

**ABSTRACTS OF PRESENTATIONS MADE AT THE  
2014 CORESTA CONGRESS IN QUEBEC, CANADA**

**AGRONOMY & LEAF INTEGRITY AND PHYTOPATHOLOGY & GENETICS**

*(in alphabetical order of first authors)*

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

**AMANKWA G.A.; MISHRA S.; SHEARER A.D.; VAN HOOREN D.L.**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. APOST02

**Evaluation of two flue-cured tobacco F1 hybrids with different sources of male sterile cytoplasm**

Hybrid cultivars are growing in importance in flue-cured tobacco production in Ontario, Canada. The first F1 hybrid cultivar was released in 2006, and today hybrids account for about half of the flue-cured tobacco acreage. Cytoplasmic male sterility (CMS) is the foundation of hybrid breeding and seed production in flue-cured tobacco. Hybrid cultivars released in Canada so far have CMS from *Nicotiana suaveolens* cytoplasm; however, efforts are underway to develop hybrids with a *Nicotiana glauca* source of male sterile cytoplasm, in case the current source of CMS may become deficient in some production factor, for example, susceptibility to a disease problem. In addition, under similar growing conditions, hybrid seed production using CMS has been found to improve under *N. glauca* compared with *N. suaveolens*. Two CMS flue-cured tobacco F1 hybrids developed from crossing an advanced breeding line (pollen parent) with a *N. glauca* source and a *N. suaveolens* source of male sterile versions of a commercial pure line variety, were evaluated in field trials along with two pure line cultivars at Delhi, Ontario, in 2011 and 2012. The purpose was to determine if the performance of these hybrids is affected by the source of CMS cytoplasm. Several agronomic traits were measured including yield, grade index, days to flowering, topping height, leaf size and total alkaloids and reducing sugars. A difference was found between the two hybrids only for the width and area of tip leaves and percent total alkaloids.

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**Excessive heat during curing influences TSNA levels in dark fire-cured tobacco**

Experiments were conducted at the Highland Rim Research & Education Center near Springfield TN in 2011 to 2013 to evaluate the effects of excessive temperatures during fire-curing on TSNA levels in dark fire-cured tobacco. Two identical barns were filled with 360 sticks each of PD7318LC dark tobacco each year. Traditional fire-curing methods were used in one barn (“normal barn”) with maximum temperatures not intended to exceed 135 °F at any time during fire curing. In the second barn (“hot barn”), fires were built to exceed 160 °F during part or much of the cure. In one set of experiments conducted in 2011 and 2012, fires in the hot barn were normal except for those occurring during the leaf drying stage, where temperatures reached 190 °F in 2011 and 166 °F in 2012. Leaf samples were collected at the end of curing in this first set of experiments. In another set of experiments conducted in 2012 and 2013, high temperature firing was used during much of the cure from the end of yellowing through leaf drying, with temperatures reaching 180 °F in 2012 and 172 °F in 2013. In this second set of experiments, leaf samples were collected after each firing and corresponded as much as possible to end of yellowing, color setting, leaf drying, finishing, and end of cure (takedown). In the first set of experiments, average total TSNA levels from the hot barn were 2.8 times higher in 2011 and 2.3 times higher in 2012 than average total TSNA levels in the normal barn each year. In the second set of experiments, average total TSNA levels from the hot barn were 4.8 times higher at the end of yellowing, 5.4 times higher after color setting, 11 times higher after leaf drying, 7 times higher at finishing, and 9.9 times higher at takedown than TSNA levels from the normal barn in 2012.

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**Evaluation of mustard bran soil amendments for the control of root-lesion nematode (*Pratylenchus penetrans*) and effects on selected soil fungi in flue-cured tobacco production in Ontario, Canada**

The root-lesion nematode (RLN) is an important pest in flue-cured tobacco production in Canada which is controlled through the pre-planting application of chemical soil fumigants. Mustard green manure and the incorporation of mustard by-products into soil have been investigated as a possible means of RLN control because of their release of allylisothiocyanates into soil. The purpose of this work was to evaluate the use of oriental mustard bran as a soil amendment for RLN control and for possible effects on soil-borne fungi. Field trials were conducted during 2011-2012 using randomized complete block designs. Oriental mustard bran was incorporated in soil at rates of 2250-4500 kg/ha prior to planting. Control plots were untreated or fumigated with Chloropicrin 100. Plots consisted of two rows of plants, each 8 m in length with a 1.168 m between row spacing and a between plant spacing of 51-56 cm. Soil from the central 4 m of the plot was sampled and used for the enumeration of RLN and soil fungi. Plants were measured for growth periodically during the season and yield was determined. Differences among treatments were greater in 2012 and there were no significant differences among treatments in 2011. In 2012, the fumigated treatment had significantly greater yield than all other treatments and the performance of mustard amendments was similar to the non-treated control. In both years and overall, the non-fumigated plots had the lowest plant yield and only the fumigation treatment significantly lowered the mid-season counts of RLN. Mustard bran amendments increased populations of *Fusarium* and *Mortierella* but did not affect *Pythium*. Mustard bran was ineffective in controlling RLN, and was not comparable to fumigation for enhancing plant yield in the presence of significant nematode pressure.

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**Screening, identification and characterisation of an *Arthrobacter* sp. strain MC-10 capable of degrading quinclorac**

The objective of this study was to screen strains able to degrade quinclorac efficiently. The strains were isolated from the perennial administration quinclorac paddy fields. The strain MC-10, which can degrade quinclorac efficiently, was screened by enrichment and selective medium. The strain was identified by morphological, physio-biochemical characteristics and 16S rRNA gene sequence analysis. MC-10 was identified as *Arthrobacter* sp. Under the optimal growth conditions with inoculum concentration of 5%, at 28 °C, pH 7 for 7 days, MC-10 had high biological activity and can degrade more than 90% of quinclorac. Degradation conditions for MC-10 are best at pH 7 and 30 °C. MC-10 can effectively degrade quinclorac when quinclorac initial concentration is within the range 1-100 ppm. And the strain MC-10 has a good ability to survive in the soil. More than 70% quinclorac in the soil can be degraded efficiently after four days of cultivation. These results indicated that *Arthrobacter* sp. MC-10 could be a promising microorganism in dealing with quinclorac pollution. Degradation of quinclorac can reduce the influence of pesticide residues in soil to the next crops, which will have a positive impact on reducing environmental and soil pollution and have positive economic and social benefits.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. APPOST03

**Agrobacterium T-DNA in *Nicotiana tomentosiformis* and other *Nicotiana* species**

*Agrobacterium rhizogenes* induces growth of aberrant roots with bacterial T-DNA; some roots can spontaneously regenerate into plants carrying T-DNA fragments. T-DNAs contain genes that are expressed in plants which normally lead to root growth and to the synthesis of novel compounds, called opines, used by the agrobacterium. Interestingly, tobacco has been found to contain such T-DNA and is therefore derived from an ancient *Agrobacterium* infection event. Few examples are known of plants with T-DNA, another one being *Linaria vulgaris*. Up to now, only a small part of the tobacco T-DNA was identified. Taking advantage of the deep sequencing technique, we sequenced the DNA of one of the two immediate ancestors of tobacco, *Nicotiana tomentosiformis*, and identified 4 T-DNA sequences: TA, TB, TC and TD, derived from four transformation events. These events could have taken place successively or in parallel. In the latter case, the T-DNAs could have been combined by crosses between different transformants. Each of the four T-DNAs consists of partially repeated sequences. The DNA sequences of these repeats were identical at the time of their introduction into the ancestors, but subsequently diverged. Since divergence is proportional to time this allowed us to reconstruct the order of T-DNA introduction. In tobacco, the TC region is absent and may have been lost. So far, we do not know whether and how the T-DNA genes modify the morphogenic or physiological properties of the plants that contain them. We found that most of the 41 potential T-DNA genes lost their function although one (*mas2'*) was shown to encode production of the opine desoxyfructosylglutamine. Some tobacco cultivars have a high *mas2'* expression, suggesting that *mas2'* plays a role in the physiology of these cultivars. Further studies are required to establish the structural and functional basis for the differences in *mas2'* expression.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. APPOST24

**The difference of cadmium tolerance in two varieties of *Nicotiana tabacum* L. and effect of exogenous abscisic acid on cadmium accumulation in *Nicotiana tabacum* L.**

Hydroponic cultures were conducted to investigate the tolerance and accumulation of cadmium (Cd) between two varieties of *N. tabacum* L., 'MS-Yunyan87' and 'MS-K326'. The effect of Cd and abscisic acid (ABA) on growth and Cd accumulation in tobacco seedlings was also investigated. A significant decrease in the dry weight, net photosynthetic rate, stomatal conductance, intercellular CO<sub>2</sub> concentration and transpiration rate was found in two varieties of *N. tabacum* L. The dry weight of 'MS-K326' was higher than that of 'MS-Yunyan87' when exposed to 50 µmol•L<sup>-1</sup> Cd. With the same treatment, net photosynthetic rate, stomatal conductance, intercellular CO<sub>2</sub> concentration and Cd accumulation in shoots of 'MS-K326' were higher than those of 'MS-Yunyan87'. The Cd concentration in shoots and roots of two varieties of *N. tabacum* L. increased with the increasing Cd treatment concentration. In all treatments, Cd concentrations were higher in the roots than that in the shoots. In the presence of 50 µmol•L<sup>-1</sup> CdCl<sub>2</sub>, exogenous ABA treatment increased the concentration in roots, reduced Cd concentration in shoots and the transport of Cd to the shoots from root. The percentage of shoot/total Cd content showed a significant relationship with the transpiration rate.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP01

**Reducing TSNAs in Burley tobaccos through alteration of the N-assimilation pathway**

There is great interest in lowering the levels of tobacco-specific nitrosamines (TSNAs) in tobacco products. Because air-cured Burley tobaccos generally contribute a disproportionate share of TSNAs in typical “American blend” cigarettes, reducing the TSNA content of this tobacco market type would be particularly welcomed. We have previously been successful in lowering the levels of the specific TSNA N-nitrosornicotine (NNN) in Burley tobaccos via the down-regulation of the genes responsible for the synthesis of its alkaloid precursor nicotine.

We have recently employed a molecular genetics-based strategy that was designed to reduce the accumulation of all classes of TSNAs in air-cured tobaccos. Burley plants were genetically transformed to express several candidate transgene constructs. T2 generation plants were grown in two North Carolina field locations in 2013 to determine their effect on TSNA formation. One construct was identified that mediated substantial reductions in all TSNA species in comparison to control lines. Averaged over both locations, total TSNA levels were reduced by over 75% in the air-cured leaf of plants expressing this specific construct. The details and implications of this strategy will be discussed.

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**The Pesticide Approval Scheme Service: reducing Zimbabwe’s environmental footprint**

The Zimbabwean tobacco industry exports most of the crop produced each year to manufacturing companies world-wide. To assist growers' select appropriate pesticides for use in tobacco and to ensure that the exported crop is free of pesticide residues, the Tobacco Research Board (TRB) introduced an Agrochemical Countenancing Scheme in 1964 and it has been operating since then. Consequently, agrochemical use on tobacco in Zimbabwe is restricted to chemicals approved by the TRB. To ensure that pesticide residues on the leaf are within the CORESTA Guidance Residue Levels (GRLs), TRB screens all tobacco agrochemicals for efficacy, application method and timing of application. Annually, well over 100 agrochemicals including new active ingredients on tobacco, those from new sources and new formulations are evaluated. Additionally, in an endeavour to reduce Zimbabwe’s tobacco production environmental footprint, TRB actively searches and screens for safer and greener alternatives to banned and phased-out agrochemicals. As a result of this comprehensive testing programme, the Zimbabwean growers can be certain that the use of all agrochemicals on tobacco will follow principles of Good Agricultural Practice (GAP). Growers are regularly updated on the Pesticide Approval Scheme Service (PASS) developments through the publication of a list of all products recommended for use, those under test and those no longer on the approved list. As a result of largely adhering to the use of agrochemicals approved by the TRB, there have seldom been reports of unacceptable residue levels on the Zimbabwean leaf. This paper provides an update on the Pesticide Approval Scheme and the latest developments in tobacco agrochemical evaluations in Zimbabwe.

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**Effect of planting date on the incidence of aphid-transmitted virus diseases on tobacco in Zimbabwe**

Work conducted at the Tobacco Research Board, in the seasons 1956-1961 showed there was a relationship between time of planting and the incidence and severity of aphid-transmitted viral diseases on tobacco. It was demonstrated that plantings up to mid-November escaped aphid infestations and the resultant diseases, while December plantings were severely affected by viruses. More recently, the introduction of new varieties, changes in climate and general non-compliance with the legislated tobacco planting and stalk destruction dates, made it necessary to investigate if the relationships between planting dates and viral diseases still held. Field trials were, therefore, conducted for three seasons with the objective to investigate these aspects. The most popular TRB variety, KRK26, was used in the trials and four planting dates ranging from mid-September to late December were evaluated. In the third year, five varieties (KRK26, KRK29, KRK66, T71 and T72) were included in the trial. Virus disease assessments were conducted starting from 6 to 16 weeks after planting. Yield data for each planting date was recorded. Results showed that PVY was the most prevalent virus disease followed by tobacco bushy top disease. Additionally, the lowest virus disease incidences were in the September and October crops while December plantings were devastated by PVY with as much as 90% of the crop infected. No differences were apparent among varieties in susceptibility to PVY, indicating that none of the Kutsaga varieties had resistance to the virus. The highest tobacco yields were recorded in the early planted tobacco and generally there was a 700-1000 kg loss in yield for every 30-day delay in planting. These results are similar to those obtained in 1963, and demonstrate that early planting remains a cost effective method of managing aphid transmitted virus diseases.

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**Development of an improved Burley variety with enhanced blue mold resistance**

Recent advances in high-throughput DNA sequencing and other genomics tools have greatly increased the ability and precision of gene characterization, even in plant species with large, complex genomes such as tobacco. A blue mold resistance (BMR) locus originating from *Nicotiana debneyi* has been deployed in several commercial burley varieties. This source of resistance has been underutilized, however, due to a yield penalty associated with plants that are homozygous at this locus, and an observed reduced efficacy when the locus is heterozygous. We subjected near-isogenic burley lines that differ at the BMR locus, as well as an accession of *N. debneyi*, to RNA-seq analysis to identify genes of *N. debneyi* origin that are located on the foreign introgression region in the blue mold resistant burley isolate. Given that introgression fragments derived from wild *Nicotiana* species frequently fail to recombine with their *N. tabacum* genomic counterparts, an additional objective involved testing whether recombination was actively occurring within the chromosomal region that contains the BMR locus. Our results showed that this region is recombinationally active, meaning that it should be possible to reduce the size of the introgression fragment, and likely reduce the associated yield drag, while retaining the BMR trait. The results of the RNA-seq analyses will be discussed within the context of our strategy to produce superior blue mold resistant tobacco cultivars.

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**Investigation of black shank resistance and agronomic performance of flue-cured tobacco lines and hybrids carrying the introgressed *Nicotiana rustica* region, *Wz*.**

Black shank, caused by *Phytophthora nicotianae*, is one of the most important diseases affecting tobacco production in the United States. Growers require genetic resistance to multiple races of *P. nicotianae* that can be combined into cultivars which also provide high yields of cured leaf with acceptable quality. We previously identified DNA markers associated with an introgressed *N. rustica* genomic region (designated as *Wz*) found to contribute to resistance to race 0 and race 1 isolates of the black shank pathogen. We used marker-assisted backcrossing to transfer *Wz* into the elite genetic background of flue-cured tobacco cultivar 'K326' and to develop nearly isogenic lines and hybrids with and without the race 0 immunity gene *Php*. These derived materials were evaluated in multiple field environments for black shank resistance, yield, and quality characteristics. To further characterize the mechanism of the *Wz* resistance, these materials were evaluated in greenhouse and growth chamber experiments for stem resistance and their tendency to succumb to disease under increasing inoculum loads. *Wz* was observed to positively affect resistance in all seven disease field environments tested. Genotypes in which *Wz* and *Php* were combined exhibited the highest levels of resistance. We observed no evidence of a negative relationship between the presence of *Wz* and yield and/or quality. Both race 0 and race 1 isolates were able to overcome *Wz* resistance in a small percentage of plants inoculated in a growth chamber, indicating that *Wz* may not confer resistance of the immunity type. Low levels of stem resistance were observed in *Wz* lines, which differs from high stem resistance observed in *Php* lines. These data suggest strong commercial value for *Wz* in tobacco breeding programs with the goal of developing high-yielding tobacco cultivars combined with resistance to multiple races of the black shank pathogen.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP24

**Effect of growth temperature on polyphenol content and metabolism in tobacco (*Nicotiana tabacum* L.) leaves**

Growth temperature for tobacco plants greatly influences growth and quality of tobacco leaves, and polyphenol content in tobacco leaves was often related to tobacco aroma flavour types. The purpose of this study was to evaluate the effect of different growth temperatures (mean temperature of 30.5, 23.5, and 16.5 °C) on polyphenol content, key enzyme activities and gene expression of polyphenol metabolic pathways in tobacco leaves during the growth and development process of tobacco plants grown in artificial climate chambers. Compared to the leaves of plants grown at a mean temperature of 23.5 °C, tobacco leaves grown in a lower temperature environment (16.5 °C) had higher chlorogenic acid, neochlorogenic acid, rutin, scopoletin, resurrection lily phenolic glycosides, caffeic acid, total phenol, total flavonoids, and lignin content. Plants grown in a higher temperature environment (30.5 °C) had lower chlorogenic acid, neochlorogenic acid, scopoletin, resurrection lily phenolic glycosides, caffeic acid, total phenol, total flavonoids, and lignin content than those grown at 23.5 °C. The activities of PAL and C4H enzymes, key enzymes in polyphenol metabolic pathways, were higher in plants grown at a low temperature (16.5 °C) than in plants grown at higher temperatures. In contrast, PPO and POD enzyme activities were lower in plants grown at low temperatures than in plants grown at higher temperatures. These results indicate that growth temperatures can significantly affect the polyphenol content of tobacco leaves and that lower growth temperatures are advantageous for the synthesis and accumulation of polyphenols.

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**Evaluation of nitrogen application timings on yield, quality, and sucker control in flue-cured tobacco**

Research was conducted in 2012 and 2013 to evaluate the effects of late season nitrogen application timing and method on yield, quality, and leaf nitrogen content. The total recommended nitrogen rate was split over up to four applications during approximately the first eight weeks of the growing season. The latest application was made at topping. Late season nitrogen applications were either applied as a “stalk rundown” or applied directly to the soil surface. Yield, quality, and leaf nitrogen content data were collected. Late season nitrogen applications did not result in adverse effects on yield or quality. Soil applied nitrogen applications late in the season increased yield compared to “stalk rundown” applications. Late season nitrogen applications increased leaf nitrogen content in both the cured leaf and green tissue samples collected one week after application. Both the 2012 and 2013 growing seasons had excessive rainfall, in some cases more than twice the normal rainfall amount for the season. With normal rainfall, it is likely the nitrogen applications at topping would result in adverse effects on leaf quality.

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**Is low-oxygen controlled atmosphere applicable to phosphine-resistant cigarette beetles?**

Phosphine fumigation has been used for disinfesting stored tobacco for many years. Nevertheless, development of resistance in the cigarette beetle *Lasioderma serricorne* (F.) has necessitated that the tobacco industry develop alternative technologies. Low-oxygen (O<sub>2</sub>) controlled atmospheres (CAs) have been the subject of intense interest as an ecologically friendly alternative to phosphine fumigation. The recommended protocols of low-O<sub>2</sub> CA for the disinfestation of stored tobacco (0.5% O<sub>2</sub>/28 °C/9 d; 0.5% O<sub>2</sub>/38 °C/4 d) were established recently based on experimental data obtained using phosphine-susceptible cultures. Hypoxia tolerance of 15 *L. serricorne* cultures with different susceptibility to phosphine (LC<sub>50</sub> for eggs at 25 °C/72 h: 6.6–845.3 ppm) was examined to ascertain whether the low-O<sub>2</sub> CA protocol is universally applicable for beetles irrespective of phosphine resistance. One hundred eggs (collected within 24 h of being laid) contained within glass tubes (50 × 6 mm ID) were placed into gas-washing bottles (1 L). Then five bottles containing the eggs were connected serially by silicone tubing and were purged with premixed gas (0.5% O<sub>2</sub> in nitrogen (N<sub>2</sub>); 75% RH). Tests were conducted in a temperature-controlled room (20 °C). The bottles were disconnected sequentially from the distal end at predetermined intervals (4, 6, 8, 10, and 12 days of exposure duration). After exposure, the egg viability was assessed as a measure of their hatching. Then the time necessary to achieve 50% lethality (LT<sub>50</sub>) was calculated. Susceptibility to hypoxia varied among cultures: 5.3–8.3 days in LT<sub>50</sub>. No correlation was found between susceptibilities to hypoxia and to phosphine. The LT<sub>50</sub> value of the phosphine-susceptible culture, which was used to establish the CA protocol, was comparable to or greater than those of other 14 cultures. Results show that the present CA protocol is also applicable to phosphine-resistant *L. serricorne*.

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### **Six decades later: tobacco research remains the backbone of the tobacco industry in Zimbabwe**

Zimbabwe was and remains a preferred source of flavour-style flue-cured tobacco leaf. This sustained demand for the golden leaf saw agriculture grow by 4.6% in 2012, with tobacco accounting for 10.7% of the GDP and constituting 21.8% of total exports. With 92 000 growers registered, approximately 166.6 million kg of flue-cured tobacco valued at \$612 million was sold in the 2013 marketing season. The economic prosperity of tobacco growers and the industry is largely supported by a vibrant, relevant, practical, cost-effective and environmentally sustainable process of insightful research, development and technology transfer. Zimbabwe exports approximately 99% of the tobacco produced, thus placing it in a highly competitive global market that requires a quality product at the right price. To meet these requirements, the Tobacco Research Board (TRB) has for the last six decades underpinned the tobacco industry in Zimbabwe by proactively and timeously providing elite tobacco varieties, tobacco research information, knowledge and technology to enable the growers to attain the best quality and highest yields of tobacco at the lowest possible cost. Over the past 60 years, some 45 varieties of flue-cured, Burley and Oriental tobacco have been developed and made available to growers. The potential yield of varieties has increased through research from about 800 kg/ha in the 1950s to 5 000 kg/ha currently. Additionally, all agrochemicals used in tobacco production in Zimbabwe are countenanced by the TRB through a Pesticide Residue Scheme Service (PASS) whose recommendations are enforced through the Tobacco Marketing and Levy Act. This ensures their safe use while minimising pesticide residues to guarantee international acceptance of Zimbabwean tobacco. TRB thus remains an integral part of the Zimbabwean tobacco industry and continues to play a major role in maintaining the good reputation of Zimbabwe tobacco. This paper will discuss in some detail TRB's journey as it continues to serve the tobacco industry.

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### **Identification of maternal haploids of *N. tabacum* aided by transgenic expression of green fluorescent protein**

Haploid plants in tobacco (*Nicotiana tabacum* L.) occur spontaneously at low frequencies due to parthenogenesis. For practical use, however, an efficient method is needed to identify them at the seedling stage. There are numerous methods for producing haploid plants in tobacco, of which the interspecific hybridization method has been widely adopted. In this method, *N. tabacum* is hybridized as a female with *N. africana*. This is a semi-lethal cross, and the majority of progeny die at the seedling stage. Surviving seedlings are a mixture of various aneuploid F<sub>1</sub> interspecific hybrids and gynogenic haploids. The separation of haploids from aneuploid F<sub>1</sub> hybrids can be aided by observations of leaf shape, trichome density, and other factors, but this process can still be an art. The objective of this study was to investigate the use of *N. africana* engineered with a *gfp* transgene driven by the CaMV 35S promoter for distinguishing haploid plants from other surviving plants derived from this cross. *N. africana* plants homozygous for a single copy of a 35S:*GFP* insertion were used as male parents in crosses with Burley tobacco cultivar, TN 90LC. Seed resulting from these crosses were germinated and all surviving plants were screened for expression of GFP, genotyped at microsatellite marker loci located across the *N. tabacum* genome, and analyzed for nuclear DNA content using flow cytometry. Results demonstrate *gfp* expression to be a useful visual phenotypic marker for identification of maternal haploids at the seedling stage. DNA marker genotyping of surviving non-haploid plants suggests the role of one or more genes on *N. tabacum* chromosome H in the lethality reaction. Other genes of *N. africana* origin may also play a role in the lethality reaction.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP20

**Comparison of transcriptomes and gene expression profiles of two chilling- and drought-tolerant and intolerant *Nicotiana tabacum* varieties under low temperature and drought stress**

Tobacco plants often encounter chilling and water scarcity conditions when grown in spring and early summer, leading to retarded growth and developmental abnormalities. The purpose of this study was to investigate comprehensive molecular mechanisms of response and adaptation of tobacco seedlings to chilling and drought stress. By newly developed and high-throughput Illumina Hiseq™ 2000 RNA-seq and Digital Gene Expression (DGE) analysis, we constructed two transcriptomes and 14 DGE libraries using the chilling- and drought-tolerant tobacco variety MS-K326 and relatively intolerant variety Yunyan203 during chilling stress at 2 °C for 48 h and air-drought stress for 48 h. From the sequencing results, 106 million 90-bp quality-reads were obtained from two transcriptomes, then assembled into 97,921 non-redundant unigenes with mean length of 653 nt. After annotation to noted databases, all unigenes were used as references to annotate DGE sequences. All DGE libraries were compared to investigate differential expression patterns between the two varieties MS-K326 and Yunyan203 under chilling and drought stress, and 63 genes were found to be differentially expressed in the chilling- and drought-treated tobacco seedlings. By investigating these commonly expressed genes and some other known stress tolerance increasing genes, we found that the stress-tolerant tobacco variety might increase its chilling and drought tolerance by (1) rationally regulating membrane fluidity via changing saturation of lipids and membrane components; (2) synthesising more macro-molecules stabilisers, and osmotic adjustment solutes, that is, osmolytes; (3) reducing reactive oxygen species production by adjusting photosynthesis components as well as eliminating reactive oxygen species by rapidly activating anti-oxidant enzymes. This study provides a global view of transcriptome response and gene expression profile differences between two tobacco varieties with different chilling and drought tolerance in response to low temperatures and drought stresses.

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**HURST P.; VELAZQUEZ S.C.; McCOY N.; MUGWAGWA I.**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP49

**Addressing hazardous child labour and creating decent youth employment in agriculture**

In 2013 ECLT described its work to generate evidence showing that making workplaces safer and healthier is key to combatting child labour in the young adult population in tobacco growing areas, comply with international law, and address the depletion of the tobacco agriculture workforce and abandonment of areas, particularly in continents where the workforce is notably young.

The ECLT Youth Employment initiative was started in 2013 following the Malawi Conference on Child Labour in Agriculture, which was conducted by the ILO, convened by the Government, and sponsored by tobacco companies and ECLT.

In the recent period, ECLT's work advanced by working with public and private social partners and tobacco companies in field experimentation gathering evidence on the necessary conditions that represent decent work for youth on tobacco farms. ECLT also began to extend the Youth Employment initiative to Zambia and Guatemala, in addition to continuing its five ongoing comprehensive projects that address the root causes of child labour in Malawi, Uganda, Tanzania, Mozambique and Kyrgyzstan.

In Malawi, at age 14 “children”, having attained the statutory minimum age for employment, have a legal/human right to be employed as long as it is not in a job with poor health and safety conditions (i.e. hard risk) where they would be classed as “hazardous child labourers”, as described in the Malawian Employment Act of 2000, Part IV, 2013. With good safety and health conditions a legally employed young person can earn money, learn skills, and contribute to family and society. Conversely, the lack of evidence on decent work for youth results in making agriculture work for youth illegal. In addition, the health and safety protection for youth improves the conditions for all workers. ECLT will present the advancement of its efforts to gather evidence.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. APPOST29

**Dissection of genetic overlaps between brown spot and black shank disease resistances in tobacco (*Nicotiana tabacum L.*) line Beinhart 1000**

Brown spot and black shank have been two serious fungal diseases, resulting in huge losses to tobacco production. Tobacco line Beinhart 1000-1 is resistant against brown spot and black shank and appropriate for the genetics analysis and resistance breeding. In this research, the F<sub>1</sub> and F<sub>2</sub> populations were constructed from a cross between Beinhart 1000-1 and susceptible varieties, G140 and Xiaohuangjin 1025. The resistance inheritance and genetic mapping was carried out.

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## JOHNSON C.S.

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP11

### **Potential new black shank fungicides for tobacco: 2009, 2011-2013 Virginia results**

Tobacco black shank, caused by races 0 and/or 1 of *Phytophthora nicotianae*, is among the most common and damaging tobacco diseases in Virginia, as well as around the world. While crop rotation reduces disease pressure effectively, cropping intervals are often too short to control disease alone. Partial black shank resistance is increasing, but many growers remain dependent upon use of a soil fungicide for consistently effective black shank control. Field trials in 2011-2013 compared black shank control on a highly susceptible Burley tobacco cultivar after application of Ridomil Gold (mefenoxam) to that after use of Revus (mandipropamid) or Presidio (fluopicolide). A 2009 test using dark fire-cured tobacco and Burley tobacco studies in 2010-2013 compared black shank control from use of Zorvec (oxathiapiprolin) to that from Ridomil Gold (mefenoxam). All experiments were arranged in a randomized complete block design with four to six replications. Percent black shank incidence was monitored bi-weekly in each trial, from transplanting until harvest. Application of mandipropamid at transplanting followed by use of mefenoxam at layby resulted in numerically higher late-season plant survival (38%) versus application of mefenoxam at both transplanting and layby (29%) or the untreated control (12%). Application of fluopicolide in the transplant water, at first cultivation, and layby also resulted in higher late-season survival (67%) compared to mefenoxam (45%) or the untreated control (19%). Application of oxathiapiprolin improved plant survival (63%) compared to use of mefenoxam (57%) or the untreated control (28%) in 2009 and 2011-2013. Late season plant survival was greater after three applications of fluopicolide (91%) than of oxathiapiprolin (62%) in 2011, but similar among the two products (42% and 32%, respectively) in 2012 and 2013.

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## KANE K.(1); CAILLETEAU B.(2)

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP13

### **Foliar applications of WG<sup>®</sup> CONTANS against *Sclerotinia sclerotiorum* on tobacco crop**

In France, the fungus *Sclerotinia sclerotiorum* affects tobacco late in the cycle, after topping. The problem has been increasing over the last 6-7 years. Rainfall is an important factor for contamination and spread. Symptoms are stem and leaf vein necrosis. At the end of the cycle, sclerotia are observed inside the stem. The disease causes a loss of yield and quality. Few active ingredients are authorised in France and they are preventive only. In order to reduce the pesticide residues and their environmental impacts, alternative methods are being tested.

From 2011 to 2013, experiments were performed at Bergerac, on a highly sensitive flue-cured breeding line in conditions of natural infestation. The tested alternative method consisted of three foliar applications of CONTANS<sup>®</sup>WG (*Coniothyrium minitans* 100 g/kg), 20 days apart, with a dose of 2 kg/ha. The experimental design had randomised complete blocks with four replicates and included an untreated control as well as the reference treatment: two applications of SWITCH<sup>®</sup> (Cyprodinil 375 g/kg + fludioxonil 250 g/kg) at a dose of 0.6 kg/ha, at the same time as the last two applications of CONTANS<sup>®</sup>WG. Treatments were applied with 300 l/ha of spray, according to good experimentation practices, starting 50 days after transplantation.

In terms of frequency of diseased plants, the alternative method produced results on average similar to the conventional method each year. However, parasitic pressure heterogeneities in natural conditions led us to repeat the experiment for one more year (2014). Furthermore, in 2014, the viability of extracted sclerotia from stems of treated and untreated plants will be checked.

CONTANS<sup>®</sup>WG is a product with known efficiency in soil treatment but it is not widely used as a foliar spray. However, this work suggests some interest in using it as a foliar spray. CONTANS<sup>®</sup>WG is a living product, storage conditions must be observed for optimal efficiency.

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**KANE K.(1); SRAKA Y.(2); LECARPENTIER C.(2); VIELLEDENT P.(2)**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. APOST17

**Impact of flowery fallows, crop rotation and intermediate nitrate trap crops on pest pressure in tobacco**

To reduce the use of agrochemicals and environmental impacts, natural pest control methods, including the introduction of flowered fallows, are being tested by ARVALIS Institut du Végétal in a long term experiment located at Bergerac, France.

A three-year rotation (Burley tobacco-corn-wheat) was carried out in six plots (width: 12.8 m, length: 30 m). The flowery fallow "Integrated Biological Protection (PBI)" + "Beetle" seed mixture was implemented for the duration of the rotation around all plots and alleys between plots. Controls, surrounded and not surrounded by fallows, and based on the same rotation, were grown in the same location, about 200 m away from the not surrounded.

Intermediate nitrate trap crops (CIPANs) were produced following wheat and ground on site one month before tobacco transplantation. From transplantation to topping, weekly observations were made in fallows and tobacco plots including controls. These mainly concerned aphids and beneficial insects, but also weeds, native flora and soil-borne pests (wireworms, cutworms).

Observations from 2012 and 2013 suggested that:

- Pests and beneficial insects are much more important in the flowered fallow than in the tobacco.
  - Tobacco plots surrounded by the flowered fallow have a lower pest pressure than controls.
  - The tobacco fertilised by biological fertilisers after CIPANs had a lower pest pressure than the tobacco fertilised with conventional fertilisers.
  - Among tested CIPANs, clover gives the most interesting results for fertilisation (yield and quality of tobacco) but it is necessary to face its regrowth.
  - The tall plants (fallow and CIPAN) are preferred nesting sites of female *Agrotis* (cutworms)
  - Some insects, usually considered as pests, are attracted by specific plant species in the flowered fallow and do not damage tobacco.
  - Observations suggest the existence of allelopathy phenomena among some weeds in tobacco plots.
  - Weeds present in the tobacco plot fertilised conventionally are different from those present in the tobacco fertilised with organic fertilisers.
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**KOGA C.(1); GARWE D.(1); MWENJE E.(2); RUKUNI D.(1)**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP34

**Striga gesnerioides on tobacco (*Nicotiana tabacum*) in Zimbabwe**

*Striga gesnerioides* is a noxious root parasitic weed of the economically important crops [legumes, cowpea (*Vigna unguiculata*), maize (*Zea mays*) and tobacco (*Nicotiana tabacum*)]. In 2002 this weed was identified as a major biotic constraint to tobacco production in Zimbabwe's Mvuma district. Yield losses due to *Striga* parasitism were as high as 100% under severe infestation. A diagnostic survey was conducted to assess the severity of the problem. Greenhouse, laboratory and field studies were carried out to screen for *S. gesnerioides* resistance among 12 flue-cured tobacco varieties. In another study potential trap crops were evaluated. This was done by stimulating germination of *S. gesnerioides* seed with exudates from the roots of host and non-host crops [common bean (*Phaseolus vulgaris*), ground nut (*Arachis hypogaea*), pigeon pea (*Cajanus cajan*) and cowpea]. From the tobacco fields assessed, 32% were infested with *S. gesnerioides*. The general trend observed was that while *S. gesnerioides* was detected in tobacco plots, there were no infestations on other crops in the vicinity namely [maize, groundnut, sorghum (*Sorghum bicolor*), cotton (*Gossypium* spp.), sunflower (*Helianthus annuus*) and the known host crop, cowpea, an indication of *Striga*'s high host-specificity to tobacco. Results from laboratory screening showed that none of the tobacco cultivars evaluated were resistant. In field trials the use of high rates of nitrogen fertilisers (50 kg N/ha) and the most tolerant cultivar (T66), reduced tobacco yield losses. In the trial screening for potential trap crops, it was observed that some of the evaluated crops could stimulate *S. gesnerioides* seed germination without causing further parasitism. This will ensure that the *S. gesnerioides* seed bank is reduced through suicidal germination of the seeds. An integrated *Striga* control program through the inclusion of trap crops in tobacco rotations and improved fertility is a possible management option for tobacco growers in Zimbabwe.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP45

**Soilless media for tobacco (*Nicotiana tabacum*) seedling production in Zimbabwe – widening the options**

It is undisputed that healthy growing seedlings are the basis of a good tobacco crop. Research done at the Tobacco Research Board for decades showed that the use of effective fumigants such as methyl bromide guaranteed the tobacco grower a weed- and disease-free medium on which tobacco seedlings could thrive. However, most of the soil fumigants are being phased out and the best alternative is the use of soilless media. In Zimbabwe, Gromix<sup>®</sup>, produced from well composted pine bark, is the most common medium used. However, the sole source of pine bark in Zimbabwe is the timber producing eastern districts of the country. To prevent reliance on a single finite source and to encourage resource-poor small scale growers to adopt the float tray system, there arose a need to evaluate alternative media based on materials already available on farms. The objectives of this trial were to evaluate the efficacy of coal rubble, composted maize (*Zea mays*) cobs, composted groundnut (*Arachis hypogaea*) shells, cattle manure and bean husks as growing media. Other materials namely, pine (*Pinus* spp.) sawdust, gum (*Eucalyptus* spp.) tree sawdust, and cotton (*Gossypium* spp.) seed hull were also evaluated as alternatives or amendments to pine bark. Seed germination percentage, seedling survival, seedling quality and transplantable percentages were determined. A 50:50 pine bark:sand mixture and 100% pine bark were used as the standards. Results showed that germination and survival was significantly poor in bean husk, cattle manure and in both 100% gum and 100% pine sawdust treatments while other media combinations were statistically similar to the standard media ( $p > 0.05$ ). Seedling stem length and diameter were also statistically similar ( $p > 0.05$ ) among the treatments. Maize cobs, groundnut shells, gum sawdust and coal rubble produced good seedlings and can be used as alternative growing media in the tobacco float seedling production system.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. APPOST05

**Biological and serological characterisation of PVY isolates from Central Europe**

Potato virus Y (PVY) is one of the most important viruses affecting tobacco. It is an RNA virus with a high mutation rate due to error-prone polymerase and a lack of proof reading mechanisms during replication. A high variability of this virus increases chances of the appearance of new highly virulent strains capable of breaking existing sources of plant resistance. Field observations confirm that PVY infection can be found on cultivars previously known as resistant. Here, we aimed at characterising new PVY isolates collected in Central Europe. After preliminary tests using Wislica and VAM, we chose for this study ten PVY isolates differing with their virulence and originating from Poland and Germany. In a greenhouse experiment, we inoculated six plants from each of the ten tobacco cultivars/breeding lines with each isolate. The tested plant material included major sources of PVY resistance known in tobacco, such as cultivars VSCR, PBD6, TN86, VAM, Wislica and a breeding line BPA. Four weeks after inoculation, disease symptoms were recorded and the presence of the virus in the leaf tissues was tested using ELISA tests with two types of Bioreba antibodies. Tested isolates belonged to two groups: PVY<sup>NTN</sup> and PVY<sup>NW</sup>. Most of them were able to cause leaf vein necrosis on cultivars VSCR, PBD6, Wislica and TN86. Few isolates caused this symptom on VAM cultivar. However, none of them caused necrotic symptoms on BPA line. This breeding line reacted only with chlorotic spots on the leaves, despite the fact that ELISA tests showed the presence of PVY in the leaves. This study shows that the BPA line is a valuable breeding material because chlorotic spots caused by PVY do not disqualify leaf material from industrial use.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. APPOST12

**Sunshine duration response on density of tobacco leaves by partial least-square regression**

Density of tobacco leaves, dry matter contents per square meter, was considered to be an important characteristic of flue-cured tobacco, which is closely connected to total sugar and nicotine, and smoking quality as well. Summer sunshine duration was one of the most important reasons that determined the accumulated sugar, nicotine and density of tobacco leaves. To establish the relationship between summer sunshine hours and cellular contents of Honghuadajinyuan leaves, partial least-square regression analysis was carried out. Twenty-nine Honghuadajinyuan samples of middle leaves from 16 tobacco planting regions of Yunnan were collected, with six replications of each, for further study. The results suggested a close association between foliage density and sunshine hours in June and July, with the coefficients 0.617 and 0.632, and  $R^2$  being 61.5%, all of which were statistically significant. Thus, as the sunshine duration increased, density of tobacco leaves rose consequently. The model of estimation and validation provided some degree of assurance about the applicability for tobacco planting patterns.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP41

**Leaf biomass and heavy metals uptake of different flue-cured tobacco varieties under heavy metal contamination**

In order to decrease the heavy metal content and improve the safety of tobacco leaves, pot experiments were conducted to study the effects of different flue-cured tobacco varieties (K326, Yunyan87, NC102, KRK26 and Hongda) on the heavy metal content of leaves. The varieties were grown in soils with added levels of cadmium, lead, mercury and chromium, and in natural soil (control). In soils with added heavy metals, the results showed that the biomass of leaves from the Hongda variety decreased while the biomass of leaves from the rest of the varieties was not affected. The heavy metal content of tobacco leaves from all five varieties increased in the soil with added heavy metals. In the different flue-cured tobacco varieties, cadmium content of leaves from Yunyan87 was the highest while Hongda had the lowest content - Hongda having a significant difference with other varieties grown in the soil with added cadmium. Meanwhile, the root cadmium content showed the same differences as the leaf content. In tobacco leaves from plants grown in the natural soil (control), lead and chromium content were found to be highest in K326, and lower in NC102 and KRK26, with all five varieties showing a distinct difference. In the soil with added lead and chromium, Yunyan87 showed the highest levels of lead and chromium, while Hongda had the lowest. However, the lead content of the tobacco leaves did not show the normal differences in the five varieties and the chromium contents in Yunyan87 were remarkably different from the other varieties. There were no significant differences in the mercury content of the varieties' leaves under natural soil and mercury-added soil - Yunyan87 and Hongda had higher levels while NC102 had lower levels. The arsenic content of tobacco leaves was found to be lowest in NC102, but there were no remarkable differences with the other varieties.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP51

**Degradation and residue levels of triadimefon and its toxic metabolite in tobacco leaf**

In order to further study about different factors of pesticide residues and dissipation characteristics of triadimefon and its metabolite, and provide scientific data for MRLs and proper use of pesticide on tobacco, the dissipation and residue of triadimefon in tobacco leaves was investigated after applications under the GAP conditions. A method for simultaneous determination of triadimefon and its metabolite triadimenol in tobacco were established using gas chromatography with electron capture detector (GC-ECD). The limits of quantification (LOQs) of the proposed method were 0.01 and 0.05 mg/kg in fresh tobacco leaves and cured tobacco leaves, respectively. The average recoveries of triadimefon and its metabolite triadimenol in fresh tobacco leaves and cured tobacco leaves were in the range of 79.8%~97.3% with relative standard deviation (RSD) of 1.5%~9.0% when spiked at 0.01~5.0 mg/kg. The results showed that the dissipation rates of triadimefon under field and storage conditions were obviously different with half-lives of 3.8~5.6 days and 157~315 days, respectively. The terminal residues of triadimefon in cured tobacco leaves were 0.33~6.44 mg/kg, 0.15~2.77 mg/kg and 2.77~2.53 mg/kg when collected 7, 14, 21 days after the last application, respectively. It was recommended that the MRL of the triadimefon in tobacco leaves be 5 mg/kg with a safety interval (PHI) of 14 days.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP38

**Identification of benzoic acid and 3-phenylpropanoic acid in tobacco root exudates (TRE) and their role in the growth of rhizosphere microorganisms**

Tobacco root exudates (TRE) play a key role in the microbial community in the rhizosphere. Tobacco bacterial wilt caused by *Ralstonia solanacearum* is one of the most serious tobacco diseases in China. In order to find out how the root exudates have an effect on the growth of pathogens and their antagonists, experiments were carried out in our laboratory. Two antagonists L-25 and L-9 with strong inhibitory effects on *R. solanacearum* *in vitro* were isolated from tobacco rhizosphere soil. High performance liquid chromatography (HPLC) was used to isolate and identify the types and contents of tobacco root exudates. Exogenous phenolic acids were used to investigate the effect of some phenolic acids, which existed in TRE, on the growth of the tobacco bacterial wilt (*R. solanacearum*) pathogen and its antagonists. Two main types of phenolic acids from tobacco root exudates with the highest concentrations, benzoic acid and 3-phenylpropanoic acid, were isolated and identified by HPLC. The concentration of benzoic acid was  $0.25 \mu\text{g g}^{-1}$  dry roots and that of 3-phenylpropanoic acid was  $1.15 \mu\text{g g}^{-1}$  dry roots. It revealed that lower concentration of benzoic acid and 3-phenylpropanoic acid promoted the growth of the pathogen and its antagonists, while higher concentration of those phenolic acids inhibited the pathogen and its antagonists. Besides, the promotion concentration of these phenolic acids for *R. solanacearum* (benzoic acid:  $3 \mu\text{g L}^{-1}$  and 3-phenylpropanoic acid:  $6 \mu\text{g L}^{-1}$ ) was higher than that for the antagonists. It was concluded that the pathogen made a better use of tobacco root exudates than antagonists, which may be the main cause of this mono-cropping obstacle.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP10

**Black shank resistance and leaf surface chemistry of flue-cured breeding lines nearly-isogenic for QTLs of Beinhart 1000 origin**

Black shank (caused by *Phytophthora nicotianae*) is among the most significant causes of tobacco yield losses in the United States. Host plant resistance is the most effective and cost-efficient means of control. Some sources of resistance have been of the race-specific type that can breakdown over time. Marker-assisted identification of resistance quantitative trait loci (QTLs) associated with partial resistance would help breeders move more robust sources of resistance into elite backgrounds.

Previous work using a Hicks  $\times$  Beinhart 1000 doubled haploid mapping population resulted in identification of Beinhart 1000 QTLs conferring moderate resistance to *P. nicotianae*. Through marker assisted backcrossing into the BC<sub>4</sub> and BC<sub>6</sub> generations, we developed nearly isogenic lines (NILs) in the genetic backgrounds of Hicks and K326 background for the two largest effect QTLs. These NILs have been evaluated for two years at two locations in black shank nurseries. The data suggest the QTLs provide moderate levels of resistance, with a synergistic effect between the two loci.

The previously characterized gene *NtCPS2* involved in the synthesis of *cis*-abienol co-segregates with the resistance QTL on linkage group 15. A closely linked but currently unknown gene is also involved with accumulation of sucrose esters on the leaf surface. Evaluation of genetic materials suggests that the two genes act in an additive manner to affect accumulation of these leaf surface chemistries.

Further efforts are in progress to fine map these QTL using additional DNA markers, as well as to characterize their correlated effects on yield and leaf quality.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP06

**Resistant cultivars: a sustainable management option for the tobacco root-knot nematode in Zimbabwe**

The root-knot nematode (rkn) is an economically important pest in tobacco. Among the effective control measures is the use of resistant cultivars. While the breeding programme at the Tobacco Research Board, Zimbabwe, since 1954 has been aimed at producing resistant cultivars, the abundance of effective fumigant nematicides resulted in this option not being fully utilised by growers. However, the recent phasing-out of fumigant nematicides and the reduction in long-term rotation options due to reduced hectares has brought to the fore the need for resistant cultivars as a rkn management tool. The objectives of this trial were to evaluate the performance of rkn-resistant cultivars when grown with no nematicides and to determine the critical rkn threshold required to cause significant yield losses for each cultivar. Four popular open-release cultivars, KRK26, KRK29, KRK64, KRK66 and two limited-release cultivars, T71 and T72 were used in this study. Field trials, set-up in a randomised complete block design and replicated three times were conducted over three seasons in lands with high rkn populations. Soil samples were collected from each of the plots from 3-14 weeks after planting and greenhouse bioassays conducted using a susceptible tomato (*Lycopersicon esculentum* cultivar Rodade). To determine the critical threshold required to cause yield loss, plants were inoculated with populations of 0, 2500, 5000 and 10 000 eggs/plant and root galling and yield assessments conducted until 16 weeks after planting. The varieties showed high levels of resistance with KRK64 and T72 having the lowest mean gall-ratings among the tested cultivars and less infective juveniles in the roots. KRK66 displayed a compensatory effect, by attaining higher yields despite root galling. Used in combination with good rotations and recommended cultural control practices, resistant varieties remain the most promising control option for root-knot nematodes in tobacco in Zimbabwe.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP08

**Root-knot nematode control on tobacco: alternatives to fumigant nematicides**

The root-knot nematode is an economically important pest of tobacco in Zimbabwe and research on management methods dates back to the 1940s when compost and rotations using sunnhemp (*Crotalaria juncea*) were investigated. In the 1950s, the Tobacco Research Board's attention shifted to evaluation of nematicides with ethyl dibromide (E.D.B) being reported as both effective and economic. In the 1960s, the relationship between rotations and fumigations was studied and it was reported that a combination of soil fumigation and grass rotations was the most effective method for controlling nematodes. While fumigant nematicides provided a reliable return on investment, awareness of potential and actual environmental damage prompted research on alternative control strategies. Here, an overview of research carried out with an objective to validate and secure economically viable alternatives for nematode control in tobacco production systems post the fumigant nematicide era is presented. Seedbed and field trials designed as randomised complete block designs and replicated three times were carried out from 2011 to 2013. Among the test products were the methyl-isothiocyanate generators (metham sodium and metham potassium) and green label alternatives namely sesamin (extracted from sesame) and Velum (fluopyram). In all tests a resistant (KRK64) and a susceptible (KM10) tobacco cultivar were included. Results showed that methyl-isothiocyanate generators were effective in conventional seedbeds. However, metham sodium displayed inconsistent performance in the field pointing to the need to perfect application methods. Sesamin and Velum performed significantly better than the standard fenamiphos in controlling root-knot nematodes in the field. While none of the tested products were as effective as the fumigants, effective root-knot nematode management can be achieved through a combination of these alternative nematicides, resistant tobacco varieties and proper rotations.

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**MASUKWEDZA R.; DIMBI S.**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP31

**From the green to the red morph – the changing faces of the tobacco aphid *Myzus persicae nicotianae* in Zimbabwe**

The tobacco aphid *Myzus persicae nicotianae* causes significant losses of tobacco, directly as a result of feeding and honeydew deposition and indirectly by transmitting several viruses, thus making it economically important in tobacco production in Zimbabwe. Until 2002, the most common colour of the tobacco aphid seen on tobacco was green but in the 2003/2004 tobacco season, red forms of the aphid were widely observed. In the 2004/2005 tobacco season, green forms of the tobacco aphid had become rare and where found, constituted very small numbers. In intensive research spanning three years, the biology (days to adulthood, days to first nymph, nymphs/day, longevity, fecundity and survival) of the red morph was compared to that of the known green aphid. Results showed that although days to adulthood were similar for the two forms, the red colour morph produced more nymphs/day than the green morph. Fecundity was 1½ to 2 times higher for the red morph than the green aphid and the red morph survived longer than the green aphid. These parameters combined to give the red morph of the tobacco aphid a greater reproductive potential and rate of population increase over the green morph thus making it a more serious pest and indicate the importance of close monitoring of populations and timely application of control measures by growers to prevent aphid population explosions leading to crop losses.

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

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP40

**Survey on heavy metal and radionuclides in soils and tobacco grown in Central Italy. Preliminary results of RI.ME.PE. project**

Heavy metal and radionuclides in food and tobacco are a major concern for public health. Previous results (96/T/35 project) indicated that heavy metal built-up in tobacco was site- and year-dependent, suggesting the importance of relating analytical parameters with traceability. A project funded by Regione Umbria-Italy is presently in progress at OPTA (Organization of Tobacco Growers, Italy). It aims at characterising tobacco for Cd, Pb, and radionuclides, under a site-oriented perspective, and to prospect solutions should any problem be found. The objective of this paper is to present the methodology and preliminary results of the study in order to grow clean leaf in the best areas. In 2013 GPS-referenced soil tests were carried out in fields far from and adjacent to busy roads, in order to study tobacco variety and phosphate. Traced samples of tobacco green and cured leaves, as well as fertilisers and agrochemicals, were tested for contaminants according to official methods. Results of the first year indicated that the soils had very low concentrations of heavy metals. Total Cd was in the range of 0.082-0.232 mg/kg, with no significant differences among areas near and far from busy roads. Total Pb (range 1.49-8.99 mg/kg) was in the higher range near busy roads. Both available Cd and Pb were 20-25% of their total concentrations. Tobacco leaves from field strips adjacent to busy roads had higher Pb concentrations than those from farther areas: 0.46 vs. 0.21 mg/kg ( $P \leq 0.05$ ). Minor differences in heavy metal accumulation were found among varieties and applied phosphate levels. Radioactivity was always negligible. Only one fertiliser had Cd and Pb at concentrations in the range of 4-6 mg/kg. Preliminary conclusions suggest avoiding growing tobacco in the parts of fields near busy roads or to develop a strategy for reducing dust contamination from these areas using windbreaks.

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**MIHAYLOVA-KROUMOVA A.; ZAITLIN D.; WAGNER G.J.**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP50

**Natural variation in the acyl moieties of sugar esters produced by glandular trichomes in species of *Nicotiana* and *Petunia***

A unique feature of the glandular trichomes of *Solanaceae* species is their production of sugar esters. These metabolites have been shown to possess insecticidal, antifungal, and antibacterial properties. Sugar esters produced by tobacco (*N. tabacum*) provide resistance to insects such as budworms, and are important precursors of the organoleptic components. Acyl moieties of the sugar esters in *Nicotiana* spp., petunia, and tomatoes vary with respect to carbon length and isomer structure (2 to 12 carbon chain length; ante-iso-, iso-, and straight-chain). The objective of this work was to catalog information about the diversity of acyl group composition among species, cultivars, and accessions in *Nicotiana* and *Petunia*. Sugar esters from individual plant species were sampled according to an established protocol, and butyl esters of the acyl groups were analysed by GC-MS. The amounts of each group were calculated in moles and expressed as mole%. We studied acyl groups in 21 accessions of *Nicotiana obtusifolia*, six accessions of *N. hesperis*, three of *N. alata*, two of *N. occidentalis*, four modern cultivars of commercial tobacco, and five petunia hybrids. We observed a total of 21 different acyl groups that were represented differently among cultivars, species, and accessions. In *Nicotiana* species, acetate and the iso- and ante-iso branched acids prevailed. Straight-chain acyl groups (2 to 8 carbons) were present in the petunia hybrids, while octanoic acid was found in *N. alata* and *N. sanderae*. Two unexpected acyl groups for the genus *Nicotiana*, 8-meNonanoate and decanoate, were found in sugar esters of *N. occidentalis* subsp. *hesperis*. Sugar esters and their acyl groups could serve as a model in which to explore the basis of phenotypic diversity and adaptation to natural and agricultural environments. Natural or modified sucrose esters may prove useful in foliar applications to protect crops against various pests, or other uses.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP07

**Comparative tests among nematode agrochemicals and alternative products on Virginia Bright tobacco**

There is an increasing interest in reducing the use of agrochemicals in tobacco, to maintain a high integrity of the leaf and accomplish the agro-environmental practices, presently permitting nematode agrochemicals only with a temporary permission. The objective of this study (started in 2012) was to compare several options for controlling *Meloydogyne* sp. infestation in tobacco, including ordinary agrochemicals as reference and alternative products. In 2013 a field experiment on 3-replicated 500 m<sup>2</sup> plots was carried out, with the following treatments, vs. the control: 1) Mocap (Etoprofos 10% microgranules, Certis Europe), 30 kg/ha at transpl. + 30 kg/ha 7 DAT; 2) Vydate (Oxamyl 5% microgranules, DuPont CropProtection), 30 kg/ha at transpl. + 30 kg/ha 7 DAT; 3) BioAct WG (*Paecilomyces lilacinus* strain 251, 1.25%, Biogard-CBC Europe), at 0.6 kg/100L H<sub>2</sub>O in drench water + 1 kg/ha in weekly microirrigation; 4) Mocap microgranules, 30 kg/ha at transplanting + Furos TwinF1 (free aminoacids 4.9% + rhizosphere bacteria 5 × 10<sup>8</sup> Ufc/g + micorrhyzae 0.1% + Trichoderma spp. 5 × 10<sup>7</sup> Ufc/g, Diagro), 15+15 l/ha in microirrigation, at 2 and 4 WAT; 5) Vydate microgranules, 30 kg/ha at transplanting + Furos TwinF1, 15+15 l/ha in microirrigation, at 2 and 4 WAT; 6) Chestnut tannin extract microgranules 40% a.i. at transplanting (20 kg/ha, Gruppo Saviola), and 75% WP (24 kg/ha in 4 treatments, Gruppo Saviola). Crop growth and yield, nematodes in soil (pre and post-harvest), and Barker's crop root infestation grade were determined (Barker, 1985). Results indicated that Vydate had a better control than Mocap, and the addition of Furos negatively affected the performances of both. Bioact gave results worse than the control. Tannin extracts gave agronomic and yield results not different from Vydate, although manifesting a modest nematicide action. Tests indicated that Chestnut tannin extracts represent the only alternative to Vydate. 2014 experiments will include two new products: Flocter (Bayer CropScience), and Azo-Nema+HP400 (Sipcam).

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## **MSANGO-SOKO K.R.**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP32

### **Field performance of soil and spray applications of Chlorantraniliprole® 5 SC against the tobacco budworm on Burley and flue-cured tobacco in Malawi**

The tobacco budworm, *Heliothis* spp., is a devastating pest of tobacco in Malawi. Past experiences and farmer reports have shown that by the time this pest is noticed, considerable damage is already done to tobacco during the early stages of infestation. There is need to manage future infestations better by combining safer and effective measures that can provide early control and detection of the pest.

In recent years, the number of conventional pesticides for budworm control has declined and their use increasingly undesirable due to build-up of resistance, concerns with chemical residues, and environmental contamination. This has pushed the need to diversify strategies to manage pests sustainably. One effort in this direction has been to evaluate effective and safer alternative insecticides that are compatible with Integrated Pest Management (IPM).

Experiments were conducted from 2011-2014 to evaluate the efficacy of a new insecticide, Chlorantraniliprole® 5 SC, with the aim of finding effective and safer alternatives for control of the tobacco budworm. A total of ten insecticides were evaluated in randomised complete blocks and levels of budworm infestation were assessed in each treatment at two, four and six weeks after planting. The results indicated that Chlorantraniliprole® 5 SC applied at planting (40 ml/10 litres – 30 ml/plant) followed by a spray (14 ml/10 litres water) at four weeks after transplanting provided effective budworm control (1%) compared to about 20% infestation in untreated plots. Chlorantraniliprole® 5 SC is a promising alternative for budworm control because it will provide the needed early protection compared to the currently recommended insecticides that are applied after infestation is noticed and without proper scouting, more often than not when it is too late. Chlorantraniliprole® 5 SC, with its long-lasting activity, and selectivity to non-target arthropods makes it an attractive option to growers and an excellent addition to IPM in Malawi.

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## **MUDZENERERE E.T.; SHAVA J.G.**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP21

### **Breeding for multiple disease resistance in dark tobacco in Zimbabwe**

The Tobacco Research Board's breeding program benefits not only Zimbabwe but the entire Southern Africa region. Recently, there has been renewed interest in the growing of dark tobacco among the regional smallholder growers and thus an increase in the need for high yielding, multi-disease resistant and superior quality varieties. However, the varieties available at the Tobacco Research Board (Western 86, CDL28, DRV1, DRV7 and DRV10) are susceptible to tobacco mosaic virus (TMV), root-knot nematodes (*Meloidogyne javanica*) and alternaria leaf spot (*Alternaria alternata*). Therefore, a breeding program aimed at developing varieties with resistance to the three diseases was initiated. A locally developed dark tobacco line with these three resistance traits DACR3-6-18, was used as a source of resistance. Trials were conducted from 2011 using two approaches concurrently. In one trial, five F1 hybrids were developed by crossing each of the five dark tobacco varieties with DACR3-6-18. In the second trial, resistance genes from DACR3-6-18 were incorporated into each of the five cultivars and the lines were advanced through pedigree selection until acceptable true breeding types were obtained. Data on fertiliser requirements, plant spacing and cured leaf chemistry on the hybrids were collected. Results showed that four of the hybrids, KDAC 008, KDFC 010, KDFC 070 and KDFC 86 had the three resistance traits and acceptable morphology. Nicotine content for the four hybrids was also acceptable, ranging from 4.5 to 5.5% of total dry matter. While the work on resistance is on-going, TRB has to date produced four varieties which have now been launched into a limited release programme. This will enable further observation on a bigger scale.

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**MUNANGA W.**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP30

**In pursuit of greener curing methods: use of the rocket barn for tobacco curing in Zimbabwe**

The number of registered tobacco growers in Zimbabwe is approximately 90 000 with 80% being in the small-holder category that relies heavily on fire wood for curing. This exerts pressure on forests as indigenous trees are cut especially for tobacco curing purposes leading to massive deforestation. Thus efficient curing systems are required to minimise deforestation attributable to tobacco curing. The so-called rocket barn was originally developed in Malawi and was designed to improve wood use efficiency in tobacco curing. Previous studies indicated that the barn reduced wood consumption by 50% when compared to existing conventional barns. The objective of this work was, therefore, to evaluate the curing efficiency of the rocket barn by establishing fire wood consumption and the curing cycle duration under Zimbabwean conditions. The conventional downdraft barn was used as a control. Tobacco leaf reaped from the same field at the same time was loaded into the barns at the same time. The same quantities of wood with similar moisture content were used for curing with careful monitoring of wood use and time. Results indicated that the rocket barn utilised only 47–50% of the wood required in a conventional system to complete one curing cycle. It was also established that there were no significant differences in the duration of the curing cycles for both barn types. Given this fuel efficiency, it was recommended that small holder farmers in Zimbabwe adopt the rocket barn as a way of minimising the inefficient use of fire wood in curing tobacco.

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**NAKAMURA T.**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP37

**Breeding of multi-disease resistant Burley variety with intensified bacterial wilt resistance**

Bacterial wilt, caused by *Ralstonia solanacearum*, is the most severe soil-borne disease in Burley tobacco cultivation areas in Japan. Development of Burley varieties with resistance to bacterial wilt began in the 1980s using Japanese domestic varieties, Hatano and Enshu, as resistant sources. As presented at the 2007 CORESTA Joint Study Group Meeting in Poland, practical Burley variety W17, which has high resistance against bacterial wilt and five other diseases, was developed using Hatano. The variety W17 was corresponding to the Michinoku 1 cultivar. At the same time, development of a bacterial wilt resistant variety corresponding to the Burley 21 cultivar, which occupies the largest Burley cultivation area in Japan, was conducted using Enshu. However, the developed variety showed only moderate resistance to bacterial wilt. This study was conducted to develop a multi-disease resistant Burley variety of which the level of resistance to bacterial wilt is high. To accomplish that objective, two breeding lines having bacterial wilt resistance derived individually from Hatano and Enshu were used for the initial three-way cross of pedigree breeding. Consequently, a new practical Burley variety 'W19' was developed with high resistance against bacterial wilt, moderate resistance against black shank and black root rot, and resistance against wild fire, TMV, and PVY.

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**OFESI H.K.T.**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. APOST16

**Addressing the deforestation dilemma in Malawi**

Tobacco plays a vital role in Malawi's economy as it accounts for two thirds of the country's foreign exchange earnings. Grown by thousands, tobacco is by far the single most important cash crop that directly employs the most people in the agricultural sector of the country. However, the sustainability of tobacco production in Malawi is threatened by the declining supply of wood to meet growing household demands for tobacco curing, domestic energy and construction materials. For almost a century, the country has tried to persuade smallholder farmers to plant trees through projects that provided advisory services, and subsidised or free tree seedlings. Every year millions of trees are planted and yet in spite of all these efforts deforestation still remains high and shortage of wood continues to worsen threatening the very fabric of Malawi's economy, tobacco. This paper examines past deforestation interventions and discusses what went wrong and draws some lessons to guide the present and future programmes by the now invigorated tobacco industry through the introduction of the Integrated Production System (IPS) that emphasises more on good agricultural practices for tobacco sustainability by, among many things, the planting and managing of trees. This paper shows that while efforts have been aimed at addressing deforestation and promoting self-sufficiency in wood resources in Malawi, the national policies, programmes and projects have not been addressing the root causes. As an organisation owned by the farmers, the Agricultural Research and Extension Trust (ARET) has started and will continue to work with other organisations by garnering support and influencing national policy changes that would promote afforestation programmes that are not only collectively decided upon by the tobacco farmers but also those that will tackle root causes of deforestation. This will ensure that Malawi tobacco remains compliant and competitive on the world market.

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**PEREZ-FERNANDEZ P.(1); SCHUSTER F.(1); CARUSO L.V.(2)**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP52

**Global crop protection: regulation, stewardship and outreach**

Crop protection has become increasingly the focus of several international regulatory agendas. In particular, initiatives to ensure that the use of registered Crop Protection Agents (CPAs) and certain residues on agricultural commodities do not exceed recognised limits at points from field to finished product are relevant. Homologa™, the Global Crop Protection Database, has become an essential monitoring tool to assist farmers, commodity suppliers, traders, manufacturers and governmental officials with their CPA management strategies. Thus, reflecting the ever-changing dynamics of the global tobacco industry, this presentation will focus mainly on the increasing importance of monitoring registration status of CPAs when growing and sourcing tobacco materials. Several strategic elements will be presented and discussed to illustrate the challenges faced by industry stakeholders with respect to tobacco leaf integrity, procurement and overall product stewardship while operating under increasingly stringent regulations.

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**PEREZ-FERNANDEZ P.; SCHUSTER F.**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. APOST26

**The growing non-tariff trade-barriers can be overcome with transparency**

While the world works on the globalisation and harmonisation of food, feed and fibre production and trade, the barriers to feeding nine billion people are increasing. Three factors drive this increase: (i) the health and environmental concerns that are related to the use of plant protection products, (ii) the improvements in monitoring techniques to ensure compliance, and (iii) the loss of biodiversity due to the introduction of invasive alien species.

As a result, the improvements in crop yield compete with the increasing losses due to intentional crop destruction and post-harvest yield losses when produce cannot be shipped. Both, scientific research and grower training, are not able to resolve these problems. Only access to information and resulting transparency can overcome these barriers to the distribution of crops through trade.

This information is gathered by Agrobase-Logigram and published in the Global Plant Protection Database (Homologa). It includes registered uses, use patterns, regulatory status, residue limits, business connections within the plant protection industry for over 70 countries and export/import statistics for agricultural produce.

The strategic analysis of this information allows (i) growers, processors and traders to identify trade-barriers based on differential residue limits, (ii) growers to identify the pesticides and use patterns that result in the residues below the legal limits, (iii) crop/pest specialists to identify pest specific active ingredients and their use patterns, (iv) companies to find business partners for the distribution of their products into specific markets, (v) food processors and traders to identify pesticides for which they can request registration in the countries from where they purchase crops for their products, etc. Numerous examples demonstrate how this kind of information has been used to overcome non-tariff trade-barriers.

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**POLLOK J.R.(1,2); JOHNSON C.S.(1,2); EISENBACK J.D.(2); REED T.D.(1)**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP05

**Reproduction of *M. arenaria* on flue-cured tobacco homozygous for *Rk1* and/or *Rk2* resistance genes, and possible effects of soil temperature on resistance**

*Meloidogyne incognita* has historically been the most significant root-knot nematode species on tobacco (*Nicotiana tabacum* L.) in Virginia. However, most commercial cultivars now possess the *Rk1* resistance gene to races 1 and 3 of *M. incognita*, and *M. arenaria* has become the most common root-knot species in Virginia tobacco fields. An increasing number of cultivars now also possess *Rk2* for root-knot resistance, but aspects of its efficacy are unknown. Greenhouse experiments were conducted in 2012-2013 to investigate whether possessing both *Rk1* and *Rk2* increases resistance to *M. arenaria* compared to either gene alone. Trials were arranged in a RCBD with six replications, and compared galling and numbers of egg masses and eggs from roots of C371G (susceptible), NC 95 and SC 72 (homozygous for *Rk1*), T-15-1-1 (homozygous for *Rk2*), and STNCB-2-28 and NOD 8 (homozygous for both *Rk1* and *Rk2*). Each plant was inoculated with 5,000 *M. arenaria* eggs and data were collected 60 days post-inoculation. Plants with *Rk1* alone reduced root galling and numbers of egg masses and eggs significantly versus C371G. Combining *Rk1* and *Rk2* further reduced galling in one of four trials, egg masses in two of four trials, and eggs in one of four trials. *Rk2* alone did not suppress galling versus susceptible C371G. Nematode reproduction was usually numerically lower on plants with only *Rk2* versus C371G, but apparent differences were only statistically significant in one of four trials. Because high soil temperatures have been associated with a breakdown in root-knot resistance, the effect of high soil temperature on the efficacy of *Rk1* and/or *Rk2* genes to *M. arenaria* are being evaluated at 20, 25, 30 and 35 °C. A better understanding of the effects of *Rk1* and *Rk2* on root-knot nematode parasitism should help plant breeders improve resistance in tobacco to *M. arenaria*.

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**PRZYBYS M.; DOROSZEWSKA T.; KORBECKA G.**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. APOST06

**Phylogenetic analysis of PVY isolates**

Potato virus Y (PVY) is an important pathogen of tobacco. It belongs to the *Potyvirus* genus in the plant virus family *Potyviridae*. In this study, a collection of fifteen PVY isolates from tobacco, originating from fields in Poland, Germany and Croatia, was included into phylogenetic analyses. These isolates were previously characterized serologically by enzyme-linked immunosorbent assay (ELISA). Genome sequences of tested isolates were compared with over 80 full-length nucleotide sequences of PVY deposited in GenBank. Aligned sequences of the tested isolates were used to construct phylogenetic trees by means of UPGMA method. In total, 96 genomic sequences were compared. These sequences were grouped into five clusters: PVY<sup>NW</sup>, PVY<sup>NTN</sup>, PVY<sup>O</sup>, PVY<sup>N/NTN</sup> and PVY<sup>C</sup>. The tested isolates were assigned into clusters: PVY<sup>NW</sup> and PVY<sup>NTN</sup>. Within these clusters nucleotide sequences similarity of tested isolates was compared with the sequences deposited in GenBank. In PVY<sup>NW</sup> cluster, the lowest similarity of 95.6% was detected between genomic sequences of Polish isolate IUNG14 and German Wilga156var. In PVY<sup>NTN</sup> cluster, the lowest similarity between the genomic sequences equalled 92.5% and it was observed between the Polish isolate IUNG9 and the Chinese SD-1. Moreover, the sequences of tested isolates were compared to genomic sequences of North American isolates and their similarity was also calculated. Within PVY<sup>NW</sup> cluster it ranged from 97.3% to 99.8%, while in the cluster PVY<sup>NTN</sup> ranged from 99.4% to 99.8%. These results indicate surprisingly high similarity of the tested isolates to North American isolates.

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**REED T.D.**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP47

**Evaluation of a prototype plastic float tray for greenhouse tobacco transplant production**

The greenhouse float system for tobacco transplant production relies on the use of expanded polystyrene (EPS) plant trays to provide necessary floatation. The system has served tobacco growers well but the use of EPS trays is problematic. Effective tray sanitation is more difficult as trays age and the standard practice of methyl bromide fumigation is being phased out. Proper disposal of unusable EPS trays are limited and present an environmental concern since the material is not biodegradable. Trials were initiated in the spring of 2014 and continued through the summer to evaluate the performance of a floating plastic (polypropylene) tray for use with greenhouse float tobacco transplant production. In addition to tray floatation, a critical design factor was compatibility with existing tray filling and seeding equipment. Tests were conducted to measure the buoyancy and floating characteristics as well as seedling growth. Trays having different cell volumes and number of cells were evaluated. Trays were tested with two soilless media having different water holding capacities. Preliminary results would suggest that the tray is a viable replacement for EPS trays for greenhouse tobacco transplant production. Although the trays are significantly more expensive than EPS trays, the plastic trays are easier and more effectively sanitized and have a much longer expected lifespan.

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**RICHMOND M.D.; BAILEY W.A.; PEARCE R.C.**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP03

**Preliminary investigation of curing environment and TSNA accumulation within barns in dark air-cured tobacco**

A graduate student project was initiated in 2012 through support from a CORESTA Study Grant to evaluate correlation between curing microenvironments within designated areas of curing barns and TSNA accumulation. Experiments were conducted in barns near Princeton and Lexington KY where curing environments (temperature and relative humidity) were monitored at 27 locations within each barn for the entire cure and compared to TSNA content from samples of low converter (LC) and high converter (HC) selections of TR Madole dark tobacco collected from each location within each barn. During the initial 2012 curing season, average total TSNA accumulation in TR Madole HC was higher with increased relative humidity and was nearly twice as high at Lexington compared to Princeton (7.16 µg/g at Lexington and 3.58 µg/g at Princeton) but during the 2013 curing season, the average TSNA accumulation was nearly twice as high at Princeton compared to Lexington (8.72 µg/g at Princeton and 4.12 µg/g at Lexington). In 2012, within TR Madole HC at Lexington, total average TSNA content increased from the top tier to the bottom tier (5.86 µg/g in the top tier, 7.19 µg/g in the middle tier, and 7.41 µg/g in the bottom tier) but at Princeton, total average TSNA content increased from the bottom tier to the top tier (4.40 µg/g in the top tier, 2.92 µg/g in the middle tier, 2.80 µg/g in the bottom tier). Similar tier trends were seen in 2013, with TR Madole HC at Lexington having highest TSNA in the bottom tier (3.60 µg/g in the top tier, 4.22 µg/g in the middle tier, and 4.53 µg/g in the bottom tier), while highest TSNA were in the top tier at Princeton (9.14 µg/g in the top tier, 9.11 µg/g in the middle tier, and 7.91 µg/g in the bottom tier).

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**RUKUNI D.; KOGA C.**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP46

**Mitigating salt injury in the tobacco float transplant production system in Zimbabwe**

The tobacco float seedling production system occasionally exposes transplants to salt injury, especially when there is excessive evaporation due to winds or high temperatures. Evaporation causes salt accumulation on the Gromix<sup>®</sup> soilless media surface. These salts cause physiological damage to young transplants and more often mortality. Three trials were conducted at Kutsaga Research Station to address this problem that affects mainly the late July, August and September-sown float seedbeds. The first trial evaluated four hydroponic fertiliser formulations (Floatfert<sup>®</sup>, Hydrofert<sup>®</sup>, Peters<sup>®</sup> and Formula 1) for potential in reducing salt injury. While the fertilizers had different salt indices (SI), all the formulations exposed the transplants to salt injury. The second trial evaluated the effect of split fertiliser application and delayed application of Floatfert<sup>®</sup> fertiliser. Floatfert<sup>®</sup> is normally applied at 7, 21 and 35 days after sowing (d.a.s.). Further splitting or delayed Floatfert<sup>®</sup> applications were tested to determine salt accumulation reduction potential. There were treatment differences in germination and survival at 21 and 28 d.a.s. when fertiliser was split-applied or when application was delayed. A third trial evaluated different mulch types (Vlei grass, [*Eragrostis lehmanniana*], perforated clear plastic, clear plastic tent and a floating-row cover) for reducing salt accumulation. Vlei grass mulch resulted in the highest germination and survival of transplants. The results from this work indicated that mitigation of salt injury during hot and windy periods requires a holistic approach that includes careful selection of fertilisers, appropriate application rates and timing and the use of a suitable mulch.

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**RUKUNI D.; MUKARATI T.H.**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP48

**Pre-chilling improves tobacco (*Nicotiana tabacum* L.) seed germination**

Tobacco seeds require light and an optimum temperature for maximal germination. Pre-chilling tobacco seeds has been successfully used to enhance germination and improve on seedling vigour and uniformity for some varieties. This study focused on evaluating the germination response of seeds of two new Kutsaga varieties, T71 and T72, after pre-chilling. The effect of moist pre-chilling was evaluated by subjecting tobacco seeds to durations of 7, 14, 21 and 28 days at 4 °C and then drying the seeds back at ambient temperature. A split plot design with variety as the main plot and priming method as the sub-plot was used with four replicates per treatment. Results revealed that pre-chilled seeds of both varieties had lower germination in darkness at constant 17 °C than when germinated under dark/light cycles at 20/30 °C alternating temperatures. However, the two varieties had disparate pre-chilling requirements; with T71, 7 days were sufficient while T72 required 14 days. The control had the least germination under both light and dark conditions. In the second experiment, pre-chilling seeds in different concentrations of 6-Benzylaminopurine (BA) or Gibberellic Acid (GA<sub>3</sub>) substrate; and subsequent exposure to dry heat treatment (40 °C) promoted germination of the two varieties at both 17 °C and 20/30 °C in both light and dark. Under dark conditions and at 17 °C, non-chilled seeds (control) of both varieties germinated poorly. Therefore, moist pre-chilling can be used as a method for enhancing tobacco seed germination under suboptimal conditions.

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**SASAKI R.; KAGAMI C.**

CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. APOST27

**How do traps placed at five-meter intervals contribute to detecting a beetle's nest?**

Pheromone traps are usually used for regular monitoring of the tobacco beetle, *Lasioderma serricorne*, to obtain seasonal prevalence of their occurrence. They are also used for detecting the source of infestation by the beetles. Traps for regular monitoring are placed at specific intervals, and those for detection are placed at narrower intervals than those of regular monitoring. These two types of monitoring are not conducted separately. After an abnormality is found in regular monitoring, the source of infestation is investigated using data from the monitoring. Namely, when a trap catches some beetles in regular monitoring, several additional traps are placed around the trap to delimit an infestation area. The aim of this study was to clarify the contribution of additional traps for detecting the source of infestation.

First, pheromone traps for the tobacco beetle (NEW SERRICO) were placed at ten-meter intervals in a warehouse (20 × 20 m). Three hundred male tobacco beetles were released from a point. The catches were counted 24 hours after release. Next, traps were placed at five-meter intervals and the same experiment was conducted. A series of these experiments was performed from three different release points. Experiments for one release point were replicated three times.

Three release points were regarded as the source of infestation, and a detection rate of each point was calculated. The rate of three release points was 0.45, 0.50 and 0.67 respectively. The average detection rate was 0.54. When the source of infestation was investigated based only on data from traps placed at ten-meter intervals, the rate was 0.24, demonstrating that the detection rate of the source of infestation was increased 2.3 fold by using additional traps at five-meter intervals.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. APPOST14

**Remote sensing: a new tool for confirming tobacco legislated dates compliance in Zimbabwe**

Aphids and the virus diseases they transmit are among the major challenges in tobacco production in Zimbabwe. One of the effective management strategies developed is strict adherence to legislated tobacco planting and stalk destruction dates. The Plant Pests and Diseases Act (Chapter 19:08) stipulates that all tobacco plants must be destroyed before 15 May, that sowing can only commence on 1 June and that transplanting can only begin on 1 September of each year. Physical monitoring from the ground has been effectively used for enforcement of these legislated dates. However, this method requires high staff and travelling costs. Thus, there is need for an effective, and accurate monitoring method to assist policing agents to ensure compliance, and remote sensing, which can provide a reliable, fast and accurate means of monitoring tobacco plantings and stalk destruction, is one such tool. The objective of the study was to evaluate three instruments, Landsat 7, Moderate Resolution Spectro-Radiometer (MODIS) and Multispectral Radiometer (MSR 5) for delineating tobacco fields between 15 March - 15 May, 15 May - 1 August and 1 August - 1 September. Normalised Difference Vegetation Index (NDVI) was obtained from tobacco fields with stalks still standing past 15 May, fields where transplanting was done before 1 September and fields where strict adherence to legislative dates had been observed. Significant differences ( $P < 0.001$ ) were noted between NDVI in fields where stalk destruction was done according to legislation and where this was ignored. Significant differences ( $P < 0.001$ ) were also noted between compliant and non-compliant tobacco fields between 1 August and 1 September. The study concluded that reliable monitoring of legislative date compliance can be achieved with remote sensing.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. APPOST13

**Improving tobacco yield estimating models through remote sensing based crop variable assessment techniques**

Yield estimation in flue-cured tobacco production is essential for effective marketing in Zimbabwe as it enables adequate planning and preparation for the expected leaf volumes for merchants, processors and exporters thus ensuring competitive prices for growers. Traditionally, yield estimates have been done using the Gavin method developed in 1984, which is based on an assessment of in-season development of tobacco variables such as leaf length, width, plant height and dry mass using destructive sampling techniques. The objective of this research was to develop quick and non-destructive methods of obtaining tobacco crop variables using ground-based sensors such as the Multispectral Radiometer (MSR 5). Furthermore, the research sought to apply the remote sensing variable estimating method to satellite platforms to allow for large scale, real time crop assessments. The research was based on previous work that determined the Normalised Difference Vegetation Index (NDVI) of tobacco varieties in response to different planting dates and fertiliser regimes. The results showed a positive linear relationship between Moderate Resolution Imaging Spectroradiometer (MODIS) and MSR 5 derived NDVI's that was sufficient for estimating tobacco crop variables and yield estimates. Five models for estimating leaf length, width, plant height, geometric mean area and plant height from NDVI obtained from MODIS and MSR 5 were developed. These models can be used to remotely determine factors that influence yield areas to enable more accurate yield estimation.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP19

**Building on historic achievements: a glance at the past decade of tobacco breeding in Zimbabwe**

Tobacco research work in Zimbabwe commenced in the 1920s and intensified in the 1950s when the Tobacco Research Board was formally constituted through an Act of Parliament. Varieties initially tested were introductions mainly from the USA, but these were not adapted to local conditions and had no resistances to the local spectrum of diseases. Thus, breeding efforts were initiated with an objective to develop locally adapted tobacco varieties with multiple disease resistance, high yield and quality. By the turn of the new millennium, the TRB had released ~40 flue-cured, Burley and Oriental tobacco varieties. Cultural control measures such as strict adherence to recommended hygiene practices remained important for the management of some diseases such as tobacco mosaic virus (TMV), resistance to which had not been incorporated into all the varieties. However, the land reform programme in the early 2000s brought in new growers for whom training on the basics of tobacco production was essential. Neglect of some recommended cultural control measures resulted in the upsurge of some diseases that had become unimportant such as TMV, and other aphid-transmitted virus diseases. As a strategy, TRB incorporated TMV resistance into all varieties which hitherto had lacked the requisite gene. In addition, efforts intensified in breeding for resistance to potato virus Y (PVY) and bushy top virus diseases as these became economically important. The introduction of the float tray tobacco seedling production system also introduced *Pythium* root rot and currently, exploration for *Pythium* resistance sources is on-going. Six decades after its inception, TRB has developed some 45 flue-cured, Burley, Oriental and dark fire tobacco varieties that are well adapted to the local environment and the Zimbabwean grower has continued to produce high yields of the flavour-style leaf, well sought after internationally.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP12

**Survival of floatbed *Pythium*-infected tobacco seedlings in the field and their yield potential**

The floatbed tobacco seedling production system has been widely adopted in Zimbabwe. However, one of the major challenges associated with this system is root rot caused by *Pythium* spp. Work done at the Tobacco Research Board, Zimbabwe showed that this disease usually affected seedlings post-emergent from 4 to 5 weeks after sowing (WAS) and in severe cases accounted for up to 75% seedling deaths when left uncontrolled. However, even after severe infestations it was observed that some seedlings survived infection and could be planted out. The objective of this study was, therefore, to investigate the suitability of seedlings surviving *Pythium* infection in the float bed, for transplanting. The persistence of *Pythium* in the root system of field plants was also studied. Additionally, the yield of the infected plants was established and compared to that of seedlings where curative and preventative fungicides had been applied to mitigate the disease. This work was carried out in two seasons, 2005-6 (sandy soil) and 2006-7 (sandy and heavy soils), at Kutsaga Research Station. Two systemic fungicides, metalaxyl and propamocarb-HCl, were applied as preventative as well as curative treatments into the pond water. An untreated control was included in both seasons. All seedlings were inoculated with *Pythium* oospores and mycelium at 7 WAS in the float bed. In both seasons seedlings were transplanted into field at 12 to 13 WAS and disease severity assessments were carried out at 5 and 7 weeks after planting (WAP) and 2, 4 and 6 WAP, respectively. *Pythium* root colonisation, plant survival, plant height and yield data was collected. Results showed that *Pythium*-infected seedlings from untreated control plots generally survived well in the field. However, the fungicide treatments improved the vigour, survival and yield of the tobacco plants.

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**Potato virus Y (PVY) incidence on tobacco in Zimbabwe – trends in the last decade**

Potato virus Y is among the economically important viral diseases of tobacco in Zimbabwe. Although first described in potato in 1931 by Smith, the first report on tobacco in then Rhodesia was in 1963 describing a new hazard in tobacco seedbeds caused by “necrotic Virus Y”. Two strains of the virus, one which produces mosaic-type symptoms and another that causes leaf and veinal necrosis, have been observed on tobacco. The mosaic strain reduces leaf quality, while the necrotic strain is very destructive leading to serious yield losses. The necrotic strain continues to be a problem in late-planted field tobacco in Zimbabwe. The Tobacco Research Board was one of the participants in the CORESTA collaborative experiment monitoring PVY incidence and characterising strains on CORESTA-prescribed and some local cultivars since 1996/7. The global CORESTA data presented by Verrier and Doroszewska in 2003 was based on data on number of plants infected by PVY and symptoms expressed (mosaic or necrotic) on resistant and susceptible cultivars. From this data the PVY incidence, the percent of *va* breaking strains within the necrotic strains and the estimated frequency of *va* breaking strains were calculated. The trials continued after 2003 and the trends from 2004 to 2013 for Zimbabwe are discussed in this paper. Results show that PVY incidence varied among seasons. As expected the resistant cultivars showed little or no expression of the necrotic symptoms. Of the local cultivars tested, TB4 (a flue-cured line) exhibited high resistance to the necrotic strain of the virus. Results also indicated that in some seasons there was an increase in the incidences of necrotic symptoms on some formerly resistant cultivars which could be attributed to PVY strains breaking the resistance. The estimated frequency of *va* breaking strains within the necrotic strains was also high in two of the peak seasons. As most local cultivars are susceptible to the necrotic strain of the virus, efforts are currently underway to utilise TB4 to breed for resistance to PVY.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP04

**Effect of nitrate levels in tobacco leaves on TSNA formation during storage**

Nitrogen oxides and alkaloids are the precursors of tobacco-specific nitrosamines (TSNAs). The objective of this study was to determine the effect of nitrate content on TSNA formation during tobacco storage. Two experiments were done: a field experiment with different nitrogen fertilisation rates and a pot experiment with different ratios of nitrogen sources (nitrate-N and ammonium-N). Burley tobacco samples from different tobacco fields with different yield levels as well as samples from lamina and midrib of both Burley and flue-cured tobacco were also collected. All samples were divided into two groups: the treatment group was stored in a temperature-controlled chamber at 45 °C for 15 days, and the control group was stored at 10 °C for 15 days. The nitrate and TSNA contents after storage were determined. The leaf nitrate nitrogen significantly increased with the amount of nitrogen fertiliser and leaf yield, and with increased ratio of nitrate-N to ammonium-N. As the nitrogen rate increased from 120 to 300 kg/ha, total TSNAs after storage at 45 °C for 15 days increased from 0.18 to 3.84 ppm. TSNAs in the 100% ammonium-N treatment were 1.08 ppm, and in the 100% nitrate-N treatment were 2.60 ppm. Total TSNAs increased from 0.18 to 2.20 ppm as yield increased from 2349 to 3255 kg/ha. For both Burley and flue-cured tobacco, midrib nitrate nitrogen and total TSNAs were much higher than in the lamina. After storage for 15 days at 45 °C, the increase of TSNAs in the midrib was significantly higher than in the lamina. For flue-cured tobacco, the increases of total TSNAs from the low temperature to the high temperature treatments in midrib and lamina were 367% and 78%, respectively. We speculate that nitrate nitrogen in tobacco leaf is closely related to TSNA formation during high temperature storage.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP42

**The effect of conveyor use during sucker control application in North Carolina produced flue-cured tobacco**

With an ever increasing focus on complete sucker control and maleic hydrazide (MH) residue in US produced flue-cured tobacco, current research efforts have been focused on practical application alternatives for producers. Initiatives have included the evaluation of reduced rates of applied MH, a larger emphasis on fatty alcohol and flumetralin based products, the screening of off-label compounds, and investigations into alternative application equipment. In 2011, conveyors (hooded spray shields) were introduced to US producers in the hope that labelled sucker control material efficacy would improve, MH residue could be reduced, and/or that less total product could be applied per hectare.

Research was initiated that same year to quantify the effects that a more concentrated application pattern using conveyors might have. Three sucker control materials (contact, flumetralin, and MH) as well as combinations of flumetralin and MH were evaluated with standard three-nozzle assemblies and with conveyors. Specific treatments evaluated included four applications of fatty alcohol materials (13.48/16.85/16.85/16.85 kg a.i. ha<sup>-1</sup>), recommended rates of flumetralin (0.67 kg a.i. ha<sup>-1</sup>), split applications of flumetralin (0.67 kg a.i. ha<sup>-1</sup>/0.34 kg a.i. ha<sup>-1</sup>), tank mix applications of flumetralin (0.67 kg a.i. ha<sup>-1</sup>) and MH (1.68 kg a.i. ha<sup>-1</sup>), and various rates of MH (0.56, 1.12, 1.68 kg a.i. ha<sup>-1</sup>). All compounds were evaluated with and without conveyors and at two different application volumes (327 and 467 L ha<sup>-1</sup>). Following final harvest, percent sucker control, green sucker weight per plant, sucker number per plant, green weight per sucker, yield, quality, and crop value were assessed.

The use of conveyors resulted in sucker control similar to, but never better than, that observed with standard three-nozzle assemblies. Additionally, the use of conveyors did not reduce MH residues when compared to three nozzle arrangements nor did it allow for reduced application rate or solution volume of any the materials evaluated.

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**Alternative fertilizer rates and programs in the north western Piedmont region of North Carolina**

In recent years input costs for macronutrients used in production agriculture have increased significantly. As a result, flue-cured tobacco farmers have been looking for ways to decrease inputs and, ultimately, overall production costs. Research conducted at North Carolina State University has indicated that rates of nitrogen, phosphorus, and potassium can be greatly reduced without affecting yield and quality. Research also indicates that alternative sources for nitrogen and potassium can be utilized without reducing yield or quality.

Flue-cured tobacco producers in the eastern Coastal Plain have been extremely quick in adopting these recommendations. As a result over 50% of the flue-cured tobacco crop receives a portion of total nitrogen from a liquid source (28%, 30%, or 32% Urea Ammonium Nitrate or 21-0-0-24S Ammonium Sulfate). Additionally, nearly 20% of the crop only receives phosphorus fertilizer in a transplant water application. Unfortunately, flue-cured tobacco producers in the Piedmont region of North Carolina have not readily adopted these same practices. As a result, demonstration plots were implemented to educate producers in this specific region.

On-farm evaluations were conducted in 2012-2014 in North Carolina. Nitrogen was applied at three rates: 56, 72 and 95 kg N ha<sup>-1</sup> (from 28% UAN) and phosphorus (from 0-46-0) was applied at three rates: 0, 28, and 56 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Potassium was applied to each plot at a rate of 84 kg K<sub>2</sub>O ha<sup>-1</sup> (from 0-0-22, K-Mag). All possible combinations of nitrogen and phosphorus rates were used. An additional treatment of 560 kg 8-8-24 ha<sup>-1</sup> at transplanting + 11 kg N ha<sup>-1</sup>, from 15.5-0-0, at layby was included as a conventional check. Plant height at layby, flower emergence prior to topping, and yield/quality/value were unaffected by fertility rate and fertilizer source. Early results demonstrate that reduced nitrogen rates, reduced phosphorus rates, and liquid nitrogen sources are acceptable in this region.

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### **Using flue-cured tobacco in a cropping rotation to reduce Palmer amaranth populations**

In recent years, the glyphosate resistant weed Palmer amaranth (*Amaranthus palmeri*) has become a major production issue in agricultural systems of the southeastern United States. It has been observed that when flue-cured tobacco is used in a cropping rotation, populations of Palmer amaranth are greatly reduced in subsequent crops.

Research was conducted in a cropping rotation of tobacco followed by cotton to quantify the effects of tillage (deep versus shallow), herbicide combinations applied pre-transplanting (clomazone @ 841 g a.i. ha<sup>-1</sup> versus clomazone @ 841 g a.i. ha<sup>-1</sup> + sulfentrazone @ 210 g a.i. ha<sup>-1</sup>), and hand weeding (hand weeding versus no hand weeding) on weed species populations. Tillage and pre-transplanting herbicide treatments imposed only occurred in year one of the rotation. Weed species identification and quantification was conducted multiple times in both crops using a 1.21 m by 1.21 m sampling square. Weed evaluations occurred prior to cultivation and/or herbicide application in each plot with hand weeding occurring once per season in designated plots. Weed species counts, total time to hand weed plots, crop yield, and crop value were recorded.

Palmer amaranth populations in tobacco production were greatly reduced in the presence of sulfentrazone + clomazone and/or deep tillage. However, by seasons end it was the presence of sulfentrazone that provided season long weed suppression, regardless of tillage system. The same observation was made the following season where Palmer amaranth populations decreased by as much as 55% where sulfentrazone and clomazone had been applied to tobacco. Hand weeding did not reduce weed populations from 2012-2013. Deep tillage also increased tobacco yield by 390 kg ha<sup>-1</sup>. Preliminary results favor the use of deep tillage in combination with the herbicides sulfentrazone and clomazone.

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### **Measurement uncertainty in agrochemical residue datasets**

ISO/IEC 17043:2010 defines Proficiency Testing (PT) as the evaluation of participating laboratory performance against pre-established criteria by means of inter-laboratory comparisons. The CORESTA/Food Analysis Performance Assessment Scheme (FAPAS) PT aims to check the accuracy of results submitted by participating laboratories, where each participant was requested to identify and quantify a list of possible agrochemical residues present in a tobacco test material. In this study, statistically sound approaches have been reviewed to evaluate measurement uncertainty in the residue testing results collected annually from the CORESTA/FAPAS PT dispatched between 2006 and 2012. The calculated z-scores were combined to preserve the identity of each individual residue data point. The relative standard error was then computed to express the percentage variation and the precision, in terms of how far a sample estimate is likely to deviate from the true and unknown population value. From the available industry PT data, several elements will be presented to express uncertainty in agrochemical residue testing results.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. APOST07

**Long-term water deficit imposed by air drought or chilling stress modifies the regulation of proline metabolism in *Nicotiana tabacum***

Tobacco (*Nicotiana tabacum*), which originated in the tropical Americas, is thought to be rather sensitive to water deficit imposed by drought, salinity or low temperatures, especially during the rapid growth period. However, many past investigations have mainly focused on the response of tobacco to a specific stress, whereas the molecular mechanism of comprehensive tolerance formation to multiple stresses has rarely been reported. Using comparative screening and comprehensive evaluation methods, our previous researches identified a tobacco variety, MS-K326, with a relatively high tolerance to both drought and chilling stresses at the seedling stage. Considering the principle role of proline in water deficit response in plants, the present research aimed at revealing the regulatory events underlying proline metabolism in tobacco, when exposed to different water deficit for a relatively long-term treatment. Tobacco seedlings were treated with air drought or chilling (2 °C) stress, for up to 144 h (6 d). It was found that free proline content significantly increased at the late stage of drought stress, whereas chilling induced a steady proline accumulation in leaves, during the whole course of treatment. Enzymatic and transcriptional analysis of key enzymes for proline metabolism revealed that proline accumulation is mainly attributed to activation of the glutamate pathway under both stresses. The inhibitory effect of chilling stress on proline catabolism also contributes to the rapid increase in free proline during the early stages of exposure. However, the relatively unchanged activity as well as slight transcriptional response of ornithine- $\Delta$ -aminotransferase (OAT) indicates that the ornithine pathway is not essential for proline accumulation under either drought or chilling stress, in our experiment system. All these findings show the novel regulatory mechanisms associated with different stresses, and may provide more information on understanding the role of proline regulation in comprehensive tolerance formation under various stress conditions.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP22

**Mapping of two white stem genes in tetraploid common tobacco (*Nicotiana tabacum* L.)**

Leaf colour is an indicator of chlorophyll (Chl) level and isolating leaf colour mutants can facilitate the understanding of Chl metabolism regulation. Here we describe an ethyl methanesulfonate-induced light colour mutant white stem 1 (*ws1*) in common tobacco (*Nicotiana tabacum* L.) that shows a phenotype highly similar to Burley tobacco (*Nicotiana tabacum* L.), a type of air-cured tobacco which has light-coloured leaves with white veins. Compared with the wild type, the light green stem of *ws1* gradually became pale white along with growth, while *ws1* leaves lost green colour rapidly which was positively correlated with the decline of Chl levels. A series of genetic analyses indicated that the *ws1* mutant phenotype was controlled by two recessive nuclear genes *ws1a* and *ws1b* which were preliminarily mapped to the intervals of tobacco simple sequence repeat markers linkage group 5 and 24 using the BC1F2 populations, respectively. The allelism test further revealed that the same two genes controlled the Burley character in Burley tobacco. Based on the Chl-deficient phenotype of *ws1* and the locations of the two genes, we hypothesised that *ws1a* and *ws1b* were paralogs of each other probably originated from the ancestral species *N. sylvestris* and *N. tomentosiformis*, respectively. Both genes might share similar biological functions and expression patterns, and play key roles in the regulation of Chl biosynthesis. These results laid a solid foundation for marker-assisted selection breeding and gene function analysis of the Burley character in tobacco.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP43

**Optimisation of canopy architecture of field-grown tobacco based on three-dimensional modeling**

Optimisation of canopy structure of field-grown tobacco is of importance for improving tobacco yield and quality. The architecture of individual K326 tobacco plants grown in an experimental field in Yuxi, Yunnan Province was measured by using 3D digitising and laser-scanning methods, and a three-dimensional (3D) architectural model of tobacco was built that can accurately represent the structure characteristics of tobacco plants grown in the field. A number of canopy architectural models were then derived by adjusting the distances between plants and rows, row direction and leaf number of individual tobacco plants. Different virtual experiments were conducted and spatial distribution of Photosynthesis Active Radiation (PAR) and potential photosynthesis rate of each virtual canopy under contrasting sky conditions were computed using a 3D canopy light interception and photosynthesis model. The simulation results showed that the net daily potential photosynthesis rate of the canopies only marginally increased after the row distance up to 110 cm and plant distance up to 60 cm. The largest PAR interception was reached when the row direction was around north-south ( $\pm 20^\circ$ ). Removing two leaves at the top and two leaves at the foot of each tobacco plant did not decrease the daily potential photosynthesis rate of the canopy, and this can benefit the growth and quality of the remaining leaves by improving their light condition. The yield of flue-cured tobacco was only reduced by 1.57%, the proportion of fine tobacco was improved by 6.36%, the value was increased by 4.45%, sugar-nicotine ratio was more appropriate, as opposed to the control. This study provides the basis for optimising plant spacing, row direction and leaf number of individual tobacco plants.

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CORESTA Congress, Québec, 2014, Agronomy/Phytopathology Groups, abstr. AP26

**Dynamic changes on gene expression of sucrose and starch pathway in the reduced starch accumulation responded to the light attenuation in *Nicotiana tabacum* leaves**

Plants are able to acclimate to highly fluctuating light environments. Light reduction through shading may dramatically decrease the soluble sugars and starch contents. Starch content may affect the tobacco leaf quality after curing. Starch content is affected mostly by light. This research will show how light intensity affects starch content as well as the reasons behind this. The hypothesis is that the reduction of starch responding to the light attenuation could be the result of changes at the gene expression level involved in the starch pathway. This research focused on starch synthesis and accumulation in tobacco leaf affected by light attenuation as well as gene expression changes in the starch synthesis pathway. Electrical Microscope (EM) and RT-PCR methods were used for the research. 100%, 85%, 70% and 55% of natural light intensity were set through shading on the field by nylon mesh. Starch accumulation in tobacco leaves decreased with the light attenuation especially under the light intensity of 70% and 55% which were observed by EM. The highest starch accumulation was observed in the sample from 85% of light intensity. In the process of plant development, the highest starch content was observed on the 105 days after transplanting. The lowest content of starch in tobacco leaf was observed in the sample under 55% light intensity. Also the starch accumulation was confirmed by chemical analysis. Results showed that the highest contents of starch were observed in samples of 85% light intensity at both two development stages (78 and 85 days after transplanting) with the starch contents of 28% and 26%, respectively. More than ten important genes involved in the synthesis of the starch pathway, which include Extracellular Invertase (INV), UDP-Glucose Dehydrogenase (UGDD), Starch Branching Enzyme (SBE), Sucrose Synthase (SudaSy), Sucrose-6-Phosphate Phosphatase (SPP2), Granulebound Starch Synthase I (GBSS I) and so on, were affected by light intensity. Our results indicated that lower light intensity decreased the synthesis and accumulation of starch in tobacco leaf. The gene expression on the genes involved in the pathway of starch synthesis may be the main reason for this reduction.

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