ABSTRACTS OF PRESENTATIONS MADE AT THE
2011 CORESTA JOINT MEETING OF THE
AGRONOMY AND PHYTOPATHOLOGY STUDY GROUPS
SANTIAGO, CHILE

(by alphabetical order of first authors)

BAILEY W.A.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 14

Use of strip-tillage systems for dark-fired tobacco production in Western Kentucky USA

Strip tillage is a form of conservation tillage that has been readily adopted by several dark-fired tobacco growers in western Kentucky. Strip tillage production has increased each year since 2007 and currently accounts for at least 15% (1325 Ha) of the total dark tobacco production in Kentucky and Tennessee. Strip tillage has been much more readily adopted by growers than no-tillage, as less plant mortality following transplanting is usually observed. Strip tillage is somewhat of a hybrid between traditional conventional tillage and no-tillage where tobacco is transplanted into cultivated strips 30 to 40 cm wide while the remainder of soil surface is uncultivated. Strip tillage provides many of the benefits of no tillage production, such as soil and water conservation, fuel and labor savings due to reduced field preparation, and reduced delays in post-transplant field operations following rains. In addition, where dark tobacco requires field wilting on the ground at harvest, strip tillage appears to have some effect on keeping tobacco cleaner. Strip tillage requires a burndown application of a non-selective herbicide such as glyphosate or paraquat, followed by use of a strip-tiller implement to form the strips. Phosphorus and potassium fertilizer can also be applied as band applications as strips are being made, and research has shown that recommended application rates can be reduced by one-third if these nutrients are applied as band applications. On most soils in western Kentucky, the use of a powered tiller implement designed to match the strips is also recommended just prior to transplanting. Research with grower cooperators has been conducted since 2008 to evaluate additional benefits to strip tillage compared to traditional, conventional tillage systems. Results of this field research suggest a 30 to 50% reduction in land preparation costs, as well as possible yield benefits in dry seasons.

University of Kentucky, Research & Education Center, 1205 Hopkinsville St., Princeton, KY 42445, USA

BERTRAND P.F.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. PPOST 01

Disease loss in Georgia grown tobacco

This presentation discusses disease losses in the flue-cured tobacco grown in the state of Georgia, USA. In Georgia, tobacco disease losses are based on formal and informal disease incidence surveys conducted throughout the season. The discussion will document the rise of spotted wilt from a "curious new disease" in 1987 to the number one cause of disease loss in all but three years from 1997 to 2010. Losses caused by other diseases are covered as well. Between 1990 and 2010 (21 years), spotted wilt resulted in significant losses in 15 years. These losses ranged from 1.0 - 20.0%. This disease occurs at random offering opportunities for adjacent plant compensation. Other diseases also cause frequent significant losses in Georgia tobacco. Black shank caused significant loss in 14 of 21 years, with losses ranging from 0.1 to 2.0% in these years. Black shank occurs in areas and % plant loss is very close to % yield loss. Root knot nematodes caused significant loss (0.1 - 1.0%) in 13 of 21 years. Other diseases have caused occasional losses. These include blue mold in 5 of 21 years. Blue mold was not reported at any level in 9 of these years. Target spot losses occurred in 3 years. Tobacco mosaic virus is seen in trace amounts in nearly every year; however, in 2000 severe losses occurred in some fields of the hybrid varieties NC-71 and NC-72. White mold, caused by *Sclerotium rolfsii*, shows up in spots every year. However, unusually high soil temperatures in May 2009 resulted in a significant loss that year.

The discussion of losses presented here deals with average statewide losses for all diseases. Most diseases, except spotted wilt, have a very unequal distribution. A few growers may have very high losses while others have no loss at all even in the worst disease years. Spotted wilt losses tend to be higher because of the way it affects all growers similarly. In the high disease incidence years, all growers suffer some loss.

The University of Georgia, Department of Plant Pathology, 2360 Rainwater Road, Tifton, GA 31793-5766, USA

BROWN A.B.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. IG 01

Producer compliance costs for Good Agricultural Practices in the U.S.

Most purchasers of U.S. tobacco now require producers to comply with some set of good agricultural practices (GAP). Each manufacturer or leaf dealer has their own version of GAP, but in general there are three categories into which GAP can be categorized: 1) crop management, 2) environmental stewardship, and 3) labor management. While there are differences, individual company GAP is similar to or based on GAP standards recommended by CORESTA. Adoption of GAP by both U.S. and other manufacturers is often driven by liability concerns, actions of advocacy groups (particularly in labor management and environmental stewardship), and regulatory action at the tobacco product level (*e.g.* in the U.S. regulation by the Food and Drug Administration). Producer compliance costs are manifested in direct costs such as upgrades to labor housing or installation of conservation practices. However, a large component of compliance costs is indirect costs, which is often difficult to measure. In economic terms these costs are categorized as transactions costs. The purpose of this study is to examine and, where possible, quantify costs associated with adoption of GAP. Where costs are not quantifiable, the relative importance and impact on management is examined. For example, increased record keeping is required for most practices. This task is either carried out by the producer or employees assisting with farm management. Increased record keeping often does not result in hiring of a new employee, but may increase time required for management or reallocation of human resources. Preliminary results indicate that the incurrence of such transaction costs is one factor in farm structural change, particularly increasing farm size, on U.S. farms. There seem to be significant economies of scale associated with many of the transactions costs due to adoption of GAP.

North Carolina State University, Raleigh, NC 27695-8109, USA

CAI Liuti(1); HU Zhongyi(1); LEI Lu(1); LI Jixin(2)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. APOST 05

Cloning and gene structure analysis of *T-psy1* from flue-cured tobacco

Carotenoid is an important precursor of tobacco aroma, and phytoene synthase is one of the key enzymes in the pathway of aroma precursors biosynthesis and accumulation in tobacco. Based on the homology cloning method, the cDNA and DNA sequence of the tobacco phytoene synthase gene (named *T-psy1*) were cloned from the *Nicotiana tabacum* cv. K326, and its sequence and structure were analysed. The results showed that the cDNA of *T-psy1* including 1521bp, which encoded a protein of 441 amino acids, its GenBank accession is HM345582, and its protein accession is ADK25054. Using the spidey tools (http://www.ncbi.nlm.nih.gov/spidey/), analysis of *T-psy1* genomic structure showed that it contained six exons and five introns. The splicing principle of its exons and introns were consistent with GT-AG, the size of the 5 introns range from 96bp to 504bp. The results of BLAST and phylogenetic analysis indicated that *T-psy1* gene had high homology with the phytoene synthase gene of ornamental tobacco and tomato, which is 95% and 91% respectively, and the *T-psy1* encoded protein had high identity with the phytoene synthase gene encoded protein of ornamental tobacco and tomato, which is 97% and 90% respectively. The genomic structure of *T-psy1* and its introns provided the sequence information for the study of the expression of *T-psy1* and the regulation of the tobacco carotenoid synthesis pathway in tobacco.

1. *Guizhou Tobacco Research Institute, Guizhou Provincial Tobacco Company, Guiyang 550081, P.R. China*

2. *Guizhou Provincial Tobacco Company, Guiyang 550003, P.R. China*

CASTANHEIRA L.C.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 18

The study and development of protection equipment for pesticide application and harvesting tobacco

Tobacco growers are exposed to pesticides during crop protection applications and also to alkaloids during harvest periods. Throughout the year 2009 a study was carried out to evaluate and analyse all aspects regarding the legislation and technical aspects of Personal Protective Equipment (PPE) available in Brazil, and the problems encountered when using referred equipments. As a consequence of the study, there is a need to develop specific equipment for tobacco workers and different equipment for pesticide application and harvesting, due to specific situations of exposure. In the field, work activities were observed for a year and fabrics were monitored together with specific materials for the manufacture of PPEs. The modeling of clothes was made respecting the anthropometric characteristics of the users. Once the protective equipments were developed, they were tested in the field. In light of comments made, and problems observed in modeling and fabrics, and also the views, criticisms and suggestions provided by the growers themselves, further amendments were made to obtain the final models. As a result, it was possible to obtain lighter, more comfortable and more efficient protection kits for pesticides application and harvest. At the same time, a set of safety procedures were established involving, in addition to protective equipment, work routines designed to reduce workers' exposure to other critical aspects, such as physical agents (air temperature, solar radiation) and tiring work.

Independent Consultant - Safety and Hygiene in Rural Labor, Campinas - SP, Brazil
SindiTabaco – Interestate Tobacco Industry Union, Brazil

CHEN Dexin(1); YANG Jinguang(1); WANG Fenglong(1); ZHOU Wenliang(2); SHEN Lili(1); QIAN Yumei(1)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 26

Development and application of immunocapture real-time RT-PCR for detection of potato virus Y (PVY) in individual peach aphid (*Myzus persicae*)

Potato virus Y (PVY) is transmitted in a nonpersistent manner by peach aphid (*Myzus persicae*), leading to many crop diseases. PVY infected tobacco plants could cause serious damage on tobacco quality and yields due to its simple transmission path and broad distribution. The large number of viruliferous peach *M. persicae* is one of the major culprits for the rampant outbreak. In the present study, immunocapture real-time RT-PCR (IC-real-time RT-PCR) was investigated to detect PVY in individual peach aphids (*Myzus persicae*) by combining ELISA and conventional RT-PCR. The results showed that the amplification curve had a flat baseline, a distinct exponential area, a large and stable slope and the coefficient of variation was very small. There was a linear relationship between threshold cycle values at which samples crossed the threshold and the logarithmic values of template concentration. Compared with DAS-ELISA, which is extensively used by many of laboratories at present, real-time PCR can be used as a new method to detect PVY components in tobacco quantitatively, as it is faster, more sensitive and specific. This assay could be directly carried out without total RNA extraction by capturing TMV particles. This effectively reduced the pollution and inhibiting effects of proteins, polysaccharide and phenol materials in the operating process. IC-RT-PCR could detect TMV particles in dry tobacco leaves, single tobacco seed, and even external exudates of tobacco plants caused by physical damage. Therefore, as a specific, rapid, sensitive and simple method for detecting slight amounts of TMV particles, IC-RT-PCR is important and scientifically significant for the prediction, forecast and prevention of tobacco virus diseases.

1. *Key Laboratory of Tobacco Quality Control, Ministry of Agriculture, Qingdao, Shandong 266101, P.R. China*
2. *Guangxi Tobacco Company Baise Branch, Baise 533000, P.R. China*

CZUBACKA A.; DOROSZEWSKA T.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 30

Evaluation of agronomic characteristics of transgenic tobacco doubled haploids

Transgenesis allows the modification of plant genomes relatively quickly and precisely. Moreover, compared with classical methods, genetic transformation significantly facilitates and shortens the time needed for breeding a new cultivar. Shortening a breeding process is possible thanks to obtaining haploids and then doubled haploids being homozygotes as regards all traits, which increases the efficiency of selection of desired recombinants.

The plant material used in the study consisted of transgenic tobacco lines containing lettuce mosaic virus coat protein gene (LMV CP) determining resistance to potato virus Y (PVY). Doubled haploids were obtained by combination of anther culture and spontaneous doubling of the number of chromosomes under *in vitro* conditions. The generation DH_2 of doubled haploid lines, obtained from hybrids coming from crossing transgenic cultivar MN 944 with cv. Wislica and hybrids obtained from crossing transgenic cv. BY 103 and its nontransgenic equivalents, were estimated by comparison with their parental cultivars. The experiment was conducted under greenhouse conditions. The evaluation of agronomic traits included elements of growth and development such as growth rate, length of vegetative and generative stages, area of leaves, height and morphology of plants. Cured leaves of DH lines were analysed as regards selected physical and chemical traits. Moreover, the pollen viability was estimated.

Individual DH lines differed among themselves and in comparison to parental cultivars as regards some traits. However, only little diversity within the lines was noticed. The DH lines characterized by the most satisfying agronomic traits may be valuable initial material in further breeding.

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

DAVENPORT S.E.; FORSYTH K.; TULLY L.; LISTER R.; LE LAY P.; MAUNDERS M.; SANCHEZ-TAMBURRINO J.P.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 03

Lowering TSNAs through nitrite reductase activity

Tobacco specific nitrosamines (TSNAs) are compounds in tobacco that have been shown to be carcinogenic. TSNAs are formed by the nitrosation of tobacco alkaloids with the main nitrosating agent believed to be nitrite.

High levels of nitrate accumulation can occur as a result of high nitrate levels in the soil. The nitrate is taken up by a nitrate transport system and enters the cytosol where it is either stored in vacuoles or reduced by the cytoplasmic enzyme nitrate reductase (NR) to nitrite. Nitrite is then reduced to ammonium by nitrite reductase (NiR) in the chloroplasts of leaves or in the plastids of non-photosynthetic organs.

By modifying the nitrogen pathway it is possible to influence the build up of nitrite in the cell by controlling the activity of NiR.

In this study an *Arabidopsis thaliana NiR* (AtNiR) was isolated and used to transform tobacco plants under the control of a constitutive promoter (CERV - carnation etched ring virus). The aim was to over express NiR in an attempt to alter the level of residual nitrite in the leaf. The expression of the introduced AtNiR protein was analysed by western blot. Further investigation of the T_1 homozygous population demonstrated an increased NiR and NR activity. Field trial results have shown a greater than 50% reduction in TSNAs from blend and smoke analysis.

Advanced Technologies (Cambridge) Ltd., Cambridge CB4 0WA, UK

DAVENPORT S.E.; LEACH G.; JONES L.; SANCHEZ-TAMBURRINO J.P.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. APOST 04

Modification of Virginia tobacco to provide burley characteristics

It is well known that burley tobacco can contain more TSNAs than Virginia types. One possible strategy to reduce TSNAs would be to generate Virginia tobacco that possesses burley characters removing the need to add burley to a blend.

One of the differences between burley and Virginia is the composition of their amino acid content. We followed a burley and Virginia (K326) crop throughout the curing process and measured the levels and changes in amino acids (and other metabolites). At the final stage of curing the amino acid profiles of K326 and burley were very different, as shown in a field study by ATC (CORESTA 2008). One of the major differences seen was in the levels of threonine and asparagine, with burley containing several times more of these metabolites than Virginia.

Therefore using transgenic approaches (GMO), we modified the amino acid profile of K326 by the introduction of different genes involved in amino acid synthesis. These included asparagine synthase, glutamine synthetase, glutamate dehydrogenase and aspartate kinase. The greatest effect was demonstrated from an overexpressed aspartate kinase (AK).

Due to the tight regulation of threonine levels within tobacco, the AK gene was de-regulated so that the threonine levels would not be limited. An AK gene isolated from *Arabidopsis* and deregulated, was inserted into K326 and the threonine levels measured. Initial work was carried out with a leaf-specific promoter which produced elevated levels of threonine (up to a 5 fold increase) in the leaf, however morphological changes occurred. Subsequently, a senescence-specific promoter was used and resulted in lines which displayed a normal K326 phenotype with increased levels of threonine and asparagine.

These lines will now undergo assessment to validate the extent of burley-like characteristics that have been incorporated, and will determine whether this leaf could be an alternative to burley tobacco.

Advanced Technologies (Cambridge) Ltd., Cambridge CB4 0WA, UK

DE BEER M.C.; TERBLANCHE J.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. PPOST 09

Black shank resistance in air-cured tobacco - South Africa

In the South African air-cured tobacco producing regions, black shank, caused by *Phytophthora nicotianae* var. *nicotianae*, is a major concern. The control of this disease is based only on crop rotation and chemical control by means of metalaxyl derivates. However these products are registered on flue-cured and not on air-cured tobacco. For the past 40 years, the air-cured tobacco producers planted only one dark air-cured cultivar, CDL 28. Although high yielding and delivering tobacco of very good quality, it has no resistance to black shank.

A new dark air-cured tobacco cultivar, LD2, with high resistance to *Phytophthora nicotianae* var. *nicotianae*, has now been released by the Agricultural Research Council of South Africa. This cultivar originated from back crossings of C11, WOSB, Irrabourbon and CDL28. After seven generations of inbreeding, it has attained homozygosity for the much needed black shank resistance as well as other desirable characteristics.

LD2 was tested over five seasons and in all dark air-cured tobacco production regions in South Africa. The cultivar was found to be widely adapted with acceptable yield, quality, income as well as total nitrogen, total alkaloids and reducing sugar concentrations. LD2 yields higher than CDL28, the current South African standard.

Agricultural Research Council - Institute for Industrial Crops, Private Bag X82075, Rustenburg 0300, South Africa

DORFEY C.(1); KÖHLER A.(2); SCHOENINGER K.(2)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 23

Spatial distribution and impact of adjacent vegetation on families of parasitic *Hymenoptera* associated with organic tobacco in Santa Cruz do Sul, RS, Brazil

The objectives of the research were to assess the fauna of *Hymenoptera* parasitoids associated with tobacco under organic management, to verify its spatial distribution within the growing area and to assess the impact of the presence of adjacent vegetation on the abundance of parasitoids. The study was conducted in a tobacco field certified as organic in Santa Cruz do Sul, RS, Brazil, in the 2008/2009 and 2009/2010 tobacco crops. Three sampling lines were determined corresponding to: (1) a line with abundant adjacent vegetation composed mainly of large trees and shrubs; (2) a line with adjacent vegetation consisting of shrubs and native tree species of smaller size; and (3) a line with adjacent vegetation composed mainly of grass, limited only by another organic tobacco field. On each line three sampling points were set ("Outside," "Border" and "Inside") in which were installed four pit-fall traps and a Malaise trap. Samples were collected weekly from November 20th, 2008 to January 29th, 2009; and from November 20th, 2009 to January 28th, 2010. A total of 31.574 hymenopterans parasitoids were collected, assigned to 10 superfamilies, 31 families, and more than 70% of them were sampled at the "Outside" and "Border" sampling points. The families *Ichneumonidae*, *Braconidae*, *Platygastridae*, *Figitidae*, *Mymaridae*, *Eulophidae*, *Encyrtidae* and *Bethylidae* accounted for approximately 90% of the total collected. It was observed that the presence of adjacent vegetation consisting of different plant species from the main crop, as well as the successional stage of growth of these plants, has an impact on populations of parasitoids and contributes to greater diversity and a more homogeneous distribution of parasitoids among the field area. Thus, the planting and or maintenance of adjacent vegetation to tobacco fields may become an alternative to improve conservational biological control in the context of the culture.

1. *JTI Kannenberg Comércio de Tabacos do Brasil Ltda, Brazil*
2. *University of Santa Cruz do Sul (Unisc), Brazil*

ELLINGTON G.H.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 34

Flue-cured tobacco curing energy consumption and technologies to improve efficiency

Bulk curing energy consumption is a significant production cost for U.S. flue-cured growers and uncertainty remains concerning future energy prices. Over the past few seasons commercially available technologies that can potentially improve the energy efficiency of existing curing barns were implemented and evaluated. Additionally, the on-farm energy consumption data collected was used to assist with quantifying a seasonal average energy efficiency growers should target.

A bulk curing barn fan motor operates for extended periods at a reduced load as the tobacco dries. As a result, significant electrical energy and cost savings can be obtained by reducing the motor speed during the curing process. Variable frequency drive (VFD) control technology was utilized during the 2009 and 2010 seasons to decrease the fan motor speed and reduce the electrical energy consumption. At each location direct energy comparisons were made with an identical barn that maintained a constant fan speed. The seasonal average electrical savings ranged from approximately 4% to 25% (70 kWh to 380 kWh) across all locations. The maximum electrical saving obtained at any location for a single cure was approximately 32%. Additionally, the cure duration was not increased and only minimal, if any, differences in the cured leaf quality were observed as a result of reducing the fan speed.

Extremely low fuel cost compared to conventional fuels is the main justification for burning wood chips and other biomass fuels for the thermal energy requirements. Although the capital cost of a commercially available wood-chip heating system is considerably higher than that of a conventional fueled system, the significant energy cost savings (70% to 90% reduction) makes the investment economically attractive. Five cures were completed during the 2009 season utilizing an automated wood-chip fueled hot water system. The fuel cost averaged approximately $99 per cure. An additional benefit of curing with hot water is the TSNA mechanism associated with utilizing conventional fuels is eliminated.

North Carolina State University, Raleigh, NC 27695, USA

FISHER C.R.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 21

IPM and pest epidemics

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

University of Kentucky, Lexington, KY 40546, USA

FONTAINE B.; BLANCARD D.; ARMAND J.M.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 20

ProDuTa & e-phytia: a new approach for tobacco diseases identification and knowledge spreading

Each year, many trials are implemented by public or private centres to improve tobacco production. Many results are published but one question remains: how to transfer this knowledge towards producers or technical services?

Nowadays, the Internet is widely used to search data that may be needed for work related purposes. ANITTA, the French National Technical Center for Tobacco Professionals, had its own website, but it was much too static and not attractive enough for tobacco growers. ANITTA and INRA (the French National Institute of Agronomy) worked on a new website project centred on tobacco diseases. In addition to the classical information on diseases, a new tool has been developed to help people make a diagnosis with pictures. Step by step, the user is directed towards the most probable disease and can reach further information (biological cycle, other symptoms, control measures…). This application is now compatible with some new media such as Smartphones, for consultation directly in the field.

Moreover, INRA developed a ludic and educational programme for testing user knowledge about tobacco diseases. This part of the website, managed by INRA, also contains information and pictures concerning weeds and pests that can be found in a crop.

On its side, ANITTA contributed to the website by providing simple tools such as water balance, nitrogen balance, income calculation, beneficial insects identification …) in order to help tobacco producers and technical services.

This website is particular in that it encourages knowledge exchange. Every user can add comments, pictures and documents, which are the responsibility of the webmaster who has to check and validate before putting them on line.

The Scientific Commission of CORESTA has undertaken, in 2011, the translation into English of the tobacco diseases section, including the diagnosis with pictures.

1. *ANITTA (Association Nationale Interprofessionnelle et Technique du Tabac), Domaine de la Tour, 769 route de Saint Alvère, 24100 Bergerac, France*
2. *INRA (French National Institute of Agronomy), Bordeaux, France*

FORTNUM B.A.; PETERSON P.D.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 12

Bacteriophage can reduce *Ralstonia solanacearum* induced stem necrosis in tobacco

Bacterial wilt caused by *Ralstonia solanacearum* is an extremely damaging disease of flue-cured tobacco in the south-eastern USA and can be spread mechanically on farm machinery. *Rolastonia solanacearum* exhibits strong tissue-specific tropism within the host, invading primarily the xylem tissue. Transmission of *R. solanacearum* with a mechanical topper or harvester can inoculate the bacterium deep within the tissue directly into the xylem elements or surrounding tissue, dramatically reducing the amount of inoculum needed to elicit disease. Surface application of bacterial disinfectants is generally ineffective due to the deep placement of the bacterium. Bacteriaphage are bacteriolytic viruses and have been observed in association with *R. solanacearum*. *Ralstonia solanacearum* infected with a bacteriophage (DILY), producing large clear plaques in culture, was evaluated as a biological control agent for pathogenic strains of *R. solanacearum* in tobacco stem tissue. Pathogenic strains of *R. solanacearum* (isolate SC 10 and NC 132, 2009) and (SC 06, SC 10 and Y3, 2010) where applied (1×10^6 cfu) in combination with DILY (1×10^8 cfu) in all possible combinations to tobacco stems during the mechanical flower removal operation. DILY reduced stem necrosis 42 and 35% when combined with a pathogenic isolate SC 10 and NC 132, respectively in 2009 ($P \leq 0.05$). DILY reduced stem necrosis 45, 16 and 26% when combined with pathogenic isolate SC 06, SC 10 and Y3, respectively in 2010 ($P \leq 0.05$). The implications for nonchemical disease suppression in mechanically transmitted *R. solanacearum* will be discussed.

Clemson University, Department of Entomology Soils and Plant Sciences, Pee Dee Research and Education Center, Florence, SC 29506, USA

GELDENHUYS C.J.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 35

Sustainable wood supply from natural forests and woodlands versus deforestation for alternative wood supplies

Harvesting poles for construction of homes and fences had been a traditional practice for centuries in many parts of Africa. The need for poles of specific dimensions and form in the growing and curing of tobacco added to this pressure on the natural forests and woodlands. The perception in many places is that the species of natural forests and woodlands grow too slow to provide in the pole needs and that fast-growing introduced tree species need to be planted to provide alternative pole resources. Perceived degraded mixed-species woodlands are cleared to grow the alien single-species stands at wide spacing. Recent observations and studies in southern African woodlands in Mozambique and Zambia showed that the fire-adapted woodland species develop underground rootstocks with stored reserves for regrowth after fire and clearing. Existing stunted seedlings show no height-growth response after single-tree selective timber harvesting from mature woodland. However, after clearing tree stands for slash-and-burn agriculture or charcoal production, the woodlands rapidly recover their biodiversity and productivity. All the tree species show remarkably fast regrowth from the below-ground root stocks. Dense regrowth tree stands develop with stem diameter growth > 1 cm/year. Selective harvesting of stems from regrowth stands will provide more growing space for faster growth of remaining stems. This would initially provide laths, then poles, then trees for house and fence construction and for poles needed for tobacco curing. The paper will provide an overview of the observations and study results, will demonstrate that such pole harvesting could sustainably supply in the pole use needs, and that woodland regrowth management will maintain tree diversity and sequester more carbon than planting of introduced tree species.

Department of Forest & Wood Science, Stellenbosch University

c/o Forestwood cc, PO Box 228, La Montagne 0184, Pretoria, South Africa

GUO Yushuang; REN Xueliang

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 29

Cloning and functional analysis of a novel DREB transcription factor from *Nicotiana*

DREB transcription factors have important roles in regulating stress responses in plants such as low temperature, drought and salinity. In this research, a novel DREB transcription factor gene named *NbDREB2a* was isolated from tobacco (*Nicotiana benthamiana*) using homology-based and RACE technology. The full-length of the *NbDREB2a* cDNA was 1191 bp and predicted to encode a protein of 330 amino acid with a consensus AP2 domain. Quantitative real-time RT-PCR analysis revealed that *NbDREB2a* is expressed ubiquitously in tobacco including leaf, root and stem and the expression level was highest in leaf. Also, *NbDREB2a* could be induced by drought, low temperature and salinity stresses, indicating that *NbDREB2a* may have important roles in plant stress responses. *NbDREB2a* protein can bind to the DRE element and activate the expression of downstream reporter genes in yeast cells. The coding sequence of *NbDREB2a* was then cloned into the binary vector pBI121 to produce pBI-*NbDREB2a* in which the *NbDREB2a* gene was expressed under the control of the cauliflower mosaic virus (CaMV) 35S promoter. The plasmid pBI-*NbDREB2a* was then introduced into *A. tumefaciens* strain LBA4404. By using *Agrobacterium*-mediated transformation, the *NbDREB2a* gene was successfully introduced into *Arabidopsis*. Independent homozygous lines with a single copy of *NbDREB2a* were allowed to grow for 3 generations for further studies. Our results showed that overexpression of *NbDREB2a* enhanced tolerance to drought and high salt stresses in transgenic *Arabidopsis* with high relative chlorophyll content.

Guizhou Tobacco Science Research Centre, Guiyang, Guizhou 550083, P.R. China

HAYES A.(1); LUSSO M.(1); HAJALIGOL M.(1); LEWIS R.(2)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 05

Reduction of nornicotine in experimental burley tobaccos and impact on N-nitrosonornicotine in lamina and smoke

The levels and stability of nornicotine in burley tobaccos have been of considerable interest because of nornicotine's role as a primary precursor in the accumulation of N-nitrosonornicotine (NNN) during and after curing. Efforts to screen foundation seed lots of commercial burley varieties have contributed to a lowering of nornicotine levels. In recent years, a further understanding of the specific enzymes that convert nicotine to nornicotine has led to both transgenic and alternative mutagenic strategies to reduce nornicotine levels by as much as 80% below the levels achievable by seed screening alone. Previous published studies evaluating these experimental burley tobacco lines have demonstrated significant reductions in lamina NNN relative to standard controls. An important extension of this work is to demonstrate that efforts to reduce nornicotine levels in experimental burley lamina translate to NNN reductions in cigarette smoke. In this paper, we will review several studies showing that reduced nornicotine leads to lower lamina NNN relative to controls. We will also present data that further demonstrates the impact of reduced nornicotine levels in experimental burley lamina on smoke NNN levels of prototype cigarettes containing these experimental burley tobaccos.

1. *Altria Client Services Inc, Richmond, VA 23219, USA*
2. *North Carolina State University, Raleigh, NC 27695, USA*

IMAI T.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 11

Susceptibility of the cigarette beetle to low-oxygen atmosphere

Phosphine fumigation has been an exclusive method for disinfesting stored tobacco. However, the situation is getting worse because of the emergence of resistance, as well as the growing public concern about toxic chemicals in the environment. Controlled atmosphere (CA) with reduced O_2 has the potential to be an alternative method; however, knowledge related to its effects on tobacco pests has not been sufficiently accumulated. This study examined basic factors affecting the performance of CA against the cigarette beetle, such as O_2 concentration, temperature and humidity, to establish an effective disinfesting procedure for stored tobacco.

Six to eight gas-washing bottles (1 L) containing 50 to 100 eggs (within 24 h after laid), 30 fourth instar larvae, 40 to 50 pupae and 30 adults were serially connected by silicone tubing. They were purged with authentic premixed gases (0.5%, 1.0% or 2.0% O_2 in N_2) at a rate of 0.2 L/min. Under these conditions, the actual O_2 concentrations measured at the distal end rose by 0.1–0.3%. Humidity was controlled by passing the gases through gas-washing bottles containing saturated solutions of NaCl (75% RH), K_2CO_3 (43% RH) or CH_3COOK (21% RH). The bottles were disconnected one by one from the distal end at given times. Tests were conducted in air conditioned rooms (15 °C, 20 °C, 25 °C or 30 °C).

Results suggest that larva is the most tolerant stage to hypoxia. At 30 °C and 75% RH, LT99 of larva was calculated to be 6.9 d at 0.5–0.8% O_2, but exceeded 20 d for O_2 above 1.0%. When temperature decreased to 25 °C, LT_{99} was extended over 25 d. Humidity influenced the efficacy against larvae and adults. LT_{99} of larva at 25 °C and 1.0–1.3% O_2 were calculated to be 24.0, 44.6 and 50.2 d at 21%, 43% and 75% RH, respectively.

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

JACK A.(1); FISHER C.R.(1); SCHOERGENDORFER A.(1); FANNIN F.F.(1); DANEHOWER D.(2); BUSH L.P.(1)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 04

TSNA accumulation in two burley tobacco cultivars

Differences in TSNA accumulation between burley genotypes can often be attributed to differences in nicotine conversion and/or differences in amount of total alkaloids. However, several researchers have observed differences in TSNAs between certain cultivars that cannot be explained by either of these factors. KT 204LC tends to have lower TSNAs than NCBH 129LC, especially under curing conditions favorable for TSNA accumulation. In a two-year study, these two cultivars were sampled throughout curing, and TSNAs, alkaloids, nitrogenous constituents and leaf surface chemicals were measured. NCBH 129LC had higher TSNAs and higher nitrite nitrogen than KT 204LC throughout the cure. The nitrite nitrogen in KT 204LC increased very little in either lamina or midrib, but the NCBH 129LC nitrite nitrogen increased rapidly from 20 days after harvest. Nitrate nitrogen was higher in NCBH 129LC. Conversion, nornicotine, total nitrogen and total alkaloids were higher in KT 204LC. KT 204LC would therefore be expected to have higher TSNAs, but it did not. Duvatriene diols were also higher in KT 204LC throughout the cure, contrary to our working hypothesis. It is likely that the lower TSNAs observed in KT 204LC are due largely to the lower nitrate to nitrite reduction in that cultivar.

1. *University of Kentucky, Lexington, KY 40546, USA*
2. *North Carolina State University, Raleigh, NC27695, USA*

JIAO Rong(1,2); LIU Guanshan(1); LIU Haobao(1); WANG Shulin(1,2); HOU Na(1,2); WANG Quanzhen(1,2); JIN Yirong(1,2); BAI Yan(1,2); FENG Guanglin(3); LIU Chaoke(3); FENG Xiangguo(3); HU Xiaoming(3)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. APOST 06

Cloning and expression analysis of osmotic stress tolerance gene *NtP5CS* in *Nicotiana tabacum*

Stress tolerance in tobacco is an important subject in tobacco production. In this study, the key enzyme of *P5CS* gene from tobacco (*Nicotiana tabacum*) induced by drought stress was cloned and its Pro synthesis,

sequence signature, evolutionary relationship and the expression profile were analysed. This effort paved the way for establishing a foundation for the research of stress tolerance in tobacco. In this study, the degenerate primers were designed to get the segment of *P5CS*, the 5' and 3' ends of the cDNA were amplified by RACE. The sequence structure, its coded proteins conserved domains and the basic characteristics of the cloned gene, were analysed using bioinformatics. Its expression patterns were studied by RT-PCR. The results showed that the cloned gene is 2,584 bp in full length and is termed as *NtP5CS* (GenBank accession HM854026). The ORF is 2160 bp in length, which encodes proteins of 719 amino acid residues. GenBank Blast analysis showed that the sequence is highly homologous with the *tomPRO2* gene from tomato in both nucleotide and amino acid levels. According to the phylogenetic tree, *NtP5CS* and *tomPRO2* may be the Ortholog gene. RT-PCR showed the *NtP5CS* can be induced by drought, high salt, low temperature, ABA, and it has the highest expression in root and leaf of tobacco's prosperous long-term and water stress condition. In conclusion, this is the first time that *P5CS* is cloned from tobacco. From its response to water stress, it may be inferred that it plays a role in resistance to osmotic stress.

1. *Key Laboratory of Tobacco Quality Control, Ministry of Agriculture, Tobacco Research Institute of CAAS, Qingdao 266101, P.R. China*
2. *Graduate School of CAAS, Beijing 100081, P.R. China*
3. *China Tobacco Chuanyu Industrial Corporation, Chengdu 610000, P.R. China*

KIM J.H.; JANG S.W.; LEE J.H.; LEE Y.G.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 25

Characterization of novel resistance-breaking isolate of PVY in Korea

Potato virus Y (PVY) occurred in PVY resistant flue-cured tobacco 'KF120' showing vein necrosis in Buron, Korea. This isolate, PVY-ToBR1, was differentiated from other PVY based on biological properties and nucleotide sequence analyses of the coat protein gene. Host range and symptoms analysis were done using sap inoculation of PVY-ToBR1 and PVY-ToJC37 on 19 indicator plants. Remarkably, the PVY-ToBR1 isolate induced distinctly different symptoms of systemic vein necrosis on PVY-resistant tobacco cultivars that have the recessive potyvirus resistance gene *va*. In RT-PCR assays with specific primers for detection of PVY, a single band of about 800bp in length was produced. The amplified DNA was cloned and the nucleotide sequence was determined. The coat protein gene of PVY-ToBR1 showed 88.4% - 99.4% and 86.6% - 99.4% identities to the 46 different PVY isolates of the Genbank Database at the nucleotide and amino acid, respectively. The Phylogenetic relationship based on nucleotide sequences of coat protein genes showed that the PVY-ToBR1 isolate clustered with PVY^{NTN} isolates. The PVY-ToBR1 isolate is more closely related to the European than North American PVY^{NTN} isolates.

Technology Research Group, KT&G Research Institute, 22 Gajeong-ro, Shinseong-dong, Yuseong-gu, Daejeon 305-805, South Korea

LEI Bo(1); PAN Wenjie(1); DING Fuzhang(1); ZHANG Kai(2); LI Jinxin(3); LU Ku(2)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. APOST 07

Comparative transcriptome of tobacco leaves in typical ecological areas of Guizhou

Flue-cured tobacco (*Nicotiana tabacum*) is a sensitive crop to ecological environment, and distinctive tobacco has always formed under special ecological conditions. In order to investigate the molecular mechanism of formation of medium and light aroma tobacco in Guizhou, the Agilent tobacco microarray and digital gene expression (DGE) methods were used for transcriptome detection of tobacco leaves in the medium aroma tobacco area Longgang and light aroma tobacco areas Weining and Tianzhu. The results showed that there were big differences among the gene expression profiles of tobacco leaves under different ecological conditions. The number of differential expressed genes (DEGs) between Weining and Tianzhu detected by microarray and DGE were 2108 and 2756, while the number between Longgang and both Weining and Tianzhu were 1723 and 1440, respectively. Gene Ontology analysis revealed that DEGs among different ecological areas were mainly involved in response to stimulus, especially stress response.

Compared with Longgang, up-regulated genes in Weining and Tianzhu were likely preferred enriched in lipid metabolic and secondary metabolism pathways, including a lot of flavor substance accumulation related genes, such as *TERPENE SYNTHASE 21, PHYTOENE SYNTHASE, LYCOPENE EPSILON CYCLASE LUTEIN-DEFICIENT 2* and *BETA-HYDROXYLASE 1*, while most down-regulated genes seemed to play important roles in response to light, temperature and radiation, such as heat shock proteins. KEGG pathway analysis indicated that the DEGs possessed important roles in carbon fixation in photosynthetic organisms, starch and sucrose metabolism, photosynthesis, glycolysis / gluconeogenesis and nitrogen metabolism pathways, basically reflecting the difference in pathways of tobacco leaves in typical ecological areas. Our study primarily revealed the effect of ecological conditions on transcriptome of tobacco leaves, and laid the foundation for research on molecular mechanism of formation of distinctive tobacco.

1. *Guizhou Tobacco Research Institute, Guiyang 550081, P.R. China*
2. *College of Agronomy and Biotechnology, Southwest University, Chongqing 400716, P.R. China*
3. *Guizhou Provincial Tobacco Company, Guiyang 550003, P.R. China*

LEI Liping(1); QIN Xiyun(1); WAN Anyun(1); CHAI Jiarong(1); XIA Zhenyuan(1); CHEN Hairu(2); YANG Genhua(2)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 09

Using direct PCR method of hyphae for ISSR Marker and ITS sequence study of *Phytophthora nicotianae*

To investigate the diversity of *Phytophthora nicotianae* in the Yunnan Province of China, the direct PCR method was used to compare the differences in inter-translation space (ITS) of ribosomal DNA of *Phytophthora nicotianae* between the isolates from different tobacco cultivated areas in Yunnan Province. The ITS sequences and ITS polygenetic tree were constructed and analysed based on 24 isolates. The results indicated that there was no difference among the ITS sequences of 28 isolates from Yunnan. The ITS sequence of isolates from Yunnan was highly homogeneous (99.60%) and the sizes of the ITS region of *Phytophthora nicotianae* strains from different sources ranged from 910 to 915bp. Based on the phylogenetic analysis of nucleotide sequences with MEGA version 4.0 of NJ (Neighbor-Joining), the strains of *Phytophthora nicotianae* from different areas were gathered. The division of isolates of *Phytophthora nicotianae* was not related to their geographical origins. Inter-simple sequence repeat (ISSR) markers were also used to study the genetic division of *Phytophthora nicotianae*. The results showed that the 28 tested isolates could be divided into 5 cluster groups (ISSR1, ISSR2, ISSR3, ISSR4, ISSR5) with the genetic similarity coefficient 0.78 as the threshold. The tested strains had abundant genetic diversities. Strains from different genetic backgrounds were notably different. There was a certain relationship between the classification of cluster groups and the sources of strains. The study confirmed that direct PCR method of hyphae is quicker and simpler than any other proposed attempts. It has important significance to rapidly detect *Phytophthora nicotianae*. While the ITS region is conservative and ITS sequence is not fit for studying the differences, ISSR marker can be used for intraspecies population studies. *Phytophthora nicotianae* in Yunnan, China can be divided into 5 cluster groups.

1. *Yunnan Academy of Tobacco Sciences, Yuxi, Yunnan Province 653100, P.R. China*
2. *Phytopathology Laboratory of Yunnan Province, Yunnan Agricultural University, Kunming 650201, P.R. China*

LUCAS P.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 37

The basis of sustainability in several agricultural production systems – what we can learn from them and apply to tobacco

Sustainability rests on the principle that we must meet the needs of the present without compromising the ability of future generations to meet their own needs. As in other sectors, sustainability in agriculture integrates three main goals: environmental health, economic profitability, and social and economic equity.

During the last decades, agricultural production showed a huge increase in some regions of the world. As an example, wheat production in France increased from 7.7 10^6 tons in 1950 to 35.8 10^6 in 2000 due to a yield progression from 1.8 t/ha to 7.3 t/ha. This has been made possible thanks to the availability and intensive use of fertilizers and pesticides and to the breeding of highly productive varieties. Meanwhile some unexpected side effects were observed such as nitrate pollution as well as pesticide contamination of soil surface and ground water. This, with increasing prices for limited fossil energy, requires renewed methods of agricultural production while maintaining quality, quantity and economic viability. Some challenges are global (fossil energy, greenhouse gas emission), others are more local (soil and water contamination, human health). Thus, depending on the objectives, solutions may only require local and individual initiatives, or may need collective and organized actions involving different stakeholders with government bodies using their convening power. Some examples will be taken from experiences on low input winter wheat production and on integrated production of field vegetables in France. Advances and limits in the achievements of the goals that were initially set up will be discussed taking into account technical (*e.g.* availability of alternatives to the use of pesticides, breeding for hardy cultivars) and economical aspects (*e.g.* market driven production and the importance of market prices, of quality standards). DEXiPM, a tool to make a multi-criteria evaluation of innovative cropping systems on the three dimensions of sustainability, and which is currently developed on different cropping systems will be briefly introduced. The relevance of these different points will be analysed with respect to tobacco production.

INRA, UMR BiO3P (Biology of Organisms and Populations applied to Plant Protection), BP 35327, F-35653 Le Rheu Cedex, France

MIELE S.(1); BARGIACCHI E.(2); MILLI G.(3)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 10

Natural chestnut tannins to enhance tobacco nutrition and resistance to nematodes

There is an increasing interest toward non-chemical inputs for tobacco, and the other major crops, in order to minimize leaf residues, environmental hazard, and crop footprint and improve manpower safety. Increasing costs of phosphate fertilizers, and reduced nematicide options under agro-environmental measures regulating cropping practices (so-called PSR 2007-2013 plan) in the EU, have made growers particularly concerned about present and future availability, and permission of use, of ordinary agrochemicals. Natural chestnut tannins (NCT), hot water-extracted from untreated wood, and membrane-concentrated to 17% DM, were investigated as a natural alternative for their starter effect and nemastat activity (*Meloidogyne* spp.). Extensive field tests on Virginia Bright tobacco were carried out in 2011 at Fattoria Autonoma Tabacchi of Città di Castello, under different conditions (soils and tobacco cvs.). Tests compared NCT with ordinary treatments, to investigate effect and costs of the different options. Early applications of NCT in transplant water, or in a mixture with the at-planting herbicide, or by other localized application methods, give an interesting starter effect, comparable to an ordinary fertilizer treatment. When conditions were prone to nematode attacks, results indicated that NCT, applied as the previously indicated treatments and microirrigation, determined a good control at costs comparable or better than ordinary agrochemicals. NCT is presently labelled in Italy as a natural soil and water corrective, with claims on their starter effect and nemastat action as secondary effects. A twin-pack NCT-azadirachtine was also developed, as their joint application enhances the nematicide and nemastat actions of the mixture quite a lot.

1. *University of Pisa, Dip. Agronomia e Gestione dell'Agroecosistema, Via San Michele degli Scalzi 2, 56124 Pisa, Italy*
2. *Consortium INSTM, Via Giusti 9, 50121 Firenze, Italy*
3. *Fattoria Autonoma Tabacchi, Via G. Oberdan 12, 06012 Città di Castello, Perugia, Italy*

MILA A.L.(1); BERTRAND P.F.(2); FORTNUM B.A.(3); JOHNSON C.(4)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. PPOST 10

Predicting tomato spotted wilt virus (TSWV) occurrence in tobacco crops in the southeast US

Tomato spotted wilt virus (TSWV) is an important disease of tobacco, reported at significant levels since early 90's. Chemical control, specifically imidacloprid (IMD) and acibenzolar-S-methyl (ASM) applications in the greenhouse before plants are transplanted, is used to manage TSWV in the US. In fields with 25% or less TSWV incidence, IMD provides efficient control. Reduced plant growth in the field has been reported with ASM and therefore its use has not been widely adapted. This issue imposes a challenge for controlling TSWV. Therefore, knowledge of expected risk for a given season and TSWV progress in a field within a season could be useful for disease management. The effect of weather on TSWV loss was investigated with logistic regression analysis. TSWV loss reported between 1993 and 2007 was used from a total of 58 counties in North Carolina that represents 95% of the tobacco counties. The results demonstrated that the sum of monthly average air temperature from December to February had a positive and the total precipitation for the same months a negative effect on TSWV loss. This model was validated in Virginia and North and South Carolina between 2009 and 2011. Predicted risk was in the range of reported one from the tobacco counties in North Carolina and Virginia but overestimated risk in South Carolina. A modified model has been implemented in Georgia since 2010. In a second step, we quantified the progress of TSWV incidence in a field. When quantifying TSWV progress, Degree-Days (DD) and the field's TSWV history were significant explanatory variables. DD with four lower thresholds (*i.e.*, 10.5, 18, 20, and 26 °C) were used. The models were validated with data collected in 2009 and 2010 in North Carolina. Three of the four lower thresholds DD had a good predictability. The models' applicability is currently under investigation with data collected in South Carolina.

1. *North Carolina State University, Raleigh, NC 27695, USA*
2. *University of Georgia, Tifton, GA 31794, USA*
3. *Clemson University, Pee Dee REC, Florence, SC 29506, USA*
4. *Virginia Tech - Southern Piedmont AREC, Blackstone, VA 23824, USA*

MILA A.L.; KATAWCZIK M.; TSENG H.; BITTNER R.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 38

Facts and challenges in managing Granville wilt (GW) in North Carolina

Granville wilt (GW), caused by the soilborne pathogen *Ralstonia solanacearum* (Rs), is a vascular disease infecting hundreds of plant species world-wide. In the United States it infects economically important crops such as tobacco, tomato, potato, pepper, and eggplant in addition to numerous weeds. Rotation with non-host crops, fumigation, and use of resistant cultivars are commonly used to manage GW in North Carolina. Long rotation has increasingly become difficult due to the large size of flue-cured tobacco farms. Recent regulations on the use of fumigants may impose further restrictions in their application on tobacco. Resistance incorporated from *N. tabacum* T.I.448A has been the only source of resistance to GW in the United States. In our laboratory studies the highest GW incidence was observed at 30 and 35 °C in both susceptible and resistant cultivars. These results verify our field observations from replicated varietal trials and suggest that resistance to GW is temperature-dependent, similar to what has been described in tomato. Changes in rotation patterns, limitations in use of fumigants and availability of varietal resistance may challenge our capability to maximize tobacco production in fields contaminated with Rs. Furthermore, the structure of Rs populations inhabiting NC tobacco fields or the effect of implementation of varietal resistance on these populations is unknown. To address this question one hundred eighty four Rs strains isolated in 2007 and 2008 from ten fields in North Carolina and several flue-cured varieties with different levels of resistance to GW were characterized for their pathogenicity and genetic profile. Fields from which the strains were isolated from had a significant effect on their pathogenecity but not the variety from which strains were isolated. Genetic variation was analyzed using repetitive sequence-based polymerase chain reaction (rep-PCR). Statistical analysis revealed that there was a 97% genetic similarity between all the strains. A subsample of 30 strains was sequenced for the *egl* and *avr* genes, genes important for Rs pathogenecity and interaction with the host plant. Strains were almost identical to each other. These results indicate a very uniform Rs population. The implication of these findings to sustain the GW management options mentioned above will be discussed.

North Carolina State University, Raleigh, NC 27695, USA

MOORE J.M.(1); BAFALLUY R.(2); LAHUE S.S.(1); TROXELL C.E.(1)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 02

Evaluation of growth, yield, grade and chloride content of flue-cured tobacco fertilized with various rates of KCl and K-Mag as potassium sources

Fertilizer laws exist in the U.S. which require manufacturers of fertilizer stipulated as "tobacco grade fertilizer" to limit the chloride (Cl) content to not more than two percent by weight. These regulations were put in place based on research which found tobacco fertilized with higher rates of Cl containing materials to cause the tobacco to be thick, hydrophilic and with poor burning characteristics.

Increasing costs of fertilizer have caused tobacco producers to consider alternative sources of K_2O for use in fertilizing tobacco. A lower cost alternative K_2O source that is readily available in Georgia is KCl, potassium chloride, also referred to as muriate of potash. While this material contains 60 percent K_2O, it also contains 47 percent chloride. However, under Georgia production conditions nearly 100 percent of the tobacco can be irrigated, and rainfall prior to the production season is usually adequate to cause some leaching of applied Cl when applied prior to transplanting and crop growth.

An experimental study was conducted on the Coastal Plain Experimental Station of the University of Georgia, near Tifton, Georgia, U.S.A. in 2010 and 2011. The objective of the study to compare the growth, yield, grade and Cl content of the cured leaf as a result of various rates of Cl applied as KCl as a part of the total fertilizer program for producing the crop. Variation among the treatments varied only slightly for growth, yield and grade, but Cl determined from the cured leaf of each of four harvests varied according to the amount of Cl applied in the fertilization program. Appearance of the growing tobacco did not differ by treatment. This work confirms the historical studies that suggest that the amount of Cl containing fertilizer materials be limited in the fertilization of flue-cured tobacco as means of limiting the Cl content of the cured leaf.

1. *University of Georgia, Tifton, GA 31793-5766, USA*
2. *SQM Brazil, Al Tocantins 75, 6 Andar Sala 608-610, CEP 06455-020 - Barueri - SP, Brazil*

MUNTHALI F.C.; MAGULU R.K.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 31

Evaluation of Super Harvest (Flumetralin + Butralin) suckeride in tobacco

Field experiments were conducted to evaluate the sucker control efficiency of the suckerides Super Harvest (Flumetralin + Butralin), Butralin and Antak; and the yield and quality of tobacco in Malawi. Non chemical treatments were also included as control treatments and consisted of hand removal of suckers with normal topping time and topped but not suckered until end of harvesting. The experiment was conducted on both flue-cured tobacco at Mwimba and burley tobacco at Mwimba and Kandiya Research Stations. The treatments were evaluated in a randomized complete block design with four replications. The gross plot size was 6 ridges x 12 plants per ridge at a ridge spacing of 1.2 m with 0.6 m spacing between plants. The net plot was 20 plants of the two middle ridges with the end plants on each side of the ridge discarded. Application of Super Harvest resulted in the best sucker control at 81% in flue at Mwimba; 77 and 69% in burley at Mwimba and Kandiya, respectively. Except for burley at Kandiya, Super Harvest with the highest degree of sucker control also gave the highest cured leaf yield of 3601 kg/ha for burley and 2840 kg/ha for flue at Mwimba. The different treatments had no influence on leaf quality and grade in burley tobacco. Tobacco treated with Super Harvest produced the highest proportion of mahogany leaf colour, while the third grade leaf was the most predominant with the nil desuckering treatment in flue-cured tobacco. Super Harvest gave the best sucker control and the highest yield in both burley and flue-cured tobacco.

Agricultural Research and Extension Trust, Private Bag 9, Lilongwe, Malawi

PEARCE R.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 36

The role of soil conservation practices in sustainable leaf tobacco production

Maintenance and protection of the soil resource is vital to the sustainability of any agricultural production system. Intensive tillage of the soil is still common in modern tobacco production systems. Tillage whether accomplished by hand tools, draft animals, or heavy equipment leads to degradation of the soil resource. Tillage introduces air into the soil promoting the oxidation of organic matter and weakening the soil structure. Tillage buries plant residue leaving the surface bare and exposed. Together, these factors increase the susceptibility to topsoil loss by erosion. Soil conservation practices such as reduced tillage, cover crop management, and crop rotation can help to slow degradation and maintain the productivity of the soil resource. No-tillage methods were introduced to modern agriculture in the late 1960s and have become widely adopted worldwide for the production of grain crops. However, application of these methods to large scale tobacco production has lagged behind due to several social and agronomic factors. There remains a prevailing myth among many tobacco growers that the soil must be stirred or cultivated to promote the growth of tobacco. Recent research and grower experience has demonstrated that with proper management tobacco can be successfully grown with minimum or no-tillage practices. Long-term field research is currently underway to assess the impact of tillage and rotation on soil properties. Just five years of continuous conventional tobacco production has resulted in lower organic matter and reduced soil aggregate size compared to rotation and reduced tillage plots. While conventional/intensive tillage is still the norm for tobacco production, methods such as no-till strip-till, and mulch-tillage are gaining favour with some growers. Increased adoption of reduced tillage methods in conjunction with crop rotation and cover crop management will slow the degradation of soil resources and promote sustainable tobacco production.

University of Kentucky, Lexington, KY 40546, USA

PEEK D.R.(1); REED T.D.(2)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 32

The status of curing burley tobacco in the United States for sustainable production

The physical and chemical quality of burley tobacco is paramount for sustaining production in the United States. As burley growers have become larger in scale the curing season has become much longer. In successive seasons, a substantial portion of the burley crop has experienced drier than normal curing conditions in conjunction with average to below average temperatures. The result is a substantial portion of the crop having a poor position in the current market and thus growers have been harmed financially. Unlike flue-cured tobacco, the nature of air-curing tobacco is predominately dependent on the weather. Therefore, in seasons of unfavorable curing weather, burley growers have little practical impact on the quality of tobacco offered for sale. The objective of this study is to demonstrate how the current burley market describes physical and chemical quality of burley tobacco and what "best curing management practices" need to be implemented to improve the curing environment and sustain burley production in the United States. Temperature and humidity data from several curing environments along with physical and chemical leaf quality evaluations will be presented.

1. *Virginia Tech, Southwest Virginia Agric. Research and Extension Center, Glade Spring, VA 24340, USA*
2. *Virginia Tech, Southern Piedmont Agric. Research and Extension Center, Blackstone, VA, 23824, USA*

PETERSON P.D.; FORTNUM B.A.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 16

The effect of growth regulators on the mechanical transmission of bacterial wilt

Mechanical transmission of *Ralstonia solanacearum* during flower and leaf removal has contributed significantly to the spread and severity of bacterial wilt of tobacco in the south-eastern USA. Mechanical topping and harvesting also coincide with the time period when maleic hydrazide (MH) is applied to arrest

axillary shoot growth. Previous controlled environment and field studies have demonstrated that MH alters the severity of bacterial wilt, especially in relation to application timing. A controlled environment study was conducted to determine the effect of MH and other growth regulator chemicals including flumetralin, IAA, IBA and NAA on the incidence and severity of bacterial wilt. Plants (cv. K346) were grown under standard agronomic practices for South Carolina. Seedlings were transferred to 15 cm pots and grown in a greenhouse on a 12-hour photoperiod artificial light supplement until they reached 30 cm tall. Plants were transferred into a controlled environment chamber at 30 °C, 68% RH on a 12-hour photoperiod. The experimental design was a randomized complete block with 3 replications and then repeated. A pathogenic strain (isolate Y3) of *R. solanacearum* was cultured on tetrazolium amended nutrient agar, re-suspended in de-ionized water at Optical $Density_{600} = 0.2 = 10^8$ cells/ml and diluted to $2x10^6$ cells/ml for inoculation. The growth regulator treatments and a water only control were applied 4 days prior to inoculation with *R. solanacearum* by misting the leaves until wetness with standardized solutions of each treatment. *R. solanacearum* inoculation simulated mechanical flower removal using a scalpel to excise the apical bud, then by pipetting 100 µl of inoculum on to the cut stem. Plants were assessed every 3-5 days for disease severity starting 7 days post-inoculation and rated on a 0 to 5 scale (0 = no visible symptoms, 5 = complete collapse of tissue). Stem necrosis was recorded on a 0 to 5 scale at the final disease assessment date. MH application reduced stem lesions, leaf wilting and stem necrosis ($P < 0.05$). Leaf wilting was reduced by IBA application ($P \leq 0.05$). Flumetralin did not reduce incidence or severity of mechanically transmitted *R. solanacearum* ($P \leq 0.05$).

Clemson University, Department of Entomology Soils and Plant Sciences, Pee Dee Research and Education Center, Florence, SC 29506, USA

PRAT M.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. IG 02

Agrochemical Analysis Sub-Group Report

The CORESTA Agrochemical Analysis Sub-Group is part of the Product Technology Study Group.

The Sub-Group's objectives are:

1) To perform regular proficiency testing of Multi-Residue Methods for the analysis of agrochemical residues on tobacco.
2) Undertake joint experiments to resolve unanswered questions arising from proficiency tests.
3) Produce and maintain a series of Technical Notes (on different agrochemical residue classes and selected individual compounds) to supplement the CORESTA Guideline N° 5 (Technical Guideline for Pesticide Residues Analysis on Tobacco and Tobacco Products) and aid in method development and improvement.

This paper will report on the Sub-Group activities in 2010 focusing on the outcome of the annual proficiency test, conducted in collaboration with FAPAS (part of the Food and Environment Research Agency, York, UK), the results of the Joint Experiment on Cyfluthrin and the degradation study on 8 agrochemicals. It will also present the various Technical Notes produced by the Sub-Group.

JT International Germany GmbH, Diedenhofener Strasse 20, D-54294 Trier, Germany

PRZYBYS M.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 24

Amino acid sequence of virus protein genome-linked (VPg) of potato virus Y (PVY) influences on overcoming of tobacco resistance

Some isolates of potato virus Y (PVY) are able to break the resistance to PVY of the main tobacco resistance sources, such as cultivars VAM and Wislica. The main aim of this study was to determine the genetic background responsible for the ability to overcome the resistance of tobacco. For this purpose, full genomes of the isolates which broke the resistance of VAM and Wislica were sequenced and compared with

the genomes of isolates that were unable to break the resistance. A mutation at the same position(s) in all resistance-breaking isolates would suggest that the change was directly linked to VAM and Wislica resistance breaking. Amino acid sequence analysis of VPg of fifteen PVY isolates revealed that amino acids residue at positions 101 and 105 are decisive in determining the ability to break the resistance of tobacco cultivars. The mutation in position 101, described for the first time in this paper, was responsible for overcoming resistance to PVY in VAM and Wislica varieties. This mutation consisted in the substitution from serine within non-breaking isolates to glycine within resistance-breaking isolates. Moreover, the studies showed breaking of resistance as a result of the mutation in the position 105 of VPg protein, which led to replacing lysine or arginine by threonine or glutamic acid. In addition, VPg amino acid sequence comparison of isolates revealed eight positions, which decide the grouping of isolates to suitable strains of virus.

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

QIN Xiyun(1); YANG Cheng(2); ZHANG Lei(3); JIANG Zili(2); FANG Qi(3); DING Ming(3); ZHANG Zhongkai

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. PPOST 05

Identification and phylogenetic analysis of 3'-part genomic sequence of an isolate of chilli vein mottle virus (ChiVMV) infecting *Nicotiana tabacum*

The genus *Potyvirus* is the largest plant virus genus. An isolate of potyvirus, designated YN75, was obtained from tobacco (*Nicotiana tabacum*) in Dali, Yunnan Province. The virions of YN75 in the crude sap of infected plants were flexuous, 730-770 nm in length, about 12 nm in diameter. Pinwheel-like inclusion bodies characteristic of potyviruses were found in the ultrathin sections of infected plants under transmission electron microscope. YN75 reacted weakly in enzyme-linked immunosorbent assay with potyvirus-specific antibodies. The 3'-part of YN75 genome was amplified by reverse-transcriptase polymerase chain reaction. The 3'-part of YN75 comprises 1188 nucleotides and has the characteristics of potyviruses. Sequence comparisons and phylogenetic analysis indicated that YN75 was an isolate of chilli vein mottle virus (ChiVMV). The coat protein of YN75 has the highest amino acid sequence identity of 91% with an Indian ChiVMV isolate. The identities of amino acid sequences of coat proteins of YN75 with other ChiVMV isolates were over 90.4%, while identities with other potyviruses were 54.2% to 67.0%. The nucleotide sequence identities of the 3'-untranslated region of YN75 with other ChiVMV isolates were between 89.0% and 90.4%, while identities with other potyviruses were 30.9% to 39.6%. This is the first report of ChiVMV infecting tobacco plants.

1. *Yunnan Academy of Tobacco Sciences, Yuxi, Yunnan Province 653100, P.R. China*
2. *Dali Tobacco Company of Yunnan Province, Dali 671000, P.R. China*
3. *Biotechnology and Genetic Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming 650223, P.R. China*

REED T.D.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 22

Integrated Pest Management strategies for tobacco production in the United States

Tobacco production remains a high value, labor intensive crop in the United States compared to other field crops. Although increases in fuel and labor costs have dramatically reduced operating margins, the profitability of tobacco production is further impacted by a wide range of pests. Such pests include various diseases, nematodes, insects, and weeds. Decades of research programs have focused on developing integrated management strategies for the most important pest species. Results of these efforts have been made available to growers through extension education programs. Integrated management programs utilize an assortment of available control strategies. Such efforts include the planting of resistant varieties, crop rotation, application of agrochemicals based on field histories and economic thresholds, as well as the use of

cultural practices to avoid pest occurrence. Examples of practical application of integrated pest management strategies will be presented and discussed as well as current gaps in available options. Currently available pest management options for organic tobacco production will be discussed. The sustainability of critical pest management strategies will be discussed in relation to the expectation of increased regulatory oversight of the tobacco grower.

Virginia Tech, Southern Piedmont Agric. Research and Extension Center, Blackstone, VA 23824, USA

SANCHEZ-TAMBURRINO J.P.; EDWARDS K.; JONES L.; DAVENPORT S.E.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 27

Tobacco Biotechnology: a tool for business solutions

Over the past 20 years, genetic modification and molecular breeding has been successfully used in many different crops to provide agronomic advantages (*i.e.*, herbicide resistance, insect tolerance). However there are many other areas where it can be used for improving a crop, from nitrogen use to drought tolerance, from increased shelf life to improved flavour.

Genetic modification and molecular breeding of tobacco provides a unique tool to quickly evaluate the function of genes within tobacco plants and their value for delivery of new improved varieties. Molecular breeding is a non-GM approach that utilises natural variation that exists within genes and which can give rise to changes in their function with respect to new tobacco types.

Here we present opportunities in tobacco genome/molecular breeding and applications of genetic modification as potential solutions to future challenges.

Advanced Technologies (Cambridge) Ltd., Cambridge CB4 0WA, UK

STEWART A.M.; FISHER L.R.; PRIEST J.A.; WHITLEY S.D.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 17

Report of 2010 pesticide residue field trials in North Carolina flue-cured tobacco

The pesticide residue field testing program at NC State University is an ongoing project that evaluates various crop protection agents (CPAs) for their maximum expected residues on cured leaf. Applications are made based on the maximum rates, timings, seasonal use, and minimum pre-harvest interval (PHI) according to current US pesticide label. Applications are made in a manner recommended on the label. Field-grown flue-cured tobacco is separated into three stalk positions and residues are measured on the cured leaf. Treatments for each CPA were arranged in a randomized complete block at two separate locations.

CPAs in the program are evaluated for three years. In 2010, four new CPAs entered the program. These were chlorantriniliprole (Coragen), flubendiamide (Belt), clothianidin (Belay), and bifenthrin (Capture). Results will be reported for these four CPAs across the two locations to show the maximum, minimum, and mean residues observed. Additionally, confidence intervals for cured leaf residues of the four CPAs will be reported.

North Carolina State University, Raleigh, NC 27695, USA

SUZUKI S.(1); NOGUCHI S.(1); YAMAGUCHI N.(1); FUJISAKI T.(2); HIYOSHI T.(1)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 28

Decrease in the carbohydrate (starch and sugar) content of flue-cured tobacco leaves by reduced expression of the genes related to starch biosynthesis

Starch and sugar (carbohydrate) contents of flue-cured tobacco leaves produced in Japan, which are higher than in other countries, are related to the low filling capacity of cut rag. Reducing the contents is expected to improve its applicability to tobacco products. For this work, we applied a genetic-engineering approach

to reduce the carbohydrate content and specifically examined starch, which is the major carbohydrate in green leaves. This report describes the growth, morphology, carbohydrate content and filling capacity of the transgenic tobacco plants with repressed expression of genes related to starch biosynthesis.

Decreased contents of starch and sugar (carbohydrate) in leaves was observed in transgenic tobacco plants with reduced amounts of protein or enzymatic activity by RNAi-mediated gene silencing of fructose - 1,6-bisphosphatase (FBP), glucose-6-phosphate isomerase (PGI), ADP-glucose pyrophosphorylase small subunit (AGPS), and isoamylase1 (isoamy1), respectively. Growth retardation and pale-yellow leaves were observed in the FBP transgenic tobacco, but PGI, AGPS, and isoamy1 transgenic tobacco showed little difference in growth, appearance, or leaf colour. The filling capacity of the cut rag from flue-cured leaves was also improved in these three transgenic tobaccos according to a decrease in the carbohydrate content.

These data suggest that the repression of starch biosynthesis in green leaves is useful to improve the filling capacity of the cut rag from flue-cured tobacco leaves produced in Japan.

1. *Japan Tobacco Inc., Leaf Tobacco Research Center, 1900 Idei, Oyama, Tochigi 323-0808, Japan*
2. *Japan Tobacco Inc., R&D Planning Div., 1-17-7 Yokokawa, Sumida-ku, Tokyo 130-8603, Japan*

TEJEDA H.R.(1); GONZALEZ R.(2); COX A.(2)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 39

Effect of high fertilizer nitrogen rates on tobacco cured leaf yield and leaf parameters associated with yield and quality in Central Chile

Tobacco growing areas in Chile are limited; however crop yield potential is high. Soil, climate, low presence of diseases and irrigation are factors responsible for high yields. Nitrogen is the main yield driver, mainly when the economic yield is a vegetal organ like leaves. A study was conducted to evaluate the effect of high fertilizer nitrogen rates upon tobacco yield, quality and related parameters. The objective was to determine profitable high yields that growers may attain by increasing fertilizer nitrogen rates and the effect on leaf parameters associated to leaf grading. Trials with flue-cured and burley tobacco were planted on growers' farms. Seven nitrogen rates, up to maximum rates of 187 and 420 kg N ha^{-1} for flue-cured and burley tobacco, respectively, exceeding the typical N rates of 140 and 300 kg ha^{-1} used for each tobacco type, were used. Trial design was a randomized complete block design with 3 replications. Other nutrients, management and irrigation practices were applied following recommendations from local BAT-CCT Tobacco Company. Soil and plant samples were analyzed at the National Institute of Agricultural Research (INIA). Total nitrogen was determined in leaf samples taken before topping. Leaf color was determined using a Hydro N-Tester, a SPAD-type color sensor distributed Hydro Agri GmbH (Yara Fertilizers). In the flue-cured trial, after reading the color, some leaves were cut to determine nitrate-N in the petiole using a Horiba Cardy Meter. In the burley trial, successive leaf color readings, total N in leaves, and commercial leaf grades were determined. High cured leaf yields resulted for the highest N rates, close to 4000 kg/ha for flue-cured and over 4000 kg/ha for burley tobacco, respectively. Maximum nicotine levels below 2.8% resulted for flue-cured tobacco. Significant regressions among N rates, measured plant parameters, yield and values for commercial quality resulted. Plant parameters measured before harvest may have some predictive value for tobacco yield and/or quality.

1. *SQM S.A., Los Militares 4290, Piso 5°, Las Condes, Santiago, Chile*
2. *Consultant Agronomic Engineer*

VARNEDORE T.(1); MOORE J.M.(2); JONES D.C.(3)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. PPOST 08

Efficacy of two new insecticides: DuPont's Coragen 1.67 SC and Syngenta's Durivo SC for control of tobacco budworm in flue-cured tobacco

Two new systematic insecticides, Coragen 1.67 SC and Durivo SC applied as tray drenches in the greenhouse, as transplant water treatments in the field, and foliar treatments were compared to several foliar

insecticides for tobacco budworm control in flue-cured tobacco on two farms in Jeff Davis County, Georgia in 2010. Both Coragen 1.67 SC and Durivo SC were controlling tobacco budworms on both the Williams and Wooten farms forty-five days after the tray drench treatments and forty days after the transplant water treatments. Even though Coragen 1.67 SC and Durivo SC transplant water and tray drench treatments were slower acting compared to the foliar treatments, they provided tobacco budworm control equal to the foliar treatments on both farms. Using Coragen 1.67 SC or Durivo SC as a tray drench or transplant water treatments saved both tobacco growers from one to two insecticide sprays across the field.

1. *Jeff Davis County Extension Coordinator, University of Georgia Cooperative Extension, Hazlehurst, GA 31539, USA*
2. *Agronomist - Tobacco, University of Georgia Cooperative Extension, Tifton, GA 31793, USA*
3. *Entomologist (Retired), University of Georgia Cooperative Extension, Mt. Vernon, GA 3044, USA*

VANN M.C.; FISHER L.R.; STEWART A.M.; SMITH W.D.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. APOST 01

Evaluation of potassium rates, application methods, and application timing for flue-cured tobacco in North Carolina

Research was conducted in 2009 and 2010 to evaluate the effects of various potassium rates and application methods on yield and quality of flue-cured tobacco. Locations were the Upper Coastal Plain Research Station in Rocky Mount, NC and the Oxford Tobacco Research Station in Oxford, NC. The purpose of this research was to evaluate current potassium rate recommendations and alternative application methods.

The first study evaluated the effects of various potassium rates on the yield and quality of flue-cured tobacco. Rates applied were 0, 75, 100, 125, 150, 175, 200, 225 lbs K_2O per acre, banded beside the plant at transplanting using 0-0-22 (K Mag). In 2009, when tobacco was grown on relative fine textured soils (sandy loam and clay loam) at the Rocky Mount and Oxford locations and with high potassium indices, no yield or quality response to potassium rate was observed. In 2010, plots were selected with more coarse textured soils with only slightly lower potassium indices. Under those conditions, yield and quality was reduced when less than 75 pounds of K_2O per acre was applied. When the potassium index was high, which is common in tobacco producing areas of NC, results from the rate studies indicate that soil texture is still an important factor in determining the amount of potassium required for optimum yield and quality. Potassium rate recommendations in NC range from 90 to 160 lbs of K_2O per acre depending upon K index and soil texture. Under the environmental conditions of these experiments, current potassium rate recommendations appear to be higher than necessary for finer textured soils with a high potassium index, but may be accurate for coarse textured soils in tobacco producing areas of NC.

The second study evaluated the effects of various potassium rates and application methods on the yield and quality of flue-cured tobacco. Potassium rates applied were 75, 125, 175, 225 lbs K_2O per acre. Application timings were: broadcast one month before planting, broadcast one week before planting, banded at planting, and two bands (1/2 after planting and 1/2 at layby). Based on yield and quality data only, it appears that under the conditions of these experiments, 75 lbs per acre of K_2O applied broadcast one month before planting, broadcast at planting, banded at planting, OR applied in split applications, provided adequate amounts of plant available potassium to optimize yield and quality. It is likely that early broadcast applications of K_2O with current rate recommendations would only be of concern with combinations of conditions that included coarse soil textures, low potassium indices, and/or excessive leaching rainfall.

North Carolina State University, Raleigh, NC 27695, USA

VERRIER J.L.; JOURDAIN M.; CAILLETEAU B.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 06

Viral infection and nicotine conversion to nornicotine in the next generation

In tobacco, nicotine demethylation to nornicotine may occur during leaf maturation and yellowing. The gene involved in this transformation is present in any plant whereas it is activated only on some, called "converters".

The appearance of such converters in the descent of non converter plants of burley inbred lines is a frequent phenomenon, thought to be linked to epigenetic events that would affect the regulation of the nicotine demethylase gene.

In order to test the effect of viral infections on such events, the following experiment was performed at Bergerac (France). In 2006, the burley inbred line TN 90LC was grown. Three weeks after transplantation the following treatments were applied, each on 25 shoots:

1. Not inoculated,
2. Mock inoculated with water,
3. CMV inoculated,
4. PVYN inoculated,
5. CMV + PVYN inoculated.

Viral symptoms were recorded on inoculated plants and ELISA tests confirmed the virus presence. Seeds were harvested from 10 individual plants in each treatment.

In 2008, respectively 100, 100 and 200 shoots from the seeds of treatments 1, 2 and 5 plants were grown. Mature middle leaves were individually harvested, air-cured, and analysed for alkaloids. The frequencies of converters (nicotine to nornicotine conversion ratio > 10%) were found to be 14, 6, and 26%.

In 2010, respectively 50, 100 and 100 shoots from the seeds of treatments 2, 3 and 4 plants were grown and analysed, and the frequencies of converters in these descents were 10, 15 and 26%.

This suggests that viral infection, in particular PVY, is associated with a higher frequency of converter plants in the descent. It fully justifies discarding virus infected plants as soon as possible during tobacco seed production.

Institut du Tabac - SEITA ITG, Domaine de la Tour, 24100 Bergerac, France

WANG Fenglong(1); YANG Jinguang(1); ZHAI Xilun(1); SONG Liyun(1); SHEN Lili(1); QIAN Yumei(1); HUANG Jin(2); WANG Yong(3)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. PPOST 03

The application of resistance signal transfer by grafting against TMV in *Nicotiana tabacum*

The tobacco virus disease caused by tobacco mosaic virus (TMV) is one of the most important diseases of tobacco in China, and no effective prevention methods are used to control the TMV virus diseases. In this study, *Nicotiana tabacum* var. *Samsun* NN and *N. tabacum* var. Yunyan 87 were chosen as rootstock and scion respectively. The results demonstrated that stress response was transferred from rootstock to scion when the rootstock was infected by TMV in advance. This indicated that the infection of TMV in rootstock leads to the scion *N. tabacum* var. Yunyan 87 acquiring the resistance against TMV. However, when rootstock and scion were infected by TMV simultaneously or later, the scion did not show resistance against TMV. The expression of PR1 gene and TMV was determined by real-time RT-PCR in three treatments. The results showed that the expression of PR1 gene increased significantly in scion when rootstock was infected by TMV in advance, and replication of TMV was then inhibited dramatically or completely. In contrast, the expression of PR1 increased little in scion when rootstock was infected by TMV simultaneously or later, and TMV replicated greatly in the scion, Yunyan 87. Therefore, the resistance signal could be transferred between rootstock and scion. This new method is useful for preventing the TMV virus diseases in tobacco production.

1. *Key Laboratory of Tobacco Pest Monitoring Controlling & Integrated Management, STMB, Tobacco Research Institute of CAAS, Qingdao 266101, P.R. China*
2. *Tobacco Corporation of Guangxi Baise, Baise 533000, P.R. China*
3. *China Tobacco Shandong Industrial Co. Ltd., Jinan 250100, P.R. China*

WELLS L.G.(1); GOUDOUNECHE J.L.(2); NOIREAU J.P.(2); SMITH T.D.(1); HARPRING M.(3)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 33

Evaluation of a mechanical burley harvesting system in France

A mechanical burley harvesting system will be used to harvest approximately 29 ha at 20 locations in France during 2011. The locations were selected from several geographic locations in France and encompass growers from four production cooperatives; Nord et Loire Tabac, Perigord Tabac, Midi Tabac and Tabac Garonne Adour. The harvesting system was initially developed by the University of Kentucky Biosystems and Agricultural Engineering Department and is manufactured and marketed by GCH International, Inc. with headquarters in Louisville, KY (USA). The system has been operating in U.S. since 2004 with approximate operating capacity of 0.2 ha/hr and annual production of 35 ha.

The system detaches, inverts and cuts notches in mature plants, then places them into slotted receivers mounted in portable steel curing racks. Each rack holds approximately 450 plants and is equipped with folding legs for support. Racks are unloaded in the field when filled while stacks of 5 empty racks are loaded onto the harvester as needed. Filled racks are eventually removed from the field to a convenient location and covered with waterproof material after 7 to 14 days of exposure to outdoor conditions. Burley cures in the racks without further attention within 6 to 8 weeks.

Operational data will be collected and analyzed to determine the efficiency and productivity of the system in France. Pertinent dimensions (length, width, row spacing, in-row spacing, plant density) will be recorded for each field harvested. Harvesting operations will be observed and time-and-motion data will be recorded. Time required for each operation (harvesting, turning, unloading and loading) will be recorded as well as time required for stoppages due to malfunctions, refuelling, repairs, adjustments, etc.

The effects of field geometries, plant factors and other conditions upon operational capacity and efficiency of the system will be reported. Special attention will be given to describing factors that can be managed by growers to maximize harvester capacity and efficacy.

1. *University of Kentucky, Lexington, KY, USA*
2. *ANITTA (Association Nationale Interprofessionnelle et Technique du Tabac), Bergerac, France*
3. *GCH International, Inc., Louisville, KY, USA*

YANG Jun; LUO Zhao-peng; JIN Li-feng; ZHAI Niu; LI Feng; XU Ya-long; ZHOU Hui-na; LIN Fu-cheng

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. PPOST 04

Molecular variation characterization of the genome 5' terminal region of PVY isolated from tobacco in China

Tobacco viral diseases, of which most are RNA viruses, are a category of infectious diseases that are distributed widely and occur severely. The natural variation (mutation) of tobacco viruses proceed quickly, and cause considerable difficulty for prediction and prevention. In order to clarify molecular variation characterization and strain separation of PVY isolated from tobacco, RT-PCR was used to amplify the 5' end of the genome of 3 PVY isolates coming from tobacco-growing areas in Shannxi and Guizhou Provinces of China, and the nucleotide sequence was analysed. The PCR fragment is about 1730nt, and located in the 5' end of the PVY genome. The sequence included complete 5' UTR and P1 gene, and partial HC-Pro gene. Shannxi 2 showed low nucleotide identity and amino acid similarity with Guizhou 1 and Guizhou 6. The major variable region was located in 3' end of 5' UTR and 5' end of P1 gene. PVY had high genetic variability compared Shannxi 2, Guizhou 1 and Guizhou 6 with 32 PVY isolates. Phylogenetic tree analysis of the 5' terminal nucleotide sequence and P1 amino acid sequence of PVY revealed the existence of three distinct groups.

Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, P.R. China

YI Jian-hua; PENG Yu; ZHOU Shu-guang; WANG Xiu-zhong; PENG Xin-hui; ZHOU Yan; ZHANG Wen-li

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 15

Nitrogen supplying capacity of field soil under long-term tobacco-rice rotation and grassplot

Field and greenhouse incubation experiments with ^{15}N labelling were conducted to explore the effects of long-term tobacco-rice rotation practice on the characteristics of soil nitrogen (N) mineralization. The results showed that, related to the effects of continuous grass cultivation (the Control), 16 years tobacco-rice rotation practice resulted in a decrease in soil physical, chemical, and biological properties, such as increased soil bulk weight and salt saturation, decreased soil cation exchange capacity, soil hydrolysis N, microbes, soil organic matter (SOM) and soil fulvic acid (FA). Under tobacco-rice rotation, N fertilizer utilization rate was 19.33% and its loss rate was 48.30%, which was 23.78% lower and 23.03% higher than that of grassplot, respectively. Tobacco-rice rotation had a negative effect on the soil organic N mineralization. During the initial incubation of 60 days, the mineralized N and mineralization rates of tobacco soil were lower than that of grassplot, while it was converse after 78 days. The supply of soil-N in tobacco field soil before the crop's topping was lower than that in grassplot, while the amount of soil-N supplied by tobacco soil was greater than that in grass after topping. On the other hand, the percentage of nitrate-N to mineralized N in tobacco field soil was less than that in grass soil. So, tobacco-rice rotation practice could decrease the soil-N supply in early growing stage of tobacco, but maintain relatively high soil-N supplying capacity in the late growing stage.

Technology Center of China Tobacco Hunan Industry Co. Ltd., Changsha 410007, P.R. China

ZAITLIN D.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 08

The genome sequence of *Peronospora tabacina*, the tobacco blue mold pathogen

Peronospora tabacina D.B. Adam (syn. *P. hyoscyami* de Bary) is the causal agent of blue mold disease, a major foliar disease of cultivated tobacco in many parts of the world. *P. tabacina* is classified as a downy mildew, a group of oomycetes that resemble filamentous fungi but are actually spore-forming protists more closely related to photosynthetic brown algae and diatoms. Many downy mildews are destructive plant pathogens with the potential to cause severe crop disease and economic losses. Like all downy mildews, *P. tabacina* is an obligate biotroph that cannot be grown in culture. Research conducted over the past decade has identified a diverse class of proteins, known as effectors, secreted by phytopathogenic microbes from three different Kingdoms (bacteria, fungi, oomycetes) that act to suppress the host defense response. Oomycete effectors are secreted into the apoplast or from specialized structures called haustoria, where they are then transported into the plant cell cytoplasm. The sequenced *Phytophthora* genomes are predicted to contain between 350 (*Ph. sojae*, *Ph. ramorum*) and >550 (*Ph. infestans*) cytoplasmic effector genes. The genome of *Hyaloperonospora arabidopsidis*, a close relative of *P. tabacina*, contains <150 predicted effector genes, however. A recent effort at the University of Kentucky has resulted in the first DNA sequence of the ~60 Mbp *P. tabacina* genome. The assembly was built from 4.73 million Roche/454 reads and 55 million Illumina 76-cycle reads, and currently consists of 35.9 Mbp of sequence in 2198 scaffolds (N50=51,722 bp). A combination of bioinformatics (*ab initio* gene prediction and effector-specific searches), and comparative genomics against the *H. arabidopsidis* and partial *Pseudoperonospora cubensis* genomes will give an estimate of the number of *P. tabacina* effector genes and enable their isolation and characterization. *P. tabacina* is only the second downy mildew genome to be completely sequenced, and will add to our understanding of biotrophy and pathogenesis in these important pathogens. The genome sequence will be made available to the research community via a BLAST server and on a genome browser showing gene predictions and annotations.

Kentucky Tobacco R&D Center, University of Kentucky, 1401 University Drive, Lexington, KY 40546, USA

ZAMBRONE F.A.D.; SELMI G.F.R.; CORREA C.L.

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 19

Evaluation of the effectiveness of the standard clothing used during tobacco harvest in a tobacco-producing region of Brazil

The level of exposure to which workers are potentially exposed depends on the specific activities that they carry out. As part of the Risk Assessment process, conducting monitoring studies in the field may reflect the real exposure conditions and provide support for better assessment of workers' safety and health protection. The object of the study was to evaluate the effectiveness of the standard clothing used during tobacco leaf harvesting process in a tobacco producing region of Brazil. To do this, the dermal exposure of workers to nicotine and cotinine was determined by passive dosimetry, following guidelines of the Environmental Protection Agency (EPA) and the Organization for Economic Cooperation and Development (OECD). Sixteen (16) workers were monitored during one working day. The dermal exposure was measured using internal dosimeters (cotton clothing, hand-washing, cleaning of face/neck), representing the workers' skin, which were then analyzed, as a means of collection. The outer clothing was an exact representation of the protective clothing used by the workers during the tobacco harvest (impermeable long-sleeved shirt and long trousers, nitrile gloves), and was worn over the internal dosimeters. The quantities of nicotine and cotinine in these matrices were measured by Ultra Performance Liquid Chromatography coupled to a Mass Spectrometry Detector. When the results were analyzed it was found that the standard clothing tested protects workers, preventing between 90 and 99.5% of nicotine exposure.

Planitox - The Science-Based Toxicology Company, Campinas-SP, Brazil
Sindicato da Indústria do Tabaco da Região Sul do Brazil, Santa Cruz do Sul-RS, Brazil

ZHANG Qi(1); CHEN Jing-yun(1); LI Wen-zheng(2); CHEN Xuan(1); LIU Jian-ming(1)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. APOST 08

Clone of glycine-rich RNA-binding proteins genes from different tobacco germplasm resources and expression analysis under different abiotic stresses

Glycine-rich RNA-binding proteins (GR-RBPs) play important roles in the post-transcriptional regulation. In this paper, 11 GR-RBPs genes (*RGP-3*) were isolated and sequenced from different tobacco varieties including cultivated tobacco and wild tobacco. Sequence homology, phylogenetic analysis and gene expression were discussed as emphases. Sequences alignment revealed that *RGP-3* in different tobacco varieties show high homology, with 100% similarity between K326 and *N. sylvestris* and the lowest similarity of 88.1% between *N. goodspeedi* and *N. repanda*. There were 426 variation sites in *RGP-3* genomic sequence, but 383 sites were located in introns. Phylogenetic trees revealed that K326 Hongda were evolutionarily closest to *N. sylvestris*, suggesting closest genetic relationship between *N. tabacum* and *N. sylvestris*. Expression analysis revealed that *RGP-3* in *N. tabacum* was strongly induced by water, weakly induced by SA, and unaffected by ABA treatment. These results will provide the basis for a wide cross to improve tobacco biotic and abiotic resistance.

1. *Shanxi Kunming Tobacco Co. Ltd, Taiyuan 030012, P.R. China*
2. *Yunnan Tobacco Research Institute, Yuxi 653100, P.R. China*

ZHANG Yanling(1); YANG Xin(2); ZHANG Shixiang(1); TIAN Yangyang(1)

CORESTA Meeting Agro.-Phyto Groups, Santiago de Chile, 2011, abstr. AP 13

Effects of humic acids on the accumulation in tobacco leaves of lead (Pb) and cadmium (Cd) found in different soils

Lead (Pb) and cadmium (Cd) are two harmful heavy metals in tobacco leaf that mainly come from soil. To explore a method to reduce lead and cadmium in tobacco leaf, pot cultivation with unpolluted and polluted soil was executed with four main tobacco growing soils (red soil, yellow soil, cinnamon soil and paddy soil) in China. The accumulation of Pb and Cd in tobacco leaf was measured and the effects of humic and fulvic

acids were discussed. The results showed that in unpolluted soils, the Pb concentration of tobacco leaf in different soils was similar despite its great difference in soil while the Cd concentration was significantly different in different soils. The higher soil Cd level and lower soil pH showed positive effects on leaf Cd accumulation. This result suggested that under unpolluted conditions, Pb absorbed by tobacco leaf was mainly related to soil properties while the absorbance of Cd was related to both soil properties and its level in the soil. In polluted soils, Pb and Cd concentrations in tobacco leaf grown in red and paddy soils were significantly higher than that in yellow and cinnamon soils, and pH became the main factor affecting their level in tobacco leaf. Under polluted conditions, Pb and Cd concentrations of tobacco leaf were decreased 17.78%~19.44% and 8.74%~13.58% in red soil and 30.22%~48.32% and 11.03%~32.84% in paddy soil, but increased 11.69%~37.54% in cinnamon soil with both humic and fulvic acid treatments except for a slight decrease in leaf Cd concentration with humic acid treatments in cinnamon soil. However, no obvious tendency was observed in yellow soil. These results suggest that humic and fulvic acid showed different effects on Pb and Cd absorption of tobacco leaf in different soils, the former mainly showed a decreased effect especially in acid soils, and the latter showed restrain and promotion effects in acid and alkaline soils, respectively. The two acids could be used as soil additives to reduce Pb and Cd concentration in tobacco leaf in polluted red and paddy soils.

1. *Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, Henan, P.R. China*
2. *China Tobacco Guangdong Industrial Corporation, P.R. China*