higher than that of flue-cured tobacco. There were significant differences in TSNA contents among curing methods for each of the two tobacco types. For Burley tobacco, the TSNA content from F+A was the highest followed by air-curing (AC), and flue-curing (FC) resulted in lowest TSNA contents. Compared with normal air-curing, F+A method increased TSNA contents by 43.2% for middle leaves and 121.3% for upper leaves. For flue-cured tobacco, the TSNA content from F+A method was also the highest, followed by FC, and the AC method produced the lowest TSNA content. Compared with normal flue-curing (FC), the F+A method increased total TSNA contents by 18.4% for middle leaves and 115.0% for upper leaves. The highest TSNA contents resulted from A+F for both tobacco types suggesting that the slow yellowing and high-temperature leaf drying was favourable to TSNA formation. This could be explained by the fact that the slow yellowing process favoured bacterial activity that reduces nitrate to nitrite, and by the fact that high temperature leaf drying promotes nitrate to become gaseous NO_x, which easily reacts with alkaloids to form TSNA.

(1) *Henan Agricultural University, National Tobacco Cultivation & Physiology & Biochemistry Research Center, Tobacco Cultivation Key Laboratory of China Tobacco, 95 Wenhua Road, Zhengzhou 450002, P.R. China*

(2) *Dali Tobacco Company of Yunnan, Dali, P.R. China*

LION K.; LUSSO M.; MORRIS W.; HART F.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 17

Tobacco specific nitrosamine (TSNA) levels of the U.S. domestic Burley crop and their relationship with relative humidity conditions during curing

From 2010 to 2014, Altria Client Services conducted a sampling program designed to evaluate tobacco specific nitrosamine (TSNA) levels in the U.S. domestic Burley crop. A representative number of bales from four distinct U.S. Burley growing regions were sampled each year in order to estimate average TSNA levels for each region. Significant variation in TSNA levels were observed both across regions within a given crop year and across crop years within a given region. Annual regional TSNA averages over the course of the study ranged from as low as 2.5 ppm up to 18.3 ppm. Relative humidity levels were investigated as a potential source of this variation since high relative humidity during curing creates an environment that favors microbial growth and consequently higher nitrite availability for the formation of TSNA. To evaluate the relationship between relative humidity conditions during curing and TSNA levels, relative humidity information was gathered from publicly available weather station data across each of the four Burley growing regions during the sampling program. Based on R-squared values, strong positive correlations were observed between relative humidity conditions during the first several weeks of curing and TSNA levels produced under those conditions.

Altria Client Services Inc., Research, Development & Engineering, 601 E. Jackson St., Richmond, VA 23219, U.S.A.

LIU Yanxia; LI Xiang

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 06

Integrated measures for bio-control of tobacco bacterial wilt and the effect on soil microorganisms

Tobacco bacterial wilt is one of the most serious problems in southwest China. Single treatment approaches are not normally effective and so developing efficient integrated measures for biocontrol of tobacco bacterial wilt is very critical. In this investigation, a three-year field trial was conducted in a severely bacterial wilt infected field in the Guizhou Province. Bioorganic fertiliser (BOF) application, deep ploughing + BOF and deep ploughing + liming + BOF were used as alternatives to control tobacco bacterial wilt and as soil treatments. The study focused on how the integrated measures could affect the soil rhizosphere microbiota by high-throughput sequencing of rhizosphere microbial genome. After three years of treatment, the control efficacy of tobacco bacterial wilt in treated soils where integrated measures were adopted was up to 61.30%. The yield and output value of tobacco leaves increased significantly. Compared with the control, the soil pathogen decreased significantly. The population of pathogens was suppressed to less than 10^7 cfu/g soil. The colonisation of antagonists was strengthened while the rhizosphere microbial structure became more balanced. Furthermore, the functional diversity significantly increased. Integrated measures could efficiently control tobacco bacterial wilt in a severely bacterial wilt infected field. Integrated measures not only increase tobacco yield and output value, but also efficiently improve soil microbial diversity, thus possessing a promising application prospect.

Guizhou Academy of Tobacco Science of CNTC, Guiyang, Guizhou 550000, P.R. China

LUSSO M.; LION K.; MORRIS W.; ADAMS A.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 20

N-nitrosornicotine (NNN) level in lamina of experimental Burley isogenic lines bred to have stable low nornicotine content and cured under conditions favorable for TSNA formation

N-nitrosornicotine (NNN) is a tobacco specific nitrosamine (TSNA) identified in cured tobacco leaf and smoke. NNN is formed through nitrosation of the tobacco alkaloid nornicotine by the nitrosating agent nitrite. Nitrite is derived from reduction of nitrate by microbial activity during curing. High relative humidity during curing creates a favorable environment for microbial growth and consequently higher TSNA levels. Although growers cannot control ambient relative humidity, they are guided to manage barn ventilation during air-curing in a way to balance between enough humidity for good quality and enough ventilation to minimize TSNA formation. Other curing practice recommendations include spacing the plants evenly on sticks, spacing sticks evenly on the rails, and avoiding packing sticks too tightly. We previously demonstrated that by stabilizing a low level of the precursor nornicotine in Burley tobacco, the levels of NNN in cured leaf and smoke of Burley cigarettes can be reduced by about 75%. Here we present the performance of experimental Burley lines stable for low nornicotine content when cured under conditions that favor TSNA formation. Control and experimental Burley lines were grown in 2014 in Virginia and cured in control and experimental barns. The control barn was housed with normal stick density and managed according to practices recommended to minimize TSNA formation. The experimental barn was packed with about 1/3 more tobacco plants than the control barn and managed to retain high relative humidity during curing. Relative humidity fluctuated between 60% and 91% in the control barn and between 78% and 94% in the experimental barn. At the end of curing, leaf samples were collected and analyzed for alkaloid and TSNA levels. NNN levels in the stable low nornicotine line were reduced by 63% and 76% in the control and experimental barns, respectively, relative to the control line.

Altria Client Services Inc., Research, Development & Engineering, 601 E. Jackson St., Richmond, VA 23219, U.S.A.

MANTCHOMBE M.K.; MSANGOSOKO K.R.; CHAMANGO A.M.Z.; GOMONDA R.W.J.; KHUMBANYIWA A.G.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 12

The Integrated Tobacco Production System (IPS) model: opportunities, challenges and future prospects for Malawi

For almost two decades, ARET has been the major player in providing research and extension services aimed at improving tobacco productivity in Malawi. However, there has been a paradigm shift following introduction of the Integrated Production System (IPS). IPS is a production model that involves establishment of contractual obligations between growers and merchants for the production and marketing of tobacco in a sustainable manner. The model was introduced in the 2012/13 season as part of a tobacco industry wide production approach to improve tobacco yields and quality, and means to control supply in relation to global requirements. Under the current arrangement, 80% of the total leaf produced is sold through the contract system while 20% still goes through auction.

Inasmuch as strides have been made since its inception, there have been benefits as well as challenges. Some notable benefits include: improved access to input loans resulting in the doubling of average tobacco yields (from 800 kg/ha to 1700 kg/ha), considerable improvement in leaf quality, improved access to production information due to multiplicity of players offering extension services, farmer compliance to global tobacco marketing requirements such as seed integrity and traceability, provision of a platform for enhanced research collaboration, provision of a framework for delivery of corporate social responsibility, among others. Among the challenges that have affected farmers negatively include: unclear contractual agreements, conflicting messages by different extension providers in the industry, late delivery and sometimes over charged inputs, introduction of unverified technologies and unclear forward pricing. In view of the foregoing, there is need for closer collaboration by all players involved to ensure that farmers are not put at a disadvantage. ARET needs to take a leading role in ensuring adherence to best practices and set standards. The IPS, if properly coordinated, would benefit growers and the nation at large.

Agricultural Research and Extension Trust (ARET), Private Bag 9, Lilongwe, Malawi

MANTCHOMBE M.K.; NYANGULU J.E.S.; CHIWAYA P.G.; MSANGOSOKO K.R.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 07

Host farmer demonstrations: a viable strategy for reaching out to growers with new tobacco technologies in Malawi

Tobacco plays a very critical role in the economy of Malawi, accounting for 60-70% of the country's foreign earnings and about 12% of its GDP. Owing to this, the Agricultural Research and Extension Trust (ARET) was established in 1995 with the mandate to generate and disseminate tobacco technologies for improved tobacco production in Malawi. This paper describes the effectiveness of host farmer demonstrations over other conventional methods as a tool for promoting and disseminating new and upcoming tobacco technologies in Malawi. With the repeal of the Special Crops Act in 1990, Malawi saw an influx of farmers venturing into tobacco production. Currently, over 200,000 smallholder farmers grow tobacco on an annual basis. Some of the methods that ARET uses to reach out to farmers include individual contacts, whistle stops, print and electronic media. Among these methods, for instance, individual visits, while being effective, reach out to fewer farmers. In contrast, host farmer demonstrations have proved to be superior in the sense that they have increased farmer participation; offers hands on, and provide a platform for farmers to learn about various new tobacco technologies, for instance, tobacco varieties, new chemicals as well as good agriculture practices (GAP). The results over the past three seasons show that 2000, 2500, and 33700 contacts in the 2011/12 season; 1500, 2000, and 23320 contacts in the 2012/13 season; and 7000, 5000, and 48,357 contacts in the 2013/14 season for individual visits, whistle stops and result demonstrations, were reached out, respectively. These figures suggest that more farmers were reached through host farmer demonstrations compared to the other two approaches confirming the effectiveness of this approach. In view of this, ARET will continue to use host farmer demonstrations as one of its key strategies in technology dissemination to tobacco farmers in Malawi.

Agricultural Research and Extension Trust (ARET), Private Bag 9, Lilongwe, Malawi

MARTELLINI B.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. IG 02

Update on the progress of the Framework Convention on Tobacco Control

The last Conference of Parties of the Framework Convention on Tobacco Control (FCTC) met in October 2014, and made decisions on taxation, product content and disclosure, electronic nicotine and non-nicotine delivery systems (ENDS/ENNDS), alternative products, liability and trade matters. These decisions will influence the industry's future research.

For product content, further work will address constituents, and also disclosure, testing and measuring cigarette emissions and contents and include them in future guidelines. Cigarette characteristics such as slim size, filter ventilation, flavour delivering capsules may be the object of next guidelines. Dependence and liability of all tobacco products will be monitored. The WHO will assess if Standard Operating Procedures for nicotine, TSNA and B[a]P in cigarettes are applicable to other tobacco products.

For ENDS/ENNDS, Parties were recommended to consider banning or regulating. The WHO was invited to consider analytical methods for contents and emissions, and to prepare a report on ENDS/ENNDS potential role in quitting tobacco usage and impact on health and tobacco control efforts.

In terms of alternative crops to tobacco, the main policy options and recommendations approved covered the inclusion of tobacco growers in policy development and the liability of the tobacco industry for health, social and environmental damages in tobacco production and in the supply chain.

In terms of trade facilitation, Parties of the FCTC were encouraged to cooperate in exploring legal options to minimise the risk of the industry making undue use of international trade and investment instruments to target tobacco control measures, and to take into account public health objectives when negotiating trade and investment agreements.

In terms of liability, the FCTC secretariat will study procedures for settling disputes on the interpretation or application of the convention, the kind of disputes that may be subject to such procedures and the interaction of the procedures with other dispute settlement mechanisms.

ULTOCO Services S.A., 82 rue de Lausanne, CH-1202 Geneva, Switzerland

MAZIER M.(1); NOGUÉ F.(2)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APW 04

TALEN and CRISPR strategies for targeted editing of the plant genome

Despite certain political concerns in some countries, transgenesis is already an indispensable technology for seed companies and public scientists to remain competitive at the international level.

Recent scientific advances in the field of transgenesis now provide answers to certain reservations expressed by citizens and blurs the border between breeding and transgenesis.

The advent of nuclease technology opens the way to extremely precise modifications of plant genomes at pre-determined sites. Among the nucleases used until now, transcription activator-like effector nucleases (TALENs), as well as the clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9) system, have proved to be particularly promising, driving to innovative applications close to revolutionising basic research and plant breeding.

In this talk, recent developments in the field of targeted genome editing technologies in plants will be covered.

(1) *INRA-UR1052-GAFL Génétique et Amélioration des Fruits et Légumes, Domaine St Maurice, 67 Allée des chênes, CS 60094, 84143 Montfavet Cedex, France*

(2) *INRA UMR1318 IJPB Institut Jean-Pierre Bourgin, INRA Centre de Versailles-Grignon, Route de St-Cyr, 78026 Versailles Cedex, France*

MICESKA G.; DIMITRIESKI M.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APOST 18

Production and quality characteristics of some new Yaka tobacco varieties

A comparative trial was carried out in 2011, in the experimental field of Tobacco Institute-Prilep, with some Yaka tobacco varieties in order to study their morphological, productive and qualitative characteristics. The trial included five new lines of the Yaka tobacco type (Yk 65-82/1, Yk 1.301-123/82, Yk 1.11-46/65, Yk 1.14-63/82, YK 1.7-65/31) and the variety Yk 125/3 as a standard. From the production and qualitative properties (d/blade, kg/ha, % of classes, and % economic impact), it was concluded that the new Yaka tobacco varieties have greater yield per plant and so per hectare and higher economic output-gross cash income per hectare was 20-80% higher compared with the control variety JK 125/3. The share of Yaka tobacco in the total tobacco production in the country is about 5%. We hope that the new developed Yaka lines and varieties will find their place in the commercial tobacco production of the Republic of Macedonia.

Scientific Tobacco Institute-Prilep, University St. "Kliment Ohridski"-Bitola, st. "Kicevski pat" bb, 7500 Prilep, Republic of Macedonia

MICESKA G.; KORUBIN-ALEKSOSKA A.; ALEKSOSKI J.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APOST 12

Variability in some old tobacco varieties in the Republic of Macedonia

The studies were made with five old tobacco varieties of the types: Prilep, Djebel and Yaka, to study their quantitative traits: height of the stalk without inflorescence, number of leaves and green mass yield per stalk. The trial was set up in the experimental field at the Tobacco Institute-Prilep in 2010, 2011 and 2012, in a randomised block design with four replications. Traditional agro-technical measures were applied during the period of vegetation.

The purpose of investigations was to evaluate the variability of the above quantitative traits typical for the old varieties by the use of biometric analysis and to give directions for their maintenance in future for selection purposes in tobacco.

The significant differences obtained between the traits of investigated variants indicate that they are different varieties adapted to agro-ecological conditions of the region. No significant differences were observed between the three years of investigation. It is an indication of highly heritable traits. The values of standard deviation and coefficient of variability are low, which is an indication of stable and homozygous genotypes. Results on the parameters of variability were lower in 2012, because the seed sown in this crop was obtained from one stalk for each variant isolated in 2010 and 2011. The lowest statistical data on variability of stalk height and leaf number in three years were registered in the varieties of type Prilep, and for green mass yield in the Yaka tobacco.

Scientific Tobacco Institute-Prilep, University St. "Kliment Ohridski"-Bitola, st. "Kicevski pat" bb, 7500 Prilep, Republic of Macedonia

MIHAYLOVA-KROUMOVA A.; ARTIOUCHIE I.; WAGNER G.J.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 07

Use of natural products from tobacco and other *Nicotiana* species to prevent black shank disease in tobacco

Black shank disease, caused by the fungus *Phytophthora parasitica* var. *nicotianae*, is a major annual threat to all types of tobacco worldwide. Thus far, the main approaches to control this disease are the creation of resistant tobacco varieties and application of fungicide treatments. Some fungicides are reported to have negative effects on the environment. The goal of this work was to test the antifungal activity of several natural products that are synthesized by tobacco and other *Nicotiana* species, and secreted to the leaf surface. We hypothesized that phylloplanin, *cis*-abienol, labdene-diol and sclareol can suppress race zero and race one-caused disease in Burley tobaccos KY14 and KY14×L8. We developed methods for leaf surface extraction, spore preparation and soil application of working concentrations of the natural compounds tested. Experiments were performed on the five to eight week-old seedlings, in triplicates. *cis*-Abienol showed high inhibitory properties toward the disease. Race zero infection was completely subdued in KY14, and race one infection was reduced by 80% and 70% in KY14 and KY14×L8, and delayed by six to ten days. Sclareol was very effective in inhibiting race zero-caused disease in both tobacco lines. KY14×L8 race one infection was inhibited by sclareol while in KY14 it was reduced by 85% and delayed by six days. Labdene-diol reduced the disease by half in eight week-old KY14 plants. Tobacco phylloplanin reduced plant infection by both races by 50% to 60% and delayed the disease by six to ten days. We consider sclareol to be the best candidate for future studies due to its antifungal properties and availability. Despite the good antifungal activity of *cis*-Abienol, it is not feasible for a large scale soil application due to the production efforts.

University of Kentucky, Kentucky Tobacco Research and Development Center, KTRDC Building, 1401 University Dr., Lexington, KY 40546, U.S.A.

MO Xiaohan; XU Ping; ZHAO Xingneng; ZHANG Lifang; QIN Xiyun; XIA Zhenyuan

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 03

The interactions between tobacco vein distorting virus and tobacco bushy top disease-associated RNA

Tobacco bushy top disease is the only plant viral disease caused by the umbravirus/luteovirus complex so far in China, resulting in significant economic losses in tobacco production in the Yunnan Province of China. Tobacco bushy top disease complex is the most complicated umbravirus/luteovirus complex discovered so far. The coat proteins of the virions of tobacco bushy top disease complex are encoded by tobacco vein distorting virus (TV DV) only, while there are five distinct viral RNA components being encapsidated. The agroclones of TV DV and tobacco bushy top disease-associated RNA (TBTDaRNA) were obtained and used as a reverse genetics platform to investigate the interactions between these two viral components through agroinfection. The agroclone of TBTDaRNA is the first agroclone of Polerovirus-associated RNA built to date. TV DV alone could infect *Nicotiana benthamiana* systemically via agroinoculation without prominent symptoms. While TBTDaRNA itself could not infect *N. benthamiana* systemically, the plants developed intensive disease symptoms when agroinoculated with TBTDaRNA and TV DV simultaneously, and the two viral components could move systemically in the plants. The results indicated that TV DV could facilitate the systemic movement of TBTDaRNA in plants, and TBTDaRNA helped TV DV in boosting the development of disease symptoms in *N. benthamiana*.

Yunnan Academy of Tobacco Agricultural Sciences of CNTC, Kunming 650031, Yunnan, P.R. China

MSANGOSOKO K.R.; MAINJENI C.E.D.; GOMONDA R.W.J.; CHAMANGO A.M.Z.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 02

Scout based application and integration of nematode resistant cultivars: an IPM strategy for reducing pesticide use in tobacco production in Malawi

Tobacco production in Malawi faces serious pest problems such as the tobacco aphid (*M. persicae*) and root-knot nematodes (*Meloidogyne spp.*). A considerable amount of pesticides are used by growers to protect tobacco from yield and quality losses resulting from these pests. The common practice is that growers apply pesticides based on routine applications. However, their high cost and increasing concerns for off-target effects, for instance, chemical residues, raise questions to re-evaluate their use. Research to evaluate the effectiveness of scout based control and a newly bred nematode resistant Burley hybrid ABH 31 was conducted at Kandiya Research Station in Lilongwe, Malawi, from 2013 to 2015. The aim of this study was to develop an IPM concept for major tobacco pests (aphids, nematodes), especially, focusing on minimising pesticide use by way of timing applications, and integrating resistant cultivars. The trial comprised four insecticide regimes: Imidacloprid at planting, scout based application, routine based application, and untreated; three nematicide regimes: Zero, half, and full recommended rate; and three Burley cultivars, ABH 31 (nematode resistant), Mkanachikhosi and KBM 33 (both nematode susceptible) were evaluated in randomised block designs. Results showed that application of insecticides based on scouting achieved the same level of control against major pests as the routine based application. The approach also reduced the number of applications by two thirds in the growing season. The integration of a nematode resistant cultivar (ABH 31) controlled root-knot nematodes even better than relying on nematicides alone. No significant differences were found on nematode galling scores when ABH 31 received full, half or no nematicide, confirming the need for an integrated management approach. These results suggest that growers can achieve the desired pest control through integrated pest management while reducing pesticide use and environmental contamination and with potential accrual of economic returns per unit area.

Agricultural Research and Extension Trust (ARET), Private Bag 9, Lilongwe, Malawi

MTONGA Y.P.; MUMBA J.B.; GOMONDA R.W.J.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 02

Evaluation of the effect of *Jatropha* seedcake as organic basal fertiliser on plant growth, yield and quality of flue-cured tobacco

Declining soil fertility and increased cost of inorganic fertilisers are a major constraint in tobacco production in Malawi. Cheaper and effective sources of nutrients would reduce the cost and improve the profit margins for tobacco. *Jatropha* is a tree of Euphorbiaceae family, seeds contain 30-35% oil, after extraction of oil the bran is the seedcake. The cake contains 4% nitrogen, 10% phosphorus, 1.48% potassium, 1.4% sulphur, 20% zinc. Experiments were conducted for two seasons to evaluate the effect of seedcake on growth, yield and quality of flue-cured tobacco at Kandiya, Mwimba and Kabwafu Research Stations. The design was randomised complete block with four replications. Treatments of different *Jatropha* application times eight weeks, four weeks before transplanting and at transplanting with seedcake totally substituting basal inorganic fertiliser SuperD (10.5 N, 24 P₂O₅ 20 K₂O 6S 0.15B). All the basal fertiliser was from seedcake, no inorganic fertiliser was applied as basal fertiliser. Two controls: Malawi recommended rate of nine by 50 kg bags/ha of SuperD top-dressed with two by 50 kg bags/ha calcium ammonia nitrate (CAN) and no fertiliser applied. The results showed that complete substitution of inorganic basal fertiliser with seedcake (88 g/station) at eight weeks, four weeks, and at transplanting and top dressed with CAN produced 16% higher yields than the Malawi recommended rate across all the sites. Seedcake produced higher proportions of the first quality grade (29%) cured leaf at all the sites. Seedcake also produced the highest proportion of orange leaf (22%) and reduced the input costs by 14%. The practice gave the larger root volumes, thicker stem diameter, wider and longer leaf. Seedcake also suppressed nematodes to scores of less than one. The results from this experiment suggest that *Jatropha* seedcake would be beneficial in tobacco production and may totally substitute basal dressing inorganic fertiliser (SuperD) in flue-cured tobacco production.

Agricultural Research and Extension Trust (ARET), Private Bag 9, Lilongwe, Malawi

**MTONGA Y.P.(1); MUMBA J.B.(1); GOMONDA R.W.J.(1); LOCKINGTON I.(2);
MACMILLAN C.(2)**

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 01

Evaluation of effect of coated fertilisers for use in flue-cured tobacco production

Two coatings, one which coats phosphate known as Avail and another called Nutrisphere-N which coats nitrogen, were used. Avail lengthened phosphate availability by protecting the phosphates from being locked up by Fe, Al, Mg and Ca. Nutrisphere-N controls losses of nitrogen by reducing volatilisation, slowing the conversion of ammonia into nitrate, which reduces de-nitrification and leaching. Experiments were conducted for two seasons to evaluate the performance of coatings on growth, yield and quality of flue-cured at Kandiya, Mwimba and Kabwafu Research Stations. The design was randomised complete block with four replications. The Avail coated SuperD and Nutrisphere coated Urea were each tested for yield and quality at rates of 100%, 90%, 75% and 50% over the Malawi recommendation (nine by 50 kg bags/ha of non-avail coated SuperD top-dressed with two by 50 kg bags/ha calcium ammonia nitrate (CAN)). Two controls: SuperD (N10.5:P24:K20%) as basal and CAN (27%N: 0P:0K) as top dressing fertiliser, the second control, Nil treatment. Results showed that coatings had positive influence on yield and quality over non-coated. Avail coated SuperD at reduction rates of 75% (75AP+C) or at 50% (50AP+C) top dressed with CAN produced higher yields and good quality tobacco across three sites. Regardless of the coatings, the Malawi recommended rate was out-performed by 24% on yield and 61% on first grade leaf. The coatings produced larger root volumes, taller plants, larger stem diameters and improved leaf characteristics, but those with Avail had an advantage over the Nutrisphere ones, it was evident that the coated fertilisers had an edge over the Malawi non-coated fertilisers. Coated SuperD when used together with CAN at reduced rates of up to 50% improves yield and quality of flue-cured tobacco. The coated fertilisers with savings of up to 50% over the non-coated amounts have significant potential savings on costs of fertilisers.

(1) *Agricultural Research and Extension Trust (ARET), Private Bag 9, Lilongwe, Malawi*

(2) *Greenbelt Fertilisers Ltd, P.O. Box 670351, Mazabuka Plant FRA Sheds, Lubombo Road,
Mazabuka, Zambia*

OFESI H.K.T.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 14

Farmer managed natural regeneration: a better option for restocking natural woodlands on tobacco farms in Malawi

Deforestation is a problem that has affected many countries in the Sub-Saharan region including Malawi. Studies have shown that Malawi's natural *miombo* woodlands are being depleted at the rate of close to 3% annually threatening the country's biodiversity and agricultural productivity. The natural woodlands have always been major sources of wood and poles used in tobacco production. Their disappearance has great ramifications on the tobacco industry and Malawi's economic development. In view of this, great efforts have been made to ensure that the deforested natural woodlands are restocked. The traditional practice has been through replanting with other indigenous or fast growing exotic tree species. Observations have shown that such efforts have yielded few positive results. Survival rates below 20% are common. Most areas remain bare despite fresh plantings. Studies have shown that most of the trees planted in these areas die or become stunted due to poor soils. Additionally, continued human activity and lack of proper care compound the problem. This has forced many to change the restocking strategy. It has now been noted that promoting natural regeneration coupled with proper care in such areas is a much faster, cheaper and more effective way of re-establishing natural woodlands. This is why ARET started promoting natural regeneration, a concept commonly referred to as farmer managed natural regeneration (FMNR), to mainly small holder tobacco farmers with great success. Natural regeneration uses the ability of indigenous trees to sprout from live stumps and roots present in the area. Shoots grow faster as they are supported by an already well developed root system. FMNR is cost effective since it requires no inputs and less labour. ARET will continue to promote sustainable and cost effective ways of dealing with deforestation in Malawi in order to sustain productivity of tobacco.

Agricultural Research and Extension Trust (ARET), Private Bag 9, Lilongwe, Malawi

PRZYBYS M.; DOROSZEWSKA T.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 21

Recombination events in PVY genomes

Potato virus Y (PVY) is one of the main pathogen that reduces tobacco crop yields worldwide. Different strains of PVY are associated with differing degrees of pathogenicity, of which the most common and economically important are known to be recombinant. Recombination is prevalent in viruses and its impact on the virulence of disease may be considerable. In order to detect recombination events, genomic sequences of 15 PVY isolates derived from Poland, Germany and Croatia were compared to 82 available genomic sequences of the PVY. Reshuffle in genomic sequences were observed in 95 isolates. The number of recombination sites of these isolates ranged from two to nine. Only in the case of two isolates belonging to the PVY^C strain there was no recombination event. Isolates belonging to the PVY^O strain always had two recombination sites. Most PVY^{NW} isolates had three or four recombination sites rarely six or seven. Most of recombination sites were found within isolates belonging to the PVY^{NTN} strain. One isolate possessed even nine recombination sites, typically 5-7. Among the fifteen tested isolates, all of them both PVY^{NW} and PVY^{NTN}, had a recombinant fragment of genome derived from New Zealand isolate. Within PVY^{NTN} isolates this fragment covered the whole of the 5' end of the genome, including P1, HC-Pro, and small part of P3 gene, whereas within PVY^{NW} isolates this fragment was smaller and comprised about half of the P1 and the whole HC-Pro gene. An exception is the IUNG 14 isolate, which despite belonging to PVY^{NW} strain, possessed fragment derived from New Zealand, such as in PVY^{NTN} isolates. Moreover, within PVY^{NW} isolates was found recombinant fragment near the 5' end of the genome derived from SASA 110 isolate (strain O). In addition, all PVY^{NTN} isolates had genome fragment derived from N 605 isolate. This fragment covered NIa-Pro, NIb and almost entire VPg and coat protein gene.

Institute of Soil Science and Plant Cultivation - State Research Institute, ul. Czartoryskich 8, 24 100 Pulawy, Poland

RISON J.L.; ROUGIER P.; FLIER W.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 23

DuPont™ Cyazypyr® a new cross-spectrum tool for sustainable control of tobacco pests

Cyazypyr® is a novel cross-spectrum anthranilic diamide insecticide discovered by DuPont that will enable tobacco growers to control several key tobacco pests. A large number of field, greenhouse and laboratory studies in many countries have shown that Cyazypyr® containing products are very effective against several species of both chewing and sucking pests in tobacco such as caterpillars, thrips, whiteflies and some aphid species.

The unique properties of Cyazypyr® allow selective control of pests, while conserving beneficial arthropods. Cyazypyr® based insecticides have an excellent eco-toxicology and environmental-fate profile. Those attributes make Cyazypyr® a valuable element of integrated control of tobacco pests, compatible with IPM programmes. Dependent on local cropping systems and temporal pest occurrence, Cyazypyr® can be applied either as a foliar spray, though soil application (pre-transplant drench), or through drip irrigation.

Cyazypyr® is part of Group 28 according to the IRAC mode of action classification scheme. Thanks to its novel and mode of action on sucking pests, Cyazypyr® has shown equal performance against susceptible and resistant pest populations. Laboratory data demonstrate that Cyazypyr® is not affected by resistance mechanisms known to confer resistance to other insecticides.

While the main route of pest's intoxication is by ingestion, contact activity of Cyazypyr® containing formulations has also been reported. The insecticide binds to ryanodine receptors present in insect muscles. The activation of those receptors causes the release and depletion of intracellular stores of calcium resulting in muscle contraction, and paralysis. Intoxication symptoms include rapid feeding cessation, general lethargy, paralysis, regurgitation (*Lepidoptera* larvae) and eventual pest mortality. These features help to prevent direct crop damage and significantly delay the transmission of some plant viral and bacterial pathogens that are vectored by thrips and Hemipteran pests such as whiteflies or aphids.

DuPont de Nemours, Défense Plaza, 23/25 rue Delarivière Lefoullon, La Défense 9, 92800 Puteaux, France

SIERRO N.; BATTEY J.N.D.; OUADI S.; BAKAHER N.; BOVET L.; WILLIG A.; GOEPFERT S.; PEITSCH M.C.; IVANOV N.V.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APW 02

The tobacco genome sequence

Nicotiana tabacum (common tobacco) is a major crop species and a model organism, for which only very fragmented genomic sequences have been available until recently. The three most commonly used tobacco types are flue-cured (or Virginia), Burley and Oriental, which are traditionally grown and harvested under different agricultural practices in over 120 countries. The tobacco plant stands out as a complex allotetraploid with a large 4.5 Gb genome, a significant proportion (>70%) of which is represented by repeats. As a species, *N. tabacum* (2n=4x=48) evolved through the interspecific hybridisation of the ancestors of *Nicotiana glauca* (2n=24, maternal donor) and *Nicotiana glauca* (2n=24, paternal donor) about 200,000 years ago. Considerable interest has centred on understanding the origin, organisation and evolution of the *N. tabacum* genome. Here we report high-quality draft genomes for three main tobacco varieties^[1]. These genomes show both the low divergence of tobacco from its ancestor genomes and display microsynteny with other Solanaceae species. We anticipate that the draft genomes will strengthen the use of *N. tabacum* as a versatile model organism for functional genomics and biotechnology applications.

[1] Sierra N, Battey JN, Ouadi S, Bakaher N, Bovet L, Willig A, Goepfert S, Peitsch MC, Ivanov NV. The tobacco genome sequence and its comparison with those of tomato and potato. *Nature Communication*. 2014 May 8; 5:3833. doi: 10.1038/ncomms4833. PubMed PMID: 24807620; PubMed Central PMCID: PMC4024737.

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

SUN Wenshu(1); SHI Hongzhi(1); ZHOU Jun(2); YANG Huijuan(1); JI Huihua(3); WANG Jun(1); JACK A.M.(3); BAI Ruoshi(2); XU Dongya(1); JIAO Zheheng(1)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 16

Effect of moisture content, nitrate and nitrite on TSNA accumulation in Burley tobacco after high temperature storage

Two experiments investigated the effects of various treatments on TSNA (tobacco-specific nitrosamines) in Burley tobacco (sealed cut rag) after high temperature storage. In Experiment 1, tobacco with five moisture contents (6%, 12%, 17%, 23%, 31%) was stored at 45 °C for 15 days. From the initial level of 0.5 ppm, TSNA increased most in the low moisture tobacco; to 5.0 and 4.2 ppm in the 6% and 12% moisture, respectively, and less in the three higher moisture treatments (1.7-1.9 ppm). In Experiment 2, nitrate (1 g / 20 g tobacco), nitrite (0.1 g / 20 g tobacco) and a water check were sprayed separately on low (8%) and high (18%) moisture tobacco before storage at 60 °C for 15 days. From an initial TSNA level of 3.8 ppm, TSNA in the water check increased after storage to 77.1 and 33.2 ppm in the low and high moisture tobacco, respectively. Nitrite sprays caused a very marked increase in both the low and high moisture tobacco, to 230.0 ppm and 207.4 ppm, respectively. The addition of nitrate caused a much smaller increase in the high moisture tobacco but a marked increase in the low moisture tobacco, to 195.1 ppm, suggesting that nitrate may be more stable in the high moisture tobacco.

- (1) *Henan Agricultural University, National Tobacco Cultivation & Physiology & Biochemistry Research Center, Tobacco Cultivation Key Laboratory of China Tobacco, Zhengzhou 450002, P.R. China*
- (2) *Beijing Cigarette Factory of Shanghai Tobacco (Group) Co., Beijing 100024, P.R. China*
- (3) *University of Kentucky, Lexington, KY, U.S.A.*

UZNAY F.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP01b

Regulatory mechanisms of Turkish tobacco market

By promulgation of new Tobacco law numbered 4733 in 2002 in Turkey; restructuring of state tobacco monopoly TEKEL with the purpose of privatization by terminating its monopoly position, discontinuing tobacco subsidy system and establishing free competition in tobacco market have mainly been aimed. Within this context, Tobacco and Alcohol Market Regulatory Authority (TAPDK), that has administrative and financial autonomous, has been established so as to fulfil regulatory and supervisory functions of TEKEL regarding to tobacco and alcohol markets in 2002.

A large number of new regulations such as By-laws, Communiques, Board Decisions and law amendments have been made within the scope of market requirements; markets have been monitoring and inspecting regularly; several joint studies and projects have been conducting on tobacco, tobacco products and alcoholic beverages markets in cooperation with public institutions, universities and private sector by TAPDK since 2002.

On the other hand, TAPDK has been following up international activities and the latest developments related to the markets of which in charge. Adaptation of EU's acqui into Turkish legislation are carried out; measures relating to tobacco control are taken; experts and directors from the Authority have been participating in meetings, congresses and working groups under EU, WHO FCTC, ISO, OIV and other relevant international bodies on a regular basis. When regulating the markets, the decisions taken in these meetings are taken into consideration.

To sum up, TAPDK has been playing an important role as a unique regulatory body in tobacco and alcohol markets in Turkey since it has been established. By presenting this paper, it is aimed to inform attendants about functioning of Turkish tobacco market from regulatory aspects.

Tobacco and Alcohol Market Regulatory Authority, Liaison Office, Kazim Dirik Dist. Fevzi Cakmak Str. No: 58, Bornova, Izmir, Turkey

VANN M.C.; FISHER L.R.; WHITLEY D.S.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 33

Conveyor use for suckercide application in North Carolina Burley tobacco production

In 2011, conveyors were introduced to the US market for use in chemical sucker control programs. Due to focused application and coarse droplet size, it was initially thought that conveyors might assist in reducing maleic hydrazide (MH) residues, reduce total solution output per hectare, and allow for material application in windy conditions. Research was conducted in 2011 and 2012 to quantify the effect of conveyor use with various combinations of MH and flumetralin in comparison to current practices used by many producers. Two rates of MH (1.68 kg a.i./ha and 2.25 kg a.i./ha) were tank-mixed with one rate of flumetralin (0.67 kg a.i./ha) and applied using a standard three-nozzle boom or a conveyor. In addition, two rates of MH (2.25 kg a.i./ha and 3.36 kg a.i./ha) served as comparison treatments for practices commonly used by many producers. Treatments were imposed at two locations in North Carolina in a randomized complete block design and replicated four times. In general, sucker control was consistently greater than 95% at all locations with all combinations of treatments. Therefore, sucker control was not improved beyond what is consistently achieved with the standard three-nozzle boom. In addition, MH residues do not appear to be influenced by application equipment, as residues were not different when equipment types were directly compared at the maximum labeled rate. Based upon this research, as well as similar results reported in flue-cured tobacco, it does not appear that conveyor use facilitates improvement in sucker control under ideal application and environmental conditions nor do the implements provide means to consistently reduce MH residues.

North Carolina State University, Department of Crop Science, Williams Hall, Campus Box 7620, Raleigh, NC 27695, U.S.A.

VANN M.C.(1); WHITLEY D.S.(1); CHEEK J.A.(1); SPIVEY B.M.(2); WHALEY W.T.(3)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 40

Tray type and soilless media brand performance in tobacco transplant production

Following the transition from outdoor seedbeds to indoor greenhouse systems in the 1980s, flue-cured tobacco seedlings have been overwhelmingly produced in expanded polystyrene (EPS) trays. In 2015, hard plastic float trays were marketed in the United States. Research was conducted in North Carolina to compare the performance of three 288 cell tray types: hard plastic, new EPS, and five-year-old EPS. In addition, four soilless media brands (Carolina Gold, Carolina Choice, Premier Pro BF, and Sunshine LT5) were evaluated within each tray type. Treatments were imposed in a single tobacco greenhouse in a randomized complete block design with a factorial treatment arrangement replicated six times. Following tray fill, percent dry cell, percent seedling emergence, and percent spiral root were evaluated from one to 18 days after seeding (DAS), depending upon parameter. Fifty days after seeding, percent total plants, percent usable plants, stem diameter, and stem height were quantified. Results indicate that new EPS trays were more likely to contain a greater number of dry cells 9 DAS, specifically when filled with Carolina Gold (9%) or Sunshine LT5 (14%) media. Reduced seedling emergence was also observed in the same treatments 13 DAS. No differences were observed in percent spiral root among tray types 18 DAS; however, spiral root was lowest in the Sunshine LT5 media (0.61%), most likely due to poor seed emergence from dry cells. Fifty DAS, older EPS trays were found to produce more total and usable plants than new EPS or hard plastic trays. In addition, the Carolina Gold, Carolina Choice, and Premier Pro BF soilless media brands produced a greater number of total and usable seedlings exhibiting preferred quality. Preliminary results indicate that there are numerous tray and soilless media combinations suitable for tobacco transplant production.

(1) North Carolina State University, Department of Crop Science, Williams Hall, Campus Box 7620, Raleigh, NC 27695, U.S.A.

(2) Johnston County Cooperative Extension, 2736 NC 210 Hwy., Smithfield, NC 27577, U.S.A.

(3) Wayne County Cooperative Extension, 208 W Chestnut St., Goldsboro, NC 27533-0068, U.S.A.

VERRIER J.L.; L'HUMEAU J.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 27

Genetic variability for upper stalk sucker growth in flue-cured tobacco and its potential use

Apart from the past use of non-flowering cultivars, there have been few reports on the use of low sucker growth varieties to help reduce the sucker control agent residues in flue-cured (FC) tobacco.

Among various FC cultivars and lines with no short photoperiod requirement, the upper stalk sucker growth (USG) propensity was investigated in a three step approach at Bergerac (France), from 2008-2014:

1. Defining the trait to be assessed,
2. Studying its variability among genotypes through different environments,
3. Testing sucker control strategies using « high » and « low » USG genotypes.

Fatty alcohols may control suckers smaller in size than approximately 3 cm. Therefore, the size of the biggest sucker in a given plant was considered, rather than their number or weight. Sucker control treatment dates are based on the flowering stage of the crop. Then, for a given plant and a given observation date, the biggest sucker size and flowering stage were recorded.

Field trials data indicate that a significant genetic variability is available for USG in flue-cured tobacco. Filler flue-cured lines, with early leaf maturity, of “European” origin, tend to be “low” compared to later maturing lines. Plant population, stalk lodging and virus infections also have dramatic impact on upper stalk sucker growth.

Selected low and high USG genotypes were tested under a top sucker control schedule based on fatty alcohols only. The sucker control was significantly better with “low” genotypes.

In general, “low” genotypes display early leaf maturity, and high sugar content. However, some of them display a more desirable equilibrium for aromatic FC style tobacco production. Breeding efforts are underway to obtain lower USG cultivars adapted to aromatic FC production.

Bergerac Seed & Breeding, Domaine de la Tour, 24100 Bergerac, France

WANG Fang(1); ZHOU Benguo(1); GAO Zhengliang(1); DONG Jianjiang(2)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 05

Discovery of RNA viruses in tobacco by high throughput sequencing of small interfering RNA (siRNA)

Sequencing and assembly of small interfering RNAs (siRNAs) from plants has been proved to be an effective way of identifying and characterising both DNA and RNA viruses. To explore the virome of a mixed tobacco sample, leaves of tobacco showing distinct symptoms were pooled for siRNAs sequencing. Assembly of siRNAs by two assemble algorithms, Velvet and Oases, was compared. The mean length of Oases contigs is 290 nucleotides (nt), which is 4.3X longer than Velvet contigs. Thus assembly of siRNAs by Oases provides advantages for obtaining the full length of viral genome and identifying novel viruses showing distant identities with known viruses. Homology search of these assembled contigs against GenBank at nucleotide and protein level revealed 12 viruses in total in the mixed tobacco sample, of which a new virus showing distant similarity with known virus, Mint virus 1, was provisionally named as Mint like virus strain AnHui (MLV-AH). The complete genome of MLV-AH comprises of 15396 nucleotides and contains nine putative Open Reading Frames (ORFs). Phylogenetic analysis of heat shock protein 70 homologs clearly placed MLV-AH alongside members of the genus Closterovirus. Bioinformatics analysis revealed the genome organisation of MLV-AH is consistent with other members of genus Closterovirus. The siRNA profiling of MLV-AH indicated (+) siRNAs and (-) siRNAs have almost equal ratio. Unlike other viruses in the sample, MLV-AH produced more abundant 22-nt siRNAs than 21-nt siRNAs and siRNAs are biased to the 3' terminal region of the genome. In this study the Closterovirus is identified for the first time in tobacco.

(1) Tobacco Institute, Anhui Academy of Agricultural Sciences, Hefei 230031, P.R. China

(2) Anhui Province Tobacco Monopoly Administration, Hefei 230022, P.R. China

WANG Fenglong(1); GAO Miao(1); QIAN Yumei(1); SHEN Lili(1); YU Guanghong(2); WANG Yaofeng(3); YANG Jinguang(1)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 10

Biological control of *Ralstonia solanacearum* using *Ralstonia* phage and the influence of diversity of soil bacteria

Tobacco bacterial wilt caused by *Ralstonia solanacearum* is one of the main diseases of tobacco. *Ralstonia* phage ϵ RS-1 was isolated from the soil of tobacco bacterial wilt nurseries from experimental fields of the Tobacco Research Institute of CAAS Institute, Jimo District, Qingdao City. ϵ RS-1 is an obligated parasite of *R. solanacearum*, and can infect and lyse *R. solanacearum* cells preventing them from dividing and reproducing and so controlling the disease pandemic. So ϵ RS-1 has real potential as a new microbial inoculant on *R. solanacearum* infected fields. We irrigated the tobacco root with the ϵ RS-1 culture (1×10^{10} pfu/mL) before artificial inoculation of *R. solanacearum*. The result showed a significant decrease in disease severity when *R. solanacearum* was co-inoculated with ϵ RS-1. The control efficiency of ϵ RS-1 against *R. solanacearum* could reach 94.87%, and was higher than control by agricultural streptomycin. Additionally, the diversity of bacterial community in treated soil by ϵ RS-1 was studied using metagenomic technique. The result showed that the soil *R. solanacearum* content is obviously lower than in the untreated control and ϵ RS-1 does have remarkable influence to the other microorganisms. In treated soil, the quantity of bacteria showed a descending trend as well as actinomyces compared to the untreated control. So ϵ RS-1 has the role of adjusting the diversity of soil bacteria.

- (1) *Key Laboratory of Tobacco Pest Monitoring Controlling & Integrated Management / Tobacco Research Institute, Chinese Academy of Agricultural Sciences, Qingdao 266101, Shandong, P.R. China*
- (2) *Baoshan Oriental Tobacco Company Ltd, Baoshan 678000, Yunnan, P.R. China*
- (3) *Qingyang Company, Gansu Provincial Tobacco, Xifeng 745000, Gansu, P.R. China*

WANG Jing; YANG Huijuan; SHI Hongzhi; LIU Guoshun

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 24

Cloning and characterisation of two members of the DanJ subfamily, NtDanJs, from *Nicotiana tabacum*

DnaJ proteins, also called J-domain proteins, function as molecular co-chaperones of Hsp70 and play an important role in protein folding, unfolding, and assembly under both normal and stress conditions as well as in cellular secretory pathways. Therefore, characterisation of a member of the DnaJ subfamily, NtDnaJ from *Nicotiana tabacum* will be meaningful to improve tobacco stress resistance. In this report, we cloned two kinds of DnaJ cDNAs sequences from tobacco K326 originally from diploid species *N. tomentosiformis* (NtDnaJ-T) and *N. sylvestris* (NtDnaJ-S), respectively. The full-length cDNA of NtDnaJ-T was 1,338 bp and contained a 1,017 bp ORF (Open Reading Frame) that encoded a protein of 338 amino acids. The 5' and 3' UTR (Untranslated Regions) were 104 and 217 bp in length, respectively. NtDnaJ-T gene had a length of 5,605 bp, and consisted of nine exons and eight introns. There were 2,020 bp in length cloned in the gene's 5' flanking region. The full-length cDNA of NtDnaJ-S was 1,318 bp and contained a 1,017 bp ORF that encoded a protein of 338 amino acids. The 5' and 3' UTR were 90 and 211 bp in length, respectively. NtDnaJ-S gene had a length of 5,946 bp, and consisted of nine exons and eight introns. There were 2,820 bp in length cloned in the gene's 5' flanking region. Both NtDnaJ-T and NtDnaJ-S contained a conserved J-domain and 4Fe-4S electron transport, showed highest mRNA expression in leaf and lower expression in root and stem, and also were shown to be regulated by light and heat shock. These results will provide an important base for DnaJ gene's function in breeding for tobacco stress resistance.

Henan Agricultural University, College of Tobacco Science, Tobacco Cultivation Key Laboratory of China Tobacco, Zhengzhou 450002, P.R. China

WANG Rui(1); SHI Heli(1); CHEN Shouwen(2)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 34

Isolation of a nicotine degradation bacterium strain and its application in tobacco stalk organic fertiliser composting fermentation

A bacteria strain which can degrade nicotine was isolated from soil collected in the Hubei Enshi area. This strain received the collection number EA-17. Based on morphological observation, physiological and biochemical analysis and 16SrDNA sequence homology analysis, the strain EA-17 was identified as *Arthrobacter histidinolorans*. EA-17 strain could grow with nicotine as the sole carbon source and within 24 hours it degraded the 0.5-4 g/L concentration of nicotine. The strain EA-17 was added to tobacco stalk as raw material in the compost fermentation, and the results showed that EA-17 could grow well in compost, nicotine degradation rate could be increased by 50% compared with the control and that the cellulose and hemicelluloses degradation rate increased 26.29% and 17.52%. Through the compost temperature, pH value, total nitrogen, water-soluble ammonium nitrogen and nitrate nitrogen content analysis showed that EA-17 had a positive effect in promoting the maturity of the compost fermentation. Isolation and application of EA-17 is an important breakthrough technology for tobacco stalk bio-organic fertiliser.

(1) *Enshi Tobacco Company of Hubei Province of CNTC, No. 119 Shizhou Road, Enshi, 44500 Hubei, P.R. China*

(2) *Hubei University, No. 368 Friendship Road, Wuchang District, Wuhan, 430011 Hubei, P.R. China*

WESTBERG H.; SPENCER A.

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 32

CPA residue levels in consecutive C48 cases of Oriental tobacco

Crop protection agents (CPA) can be applied to tobacco at various stages of the production cycle and if CPA residues are present in the product, they can be topic of concern. After tobacco is processed it is normally packed into cases or bales for shipment. This stage is a common point where CPA residue samples are collected and sent to a laboratory for analysis. The objective of this study was to test multiple positions within the case of tobacco to determine if the CPA residue levels were homogenous throughout the case. Three consecutive cases from a production run were tested to see if the CPA residue levels varied between cases. Twenty samples were taken from each of the three cases and tested in triplicate for common CPA residues found in that region. A previous study which sampled only one case revealed that pendimethalin was only found in two of the twenty samples taken from the case while all the other detected residues were consistently found in all twenty samples. A better understanding of CPA residue variances within and across cases may lead to more informed decisions about tobacco lot acceptability.

Global Laboratory Services, 2107 Black Creek Rd, Wilson, NC 27893, U.S.A.

YANG Huijuan; HUANG Huagang; SHEN Yan; YU Qiwei; SHI Hongzhi

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 19

Differential expression of pivotal genes involved in carbon and nitrogen metabolism pathways in climatic responses for different flue-cured tobacco cultivars

Henan and Guizhou are two major flue-cured tobacco growing areas in China, with distinct climatic conditions. Higher characteristic temperatures and longer sunlight periods in Henan and relatively low temperatures and shorter sunlight periods in Guizhou during the maturing stage of flue-cured tobacco. Flue-cured tobacco from Henan typically has a stronger aroma and it is more irritant compared to the tobacco from Guizhou. To elucidate the molecular changes in the tobacco grown in the two different areas, leaf samples were collected at the mature stage and gene expression involved in the carbon and nitrogen metabolism pathway were examined using PCR methods. Seven genes which included ZDS (Zeta-carotene desaturase), CHL (Chlorophyllase), GS1-3 (Glutamine synthetase), Nir-3 (Nrite reductase 3), Hex (Hexokinase), SPP2 (Sucrose-6-phosphate phosphatase) and TPP (Trehalose-phosphate phosphatase) were identified as differently expressed between two cultivars and all of them expressed at higher level in Jiucanping 2 compared with that in Bina 1. Compared with the plants grown under Baofeng environment both Nir-3 gene in Bina1 and Hex gene in Jiucaiping 2 expressed at a lower level as well as CSIE (Cellulose synthase-like protein) gene expressed at a higher level in both cultivars. Results showed that some pivotal genes expressed at lower level in Bina 1 indicating that physiological activities in the corresponding metabolism pathway were much weaker compared with that in Jiucanping 2. The nitrogen metabolism pathway in Bina 1 and sugar metabolism pathway in Jiucaiping 2 were affected mostly by environment. Results also indicated that higher temperature was conducive to nitrogen metabolism.

College of Tobacco Science, Henan Agricultural University, Key Laboratory of Tobacco Cultivation of China Tobacco Industry, Zhengzhou 450002, P.R. China

YE Xie-feng(1); LI Zhipeng(1); YU Xiaona(1); NIU Yude(2); LI Xianfeng(2); GUAN Saisai(1); ZONG Shengjie(1); MENG Qi(1)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 39

Effect of biochar amount on tobacco-planted soil carbon pool and quality of flue-cured tobacco leaves

Biochar application is one of the hot topics in plant nutrition. The level of the soil organic carbon pool has become a limiting factor in many tobacco planted areas of China. The objective of this study was to explore the application of biochar on the tobacco-planting soil carbon pool and the influence on tobacco leaf quality. A field experiment was carried out to study the effect of peanut-shell-biochar amounts on the tobacco-planted soil carbon pool and flue-cured tobacco leaf quality in 2013 at Hanzhong in Shanxi Province. The variety tested was flue-cured Yunyan 97. Peanut shells were carbonised in hermetic hypoxia equipment under 400 °C. The total carbon and total nitrogen content of peanut-shell-biochar was 420.3 g/kg, 28.2 g/kg respectively, and C/N ratio was 14.9. The cation exchange capacity content of the biochar was 93.91 cmol/kg and its pH value was 7.17. The alkaline functional groups, the acidic functional groups and the phenolic hydroxyl group content of the biochar were 1.90 mmol/g, 2.00 mmol/g and 1.40 mmol/g respectively. Results showed that: (1) Adding biochar can increase soil total carbon, easy oxidation active organic carbon, dissolved organic carbon, organic carbon mineralisation rate, soil carbon activity, soil carbon activity index and soil carbon pool management index. (2) Adding carbon can increase Nic, K⁺ and Cl⁻ content of cured-leaves and their contents increased with the increase of biochar amount. Adding appropriate amount of biochar can improve the sensory evaluation quality of cured-leaves. (3) The treatment of conventional fertilisation with biochar amount 600 kg/hm² is the best. (4) Excess amount of biochar may cause negative influence on flue-cured tobacco leaves and we need to carry out further research on increasing application of biochar with reduced nitrogen fertiliser technology.

- (1) *Henan Agricultural University, National Tobacco Cultivation and Physiology and Biochemistry Research Centre, Key Laboratory for Tobacco Cultivation of Tobacco Industry, Tobacco Science College of Henan Agricultural University, Zhengzhou 450002, P.R. China*
- (2) *Hanzhong Tobacco Company of Shanxi, Hanzhong 723000, P.R. China*

YOKOI M.(1); SHIMODA M.(2)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APOST 37

Extraction of volatile flavour compounds from tobacco leaf in a low-density polyethylene membrane

Tobacco leaves have various aromas. Many of the volatile flavour compounds in dried leaves have been investigated and reported. Although fresh tobacco leaves have their own aroma, post-harvest processes, such as heating and drying, generate flavour compounds and changes of their aroma. Flavour compounds tend to be low molecular weight and hydrophobic, therefore the extraction of flavour compounds through low-density polyethylene (LDPE) membrane could be considered a simple and rational way to prepare tobacco aroma concentrates. The objective of this study was to analyse the volatile flavour compounds of a fresh leaf at the initial stage of the post-harvest process (withering) via LDPE membrane extraction method. Virginia tobacco (Tsukuba #1) was grown in a greenhouse and harvested at the season between flower budding and budding. Subsequently, the tobacco leaf was withered under constant condition: 35 °C, 60% relative humidity (RH). Volatile flavour compounds were extracted from the tobacco leaf in an LDPE membrane pouch and analysed by GC/MS. LDPE membrane extraction showed that aldehydes, such as cis-3-hexenal and 2,4-heptadienal, decreased, and benzyl alcohol and phenylethyl alcohol increased during an initial period of withering the fresh leaf. In addition, this method allowed the analysis of volatile compounds in three different dried leaves, Virginia, Burley, and Rustica. The LDPE extraction method was useful for the analysis of the volatile flavour compounds in the tobacco leaf. An increase of alcohols seems to be a result of the degradation of glycosides in the fresh leaf. However, well-known volatile compounds such as carotenoid degradation compounds did not appear during the treatment. In order to achieve them, fresh leaves should be put at a higher temperature and/or dried.

(1) *Japan Tobacco Inc., Tobacco Science Research Center, 6-2, Umegaoka, Aoba-ku, Yokohama, Kanagawa 227-8512, Japan*

(2) *Kyushu University, 6-10-1, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan*

YUN Fei; LIU Guoshun; SONG Jing; WANG Hui; ZHANG Jun; GUO Chaochao

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APOST 35

Regulating effects of calcium salts on the photosynthetic carbon assimilation and accumulation of photosynthetic products in flue-cured tobacco under different light circumstances

The regulating effects of exogenous calcium salts on photosynthetic characteristics and products under different conditions of light intensity is revealed in this paper. With Zhongyan 100 as experimental material in this soil-culture pot experiment, two elements including four kinds of light intensity and three types of calcium salts were conducted to study the effects of the cooperation of light and calcium salts on the photosynthetic carbon assimilation, chlorophyll fluorescence characteristics and the accumulation of dry matter in flue-cured tobacco, in order to ascertain the mechanism of calcium salts on the absorption and utilisation of light energy. The results indicated that the content of pigments (chl_a, chl_b, chl_{a+b}, car) was significantly increased with the increase of shading and the application of calcium salts. The values of net photosynthetic rate (P_n), transpiration rate (E), stomatal conductance (G_s) was decreased with shading, however, the intercellular CO₂ concentration (C_i) was increased. Moreover, there was a positive effect of calcium salts on the parameters of gas exchange. The photochemical quenching coefficient (q_P), maximum photochemical efficiency (F_v/F_m), actual photochemical efficiency (ΦPSII), PSII potential activity (F_v/F_o) and the electron transmit rate (ETR) were improved with the increase of calcium salts and shading. The calcium salts could promote the photosynthetic efficiency and improve the photosynthetic rate under low light conditions. The photochemical efficiency and photosynthetic capacity were promoted with the proper application of calcium salts [Ca(NO₃)₂ in this experiment], meanwhile, the efficiency of light energy utilisation and the amount of dry matter accumulation in flue-cured tobacco could be improved under 70% natural light intensity.

Henan Agricultural University, College of Tobacco Science, National Tobacco Cultivation and Physiology and Biochemistry Research Center, Zhengzhou 450002, P.R. China

ZHAN Huaixu; WANG Fenglong; SHEN Lili; YANG Jinguang; QIAN Yumei; CHEN Dexin

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 04

Selection and identification of a tobacco endophytic bacteria strain L1 with inhibitory effect against TMV

The objective of this study is to screen a biocontrol bacterial strain with significant inhibitory effect against TMV (Tobacco mosaic virus). The strains were isolated from leaves of tobacco var. Luyan 1 resistant to TMV. The anti-TMV strain was tested by half-leaf method on hypersensitive plants of *N. tabacum* var. Samsun and identified by the morphology, physiological and biochemical tests and sequence analysis of 16S rDNA. The effect of the bacterial strain on the virus particle was firstly observed in liquid fermentation and transmission electronic microscope. The results were also verified by SDS-PAGE analysis. Additionally, indoor pot tests were conducted with three treatments. (I) The fermentation liquid was firstly sprayed on NC89 leaves 24 h before inoculating the virus to NC89. (II) Virus was inoculated to NC89 24 h before spraying fermentation liquid. (III) The mixture was inoculated to NC89 after the fermentation liquid with virus juice for 30 min. Six strains with an inhibitory effect against TMV were screened from 53 endophyte strains. The pot test results showed that the antiviral rate of strain L1 was 82.61% and further study was required. Strain L1 was gram-negative and identified as *Alcaligenes faecalis*. It was observed that *Alcaligenes faecalis* L1 could disperse TMV particles and disrupt the particles into small fragments. However, strain L1 could not affect the coat protein of TMV based on SDS-PAGE analysis. The pot test results showed that strain L1 could achieve ideal TMV control efficiency with inhibition rate of treatment I, II and III of 34.58%, 21.81% and 82.81%, respectively. The selected strain L1 could be used as an antiviral agent for future study and application.

Qingzhou Tobacco Research Institute of CNTC, No. 11 Keyuanjingsi Road, Laoshan District, Qingdao, Shandong 266101, P.R. China

ZHANG Jiguang(1); ZHANG Zhongfeng(1); YAO Zhongda(2); ZHANG Fujian(2); GUO Dongfeng(2); JI Xuejun(3); ZHU Qifa(3); XUE Lin(3); SHEN Guoming(1)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APOST 06

Materials, energy and value flows analysis of different tobacco planting patterns in Southern Anhui area

The reasonable choice of tobacco planting pattern is very important to improve the efficiency of tobacco field ecosystems and to promote the sustainable development of modern tobacco agriculture. In this study, the materials, energy and value flow characters of four tobacco planting patterns in Southern Anhui area were investigated, and the NPK input and output status, energy structure and value conversion efficiency were analysed and discussed by using the method of field investigation. The results showed that all the planting patterns should decrease the amounts of N, P fertiliser and increase the fertiliser use efficiency. And meanwhile, the K fertiliser application and straw returning should be emphasised in the three tobacco rotation patterns. The ratio of organic energy to inorganic energy was highest (0.71) in the tobacco among all crops planted, and the artificial energy was highest in the whole energy components. The fertiliser energy and agricultural machinery energy was the second. The tobacco-rice multiple cropping had the lowest energy ratio of output to input, and the planting pattern of tobacco-rice → wheat-rice → tobacco had the highest value in the four tobacco planting patterns. Moreover, tobacco-rice multiple cropping had the highest net income per hectare, but the planting pattern of tobacco-rice → wheat-rice → tobacco was the best of the four patterns from the perspective of ratio of nutrients input to output, energy efficient utilisation and the ratio of value input to output. So, it should be adapted to spread in a larger area in the South Anhui Province.

(1) *Tobacco Research Institute, Chinese Academy of Agricultural Sciences, Key Laboratory of Tobacco Biology and Processing, Ministry of Agriculture, Qingdao 266101, P.R. China*

(2) *China Tobacco Anhui Industrial Co. Ltd., Hefei 230088, P.R. China*

(3) *Anhui Wannan Tobacco Leaf Co. Ltd, Xuancheng 242000, P.R. China*

ZHANG Jixu(1,2); WANG Rui(3); LIU Dehu(4); ZHANG Jiguang(1); GAO Lin(1); DAI Yanchen(1); ZHA Ting(1); MENG Guixing(3); XIANG Bikun(3); SHEN Guoming(1)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 36

Effects of straw biochar on the growth of flue-cured tobacco and soil nutrients

To investigate the effect of biochar application on tobacco, pot experiments were conducted to study the impacts of applying straw biochar at different levels on tobacco growth and soil nutrients. The results showed that the growth of above-ground organs of tobacco was promoted by biochar application in the soil at moderate levels (0.2-1.0%) while inhibited by high level (5%) biochar application. Compared with the above-ground parts, the growth of the root system was promoted by biochar application at all levels tested, with the 5% biochar treatment resulting in the highest root/shoot ratio. This treatment also delayed the aging of the root system including lateral roots. The length of primary roots, first and second-order lateral roots and the root/shoot ratio of tobacco plants increased at early stages then decreased at late stages of development in all treatments. Moreover, with the increase of biochar application level, soil bulk density decreased while an increase was observed in soil pH and soil nutritional indicators including Organic carbon, alkali-hydrolysable nitrogen, available P and rapidly available K, with the soil nutrient level reaching the highest in the 5% biochar treatment. Our results suggested that biochar application in tobacco fields at appropriate levels (0.2-1.0%) would lead to an increase in soil nutrients and promote tobacco growth. Further field studies are needed to test the effect of biochar application in different types of soils with various physical and chemical properties.

- (1) *Tobacco Research Institute, Chinese Academy of Agricultural Sciences, Key Laboratory of Tobacco Biology and Processing, Ministry of Agriculture, Qingdao 266101, Shandong, P.R. China*
- (2) *Qingdao Agricultural University, Qingdao 266109, Shandong, P.R. China*
- (3) *Enshi Branch of Hubei Tobacco Company, Enshi 445000, Shandong, P.R. China*
- (4) *Qingzhou Cigarette Factory, China Tobacco Shandong Industrial Co., Ltd., Shandong, Qingzhou 262500, P.R. China*

ZHANG Limeng(1); JI Sigui(1); JIAO Yongge(1); LI Jiangzhou(1); GU Xinghui; CUI Yonghe; ZHAO Jinlong; ZHU Shusheng(2); YANG Min(2)

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 09

Inhibitory activity of maize root exudates against *Phytophthora nicotianae* and antifungal compounds analysis

In this study, we observed the interaction between maize root and zoospores of *Phytophthora nicotianae*, the inhibitory activity of maize root exudates against *P. nicotianae* and the antifungal compounds in the maize root exudates were tested and analysed using HPLC-MS. The results indicated that maize root could not only attract zoospores into the rhizosphere, but also secret some substances to inhibit the swimming of zoospores or cause the rupture of cystospores. Further studies indicated that maize root exudates showed inhibitory activity against the release of zoospores from sporangia, the germination of cystospores, and the hyphal growth. The inhibition rate was 73.8%, 87.2% and 55.2%, respectively, when the root exudates concentration was 0.90 mg/mL. DIMBOA, 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one and its degradation substance MBOA (6-methoxy-2-benzoxazolinone) were identified in root exudates by HPLC-MS. MBOA also showed antifungal activity against the hyphal growth of *P. nicotianae*. The inhibition ratio reached 90.94% when the concentration of MBOA in media was 0.30 mg/mL. In summary, maize root can not only attract the zoospores of *P. nicotianae*, but also secret the antifungal compounds DIMBOA and MBOA. These results support maize and tobacco crop rotation as a theoretical control method for tobacco black shank in tobacco growing.

- (1) *Yunnan Tobacco Company, Yuxi Branch, Yuxi 653100, P.R. China*
- (2) *Key Laboratory for Agro-biodiversity and Pest Control of Ministry of Education, Yunnan Agricultural University, Kunming 650201, P.R. China*

ZHANG Songtao; ZHANG Kangxu; WANG Yeqing; CUI Hong; YANG Yongxia; JIA Hongfang; ZHANG Hongying

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. APPOST 23

Study on GCN2 and AZI involved in plant priming

It was shown that higher plants could adopt the “priming” mechanism to “recall” the prior exposure to a biotic or an abiotic stress, so they could respond more quickly and effectively when exposed to the stress again. However, the molecular regulation mechanism of priming is still unclear. In the “priming” process, the kinase GCN2, which could phosphorylate eukaryotic initiation factor eIF2 α , and a predictive lipid transfer family protein AZI1 might be involved in the regulation of “priming” and signal transduction. To test this hypothesis, we cloned the *NtGCN2* by rapid amplification of cDNA ends (RACE) and *NtAZI1* from *Nicotiana tabacum* K326. Also, the Azelaic Acid (AZA) was used to trigger the defense system of *Nicotiana tabacum* K326, the transcriptional level of *NtGCN2* and *NtAZI1*, and the accumulated level of endogenous azelaic acid were detected by qPCR and GC-MS, respectively. Our primary results showed that gene expression of *NtGCN2* and *NtAZI1* could be induced by AZA application after 12h and 24h, respectively. Further, the endogenous azelaic acid was also detected by GC-MS, the content of AZA in the treated leaf was up to 29.29 $\mu\text{g/gfw}$ at 24h, while this content of AZA was not detected in the treated leaf at 0h and 12h. These results indicated that when the plant was under stress, the defense system could activate GCN2 to downregulate the globe protein synthesis, then AZI mediated signal transduction to increase the AZA content to response to plant resistance. This study suggests that *NtGCN2* and *NtAZI1* might be involved in the regulation of tobacco “priming”, thereby providing some clue for the mechanism of plant “priming”.

Henan Agricultural University, College of Tobacco Science, Tobacco Cultivation Key Laboratory of China Tobacco, Zhengzhou 450002, P.R. China

ZOU Yong; YE Xiaoqing; YU Zhihong; CHEN Yufeng; WANG Wei; CHEN Jianjun

CORESTA Meeting, Agronomy/Phytopathology Groups, Izmir, 2015, abstr. AP 35

Measuring canopy nitrogen nutrition in tobacco plants using hyper spectrum parameters

The aim of this paper is to study the quantitative relationship between above-ground nitrogen accumulation (ANA) of flue-cured tobacco and canopy spectral parameters, and then build a monitoring model for ANA of flue-cured tobacco based on various spectral indices. Tobacco was grown in the field under different nitrogen fertilisation levels. Canopy spectra data at different growing stages was collected and biomass and nitrogen content in different parts of plants was determined. The correlation analysis of ANA of flue-cured tobacco and multiple spectral parameters showed that nitrogen accumulation increased as nitrogen fertilisation level increased, meanwhile, nitrogen fertilisation level too low or too high would prompt nitrogen transfer from leaf to stem and root. The correlation between ANA and canopy reflectance was significantly negative ($P < 0.05$) at visible band (460-710 nm) and positive at band (760-1300 nm). Leaf nitrogen accumulation (LNA) was highly correlated with ANA, and quantitative equations were established between spectral parameters and ANA, and monitoring models on ANA were established using canopy. Spectral parameters, such as ratio vegetation index (RVI (680,810)), difference vegetation index (DVI (680,810)), normalised difference vegetation index (NDVI (680,810)), enhanced vegetation index (EVI810), soil-adjusted vegetation index (SAVI) and optimisation of soil adjusted vegetation index (OSAVI), all could be used to predict ANA, and the prediction accuracy R^2 were 0.901, 0.792, 0.839, 0.929, 0.921, 0.930 respectively, and root-mean-square error (RMSE) were 0.264, 0.245, 0.193, 0.188, 0.202, 0.260 respectively. Regression analysis of the relation between red edge amplitude ($D\lambda\text{Red}$) and ANA of flue-cured tobacco showed good inversion, in which the regression equation was $y = 8.376 \times 2.0901 - \text{RMSE} = 0.197$. It could be concluded that ANA in flue-cured tobacco could be monitored effectively by key vegetation indices, especially EVI810, OSAVI and $D\lambda\text{Red}$.

(1) *Shenzhen Tobacco Ind. Co., Ltd. of CNTC, Shenzhen, Guangdong 518109, P.R. China*

(2) *Shenzhen Boton Flavors & Fragrances Co., Ltd., Shenzhen, Guangdong 518051, P.R. China*

(3) *Tobacco Laboratory, South China Agricultural University, Guangzhou, Guangdong 510642, P.R. China*