ABSTRACTS OF PRESENTATIONS MADE AT THE
2010 CORESTA CONGRESS IN EDINBURGH, SCOTLAND
AGRONOMY AND PHYTOPATHOLOGY

(in alphabetical order of first authors)

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Evaluation of insect field scouting programs and use of Rynaxypyr (Coragen®) insecticide for reducing pesticide inputs in dark tobacco.

Field insect scouting programs and insecticide treatments were evaluated with the objective of reducing the number of insecticide applications made to dark tobacco. Grower surveys conducted in 2006 and 2007 showed that dark tobacco growers in Kentucky and Tennessee, USA, apply an average of four foliar insecticide applications per season. Most growers do not use structured field scouting programs to determine insect levels throughout fields and the need for insecticide applications. Our research has shown that when field scouting programs are implemented with field surveys done every 10 to 14 days and insecticides applied only when economic thresholds are reached, foliar insecticide applications can be reduced to two applications per season without resulting in adverse effects on dark tobacco yield or quality. Most foliar insecticide applications are targeted at control of *Lepidoptera* species such as tobacco budworm and tobacco hornworm. Chloranthraniliprole (Rynaxypyr, Coragen®) insecticide was registered in dark tobacco for tobacco budworm and hornworm control in 2010. Rynaxypyr has some systemic activity in the tobacco plant and thus has the potential to provide extended control of budworm and hornworm when used as an in-furrow treatment at tobacco transplanting. Therefore, Rynaxypyr has the potential to further reduce the number of insecticide applications needed in dark tobacco when used in combination with field scouting programs. Field research was conducted in 2009 and 2010 to evaluate Rynaxypyr in transplant water and foliar applications compared to standard insecticides. The effect of field scouting and Rynaxypyr use on the number of insecticide applications needed in dark tobacco will be discussed.

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Weather based prediction of spotted wilt in flue-cured tobacco in Georgia.

Spotted wilt first appeared in Georgia in 1986. By 1995 tomato spotted wilt virus (TSWV) had become the leading cause of disease loss in Georgia tobacco. Attempts to predict spotted wilt incidence based on winter cold and its assumed influence on mortality of TSWV and its thrips vectors have not been successful. Recent studies of weather influence on thrips abundance in North Carolina have found winter heat units (10.5 C base degree days) and rainfall in March were highly related to catch numbers of tobacco thrips, the principle vector of spotted wilt in tobacco. Historic weather records stored in the Georgia Automated Environmental Monitoring Network were used to create 18 heat unit and rainfall perimeters for each year from 1995 to 2009. This data set was regressed against the mean spotted wilt incidence developing in untreated plants each year in University of Georgia trials conducted for the same time period. Simple correlation found January degree days had the best relationship to annual spotted wilt incidence ($R2 = 0.52$; $pr > f = 0.004$). When this variable was plotted versus spotted wilt incidence by year the relationship appeared poor from 1995 to 1998 but very good after 1998. The correlation between January heat units and spotted wilt incidence from 1999, 2009 was better ($R2 = 0.63$; $pr > f = 0.004$). A stepwise regression of all variables versus spotted wilt incidence was run. January degree days, mean winter temperature and number of days with rain (>0.04 cm) in March contributed to the model ($R2 = 0.90$; $pr > f = 0.007$). The better fit of a

weather based model on spotted wilt from 1999 to 2009 as opposed to 1995 to 2009 suggests the range of TSWV was continuing to expand from 1995 to 1998.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP01

Nicotine is enantioselectively demethylated by cytochrome P450 enzyme CYP82E4.

Nicotine and nornicotine are two of the dominant alkaloids found in tobacco (*N. tabacum* L.), and known precursors to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine (NNN). In tobacco nicotine is N-demethylated to nornicotine by nicotine demethylase enzymes, which belong to a family of cytochrome P450 (CYP) proteins. CYP82E4 (E4) and CYP82E5 (E5) are two nicotine demethylases which have been reported to be active in tobacco. Both nicotine and nornicotine have two enantiomers which differ from each other at the 2' position on the pyrrolidine ring. Naturally occurring nicotine is almost exclusively (-)-2'-S-nicotine, and (+)-2'-R-nicotine accounts for less than 1% of total nicotine, while after demethylation 4 to 75% of nornicotine is (+)-2'-R-nornicotine and the percentage varies among lines and tissues. The reason for this high and variable percentage of (+)-2'-R-nornicotine resulting from low and constant (+)-2'-R-nicotine substrate is unknown. One hypothesis is that (+)-2'-R-nicotine is preferentially demethylated to (+)-2'-R-nornicotine. The objective of this study was to investigate the enantioselectivity of the E4 demethylase, the enzyme reported to be responsible for the majority of nicotine demethylation occurring in the plant. Using microsomes prepared from yeast over-expressing the *E4* gene as a source of demethylase enzyme, the kinetics of enzyme enantioselectivity were determined by measuring demethylase activity at several concentrations of 2'-^{14}C- R, S or racemic nicotine. These enzyme assays demonstrated that the E4 enzyme demethylated (+)-2'-R-nicotine 3-fold faster than (-)-2'-S-nicotine, and exhibited a 10-fold lower *K*m (Michaelis constant) for (+)-2'-R-nicotine than (-)-2'-S-nicotine, which means E4 has greater affinity for (+)-2'-R-nicotine. Our results clearly demonstrate the enantioselective preference of the E4 demethylase for (+)-2'-R-nicotine, which may partially explain the high percentage of (+)-2'-R-nornicotine in the plant produced from low (+)-2'-R-nicotine substrate.

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***T-srd*, a tobacco mutant with characteristics of abundant sucker and dwarfness.**

A dwarf mutant with abundant suckers was observed in populations of the second generation of *Nicotiana tabacum* (L) cv. GT11, which experienced space mutation in a recoverable satellite, the Chinese third unmanned spacecraft "Shen-Zhuo III", and expressed readily in the field, then named as *T-srd* (Tobacco of Sucker-Rich and Dwarf mutant). The suckers of *T-srd* emerged at the first leaf node 5 weeks after sowing. In the field, the emergence of suckers was orderly from bottom to top, and in the grasp of the apical bud, which had no sucker until the node lay under half of the stalk height at first flower stage. At 90 days after transplanting, the plant height, length of internode, stem girth in *T-srd* were lower than those in GT11. There was no sucker-rich and dwarf phenotype in F1 population, and the segregation ratio in F2 population of seedling between sucker-rich and sucker-free phenotype corresponded with Mendel's Law based on the Chi-square test. Those results suggest that the sucker-rich and dwarf trait is a qualitative character that belongs to the recessive mutation of a single gene. Amplified fragment length polymorphism (AFLP) analysis showed that the difference between *T-srd*

and GT11 existed on a molecular level. This mutant provides a useful material for research into sucker regulation in tobacco.

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Establishment and application of multiple RT-PCR for detection of TMV, CMV and PVY in tobacco.

The sequences of tobacco mosaic virus (TMV), cucumber mosaic virus (CMV) and potato virus Y (PVY) in the GenBank database were aligned using DNAMAN 6.0 version software, and the primers were designed on the conserved regions. And the multiple RT-PCR for detection of TMV, CMV and PVY were established in this study. The PCR products were purified and sequenced, BLAST analysis showed that these PCR products were TMV, CMV and PVY respectively, these homology were above 98%. A total of 132 tobacco samples collected from Baise of Guangxi province were analyzed by multiple RT-PCR to detect TMV, CMV and PVY, and these results indicated that positive rates of TMV, CMV and PVY were 91.7%, 18.2% and 55.3%, respectively. Multiple RT-PCR was simple, rapid and economic in comparison with conventional RT-PCR. The limitation of detection of Multiple RT-PCR was 10^4 times higher than that of ELISA, and was more specific and accurate than ELISA. Therefore, multiple RT-PCR could sync detect TMV, CMV and PVY in one RT-PCR assay that showed most important significance for prediction, forecast and prevention of tobacco virus diseases.

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***NtCDPK12*, a gene encoding calcium-dependent protein kinase, was induced by high-salt and drought in *Nicotiana tabacum*.**

Calcium-dependent protein kinases (CDPKs, EC 2.7.1.37) are the crucial Ser/Thr protein kinases in plants and play an important role in plant Ca^{2+} signal transduction. A full-length CDPK gene, *NtCDPK12* (GenBank accession no. GQ337420), was isolated from common tobacco (*Nicotiana tabacum*) leaves by rapid amplification of cDNA ends (RACE). The *NtCDPK12* cDNA is 1816 bp long and contains an open reading frame (ORF) of 1461 bp encoding 486 amino acids. Sequence alignments indicated that *NtCDPK12* contains all conserved regions found in CDPKs and shows a high level of sequence similarity to many other plant CDPKs. The results of real-time quantitative reverse transcription-PCR (qRT-PCR) showed that *NtCDPK12* was highly expressed in stems and increased in roots treated with high-salt or subjected to drought stress, which indicated that *NtCDPK12* was induced by high-salt and drought stresses.

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Combination of different sources of resistance to PVY in tobacco double haploids.

Potato virus Y (PVY), especially its necrotic strains, is a pathogen that causes significant reduction of tobacco yield in Poland as well as in many other countries all over the world. There are some sources of resistance that could be applied in breeding. Cultivar Virgin A Mutant (VAM) is the most often used source of resistance that has been a result of deletion within gene of susceptibility. The similar type of resistance is present in Polish cultivar Wislica. The best source of resistance is wild species *Nicotiana africana* resistant to all tested PVY isolates. In the studies, line BPA, which has been previously obtained by crossing *N. tabacum* and *N. africana*, was used. This line shows a kind of resistance called tolerance. Another source of resistance are transgenic lines: MN 944 LMV (containing gene of lettuce mosaic virus coat protein) and AC Gayed ROKY (containing antisense gene of PVY replicase). The aim of the study was increasing resistance to PVY by the combination of different sources of resistance in one genome. Therefore, tobacco hybrids coming from the crossing between transgenic line MN 944 LMV and line AC Gayed ROKY, between MN 944 LMV and breeding line BPA, as well as between BPA and cv. VAM, between cv. Wislica and AC Gayed ROKY were obtained. The hybrids were tested as regards resistance to PVY and then used for producing haploids by androgenesis. Next, doubled haploids were obtained by regeneration from stem fragments of resistant haploids. The ploidy level of plants was estimated by flow cytometry and microscopic analyses. Depending on lines, the doubled haploids rate varied from 29,8% to 53%. Resistance of doubled haploids was determined by biological and ELISA tests. The results showed that the most efficient protection against PVY was provided by combination of two transgenes.

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Parasitoid wasps applications in Insect Pest Management in tobacco (*Nicotiana tabacum* L.) growing sites in South Brazil.

This study aims to inventory the biodiversity of hymenopterans parasitoids associated with tobacco according to the cultivation method (organic and conventional); examine the host-parasitoid relationships in tobacco crops; check the effect of adjacent vegetation on populations of parasitoids and determine parasitoid species with potential applicability in IPM programs. In the 2007/2008, 2008/2009 and 2009/2010 tobacco harvests Malaise and Pit-Fall traps were installed in 12 organic and conventional tobacco fields in Southern Brazil. In the 07/08 tobacco harvest, in the states of Paraná and Rio Grande do Sul, a total of 179.551 arthropods were sampled distributed in 29 taxa. The order Hymenoptera was represented by 5.305 individuals (except Formicidae) belonging to 36 families. The occurrence of similar parasitoid families in both regions was recorded, showing that despite the climatic characteristics, there is a unique fauna of parasitoids associated with tobacco. In the 08/09 harvest 260.936 arthropods were sampled, distributed in 26 taxa. Hymenoptera corresponded to 22.432 individuals (except Formicidae) belonging to 47 families, of which 98.4% are parasitoids, thus presenting a high diversity of native natural enemies occurring associated with organic and conventional tobacco crops. Regarding the 09/10 tobacco harvest, already 30% of the collections were identified, totalizing 98.365 arthropods distributed in 30 taxa, of which 7.081 are hymenopterans belonging to 33 parasitoid families. Field and laboratory observations showed the association of parasitoid wasps' species parasitizing eggs, larvae and/or adults of specific tobacco pests, enabling the elaboration of new IPM techniques to be tested in the 2010/2011 tobacco harvest. The increase in the number of individuals collected in the border areas of the tobacco fields support the effect of adjacent vegetation on insect populations, providing alternative food resources, shelter and nesting sites, showing the potential of specific native vegetation in the IPM of tobacco produced organically.

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Characterization of black shank resistance transferred from *Nicotiana rustica* to *Nicotiana tabacum*.

Black shank, a root and stem disease caused by the oomycete *Phytophthora nicotianae*, is one of the most costly pathogens affecting tobacco production. Two sources of resistance are currently used in commercial tobacco varieties. Resistance derived from the cigar tobacco cultivar 'Florida 301' provides varying levels of partial resistance to both Race 0 and Race 1 but appears to be negatively correlated with yield in flue-cured tobacco. Resistance transferred from *Nicotiana plumbaginifolia* and *N. longiflora* in the form of a completely dominant gene, Ph, confers immunity to Race 0, but provides no resistance to Race 1. Additional sources of resistance would be of value to extend the range and level of resistance to this pathogen. A gene tentatively designated as 'Wz' may have been inadvertently co-transferred from *N. rustica* to *N. tabacum* when wildfire and angular leaf spot resistance was intentionally introgressed by researchers in Zimbabwe. A number of commercial and experimental tobacco cultivars possessing this currently uncharacterized gene have exhibited very high levels of resistance to both Race 0 and Race 1 in field experiments in the U.S. A doubled haploid mapping population of 71 lines derived from a cross between a breeding line (called 'Wz') carrying the introgressed *N. rustica* region and black shank-susceptible flue-cured tobacco cultivar NC 55 has been generated. The population is being evaluated for black shank resistance in field and race-specific growth chamber experiments. Preliminary data on the inheritance and race-specificity of resistance in this population will be presented. Using bulked segregant analysis, molecular markers associated with the introgressed region will be identified. The overall objective of this research is to evaluate the potential utility of this resistance mechanism in a breeding program and to identify associated markers to aid in development of tobacco cultivars with increased levels of resistance to black shank.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP16

Isolation, identification and characterization of a nicotine-degrading bacterium *Acinetobacter* sp. ND12.

A novel bacterium, strain ND12, was newly isolated from the tobacco plantation soil and identified as *Acinetobacter* sp. ND12 based on morphology, physiological tests, 16S rRNA sequence and phylogenetic analysis. The isolate could utilize nicotine as the sole source of carbon and nitrogen. 1.0 g/L nicotine was degraded by *Acinetobacter* sp. ND12 within 14 h at 28 °C and pH 6.0. The optimum nicotine concentration for the growth of strain ND12 was 2.5 g/L. The resting cells of *Acinetobacter* sp. ND12 could decompose 90% nicotine in upper tobacco leaves after treating for 11 h. These results suggested that the strain ND12 may be useful for reducing the nicotine concentration in tobacco leaves and nicotine-polluted environments.

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GAP farm certification programs for tobacco in the United States, what is the potential?

Good Agricultural Practice (GAP) certification programs are common in the United States for vegetable producers, but have not yet been developed for tobacco producers. GAP programs provide

numerous benefits to both the grower and the industry because they promote responsible production of quality tobacco while protecting the environment. Most GAP programs for tobacco include components of soil and water management, crop production and cultural practices (CPAs, IPM, variety selection, fertilization, crop rotation, etc.), curing management and on-farm storage, and product integrity. Such topics are well discussed in CORESTA Guide No. 3. GAP programs can also be used to promote awareness of local, state, and federal regulations that commonly apply to farming operations in the United States and provide educational and training opportunities for producers. At the time this abstract is written, an industry-wide stakeholder meeting to discuss the potential for GAP programs for flue-cured tobacco has been scheduled, but the meeting has not yet occurred. The presentation will discuss the future outlook for GAP certification programs for flue-cured tobacco in the United States based on the outcome of this meeting.

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Potential applications of 'GM' technology in the production of tobacco for its traditional uses.

The flue-cured and Burley tobacco industries have thus far relied solely on the intrinsic gene-pool of the tobacco plant itself for traits that enhance yield, disease resistance, leaf quality etc. Meanwhile, over the last 25 years the tobacco plant has been widely used as a convenient research species for developing transgenic (genetically modified; GM) strategies that employ genes from other sources for crop improvement, resulting in the demonstration of many new performance and input traits of potential use for tobacco production. We are conducting a comprehensive survey of this work, and also considering how and whether GM tobacco could be safely and securely produced in the open-field environment without compromising production of the conventional, non-GM crop. We anticipate that this study will be valuable to growers and their organizations as a base of knowledge concerning those GM technologies that might be employed in traditional Burley and flue-cured varieties in the future. Our progress to-date has comprised extensive literature searches in several scientific/technical databases, followed by sorting and filtering of the many thousands of publications to remove replicated material. Patents, government-regulated field trials, and interviews with industry and growers are also contributing useful information. A classification format has been designed that will enable the final search results to be interpreted easily in terms of what traits have been demonstrated in tobacco plants through GM technology, how effective they are, whether they have been expressed in commercial tobacco varieties and field-tested, etc. Our findings will be summarized in a report which will be openly available on-line.

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Functional avrA gene in *Ralstonia solanacearum* can elicit a defense reaction in tobacco stem tissue to pathogenic strains.

Bacterial wilt caused by *Ralstonia solanacearum* is an extremely damaging disease of flue-cured tobacco in the southeastern USA and can be spread mechanically on farm machinery. Tobacco roots contain naturally occurring resistance genes to the tomato strain of *R. solanacearum*. Tomato strains of *R. solanacearum* contain an avirulence gene avrA, which elicits a defence reaction in tobacco. Pathogenic tobacco strains of *R. solanacearum* contain the avrA gene, which has been mutated by a random insertion, blocking gene function and subsequent host recognition and a defence reaction (gene for gene theory). The present study evaluated the ability of tomato strains of *R. solanacearum*

with a functional avrA gene to turn on the resistance gene in tobacco stem tissue and block infection by a pathogenic tobacco strain of *R. solanacearum*. Pathogenic strains on tobacco (isolate SC10 and NC 132) were applied (1 x 10^6 cfu) in combination with tomato strains (SC11 and SC12, 1 x 10^8 cfu) in all possible combinations to tobacco stems during the flower removal operation. The tomato strains elicited a defence reaction in tobacco stem tissue and reduced stem necrosis averaged over strains from 4.5 to 1.6 on a 0-5 scale ($P < 0.001$) and effectively blocked disease development. Stem necrosis could be observed following inoculation with a pathogenic tomato strain but was limited to tissue immediately adjacent to the inoculation point. The implications for non-chemical disease suppression in mechanically transmitted *R. solanacearum* will be discussed.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. APPOST14

***Aloe vera* aqueous extracts: Potential explanation at molecular level of its allelopathic effect over *Nicotiana tabacum* plants.**

Positive allelopathic effects of *Aloe vera* extracts for medical, pharmaceutical and cosmetic applications on humans have been well documented over many years. However its vegetal applications are not so well described. More than 10 years ago, Pinar del Rio University started research to study this issue on some forestry and non-forestry crops. In this sense different beneficial effects were identified in non-forestry species such as: increase of root development, increase of pathogen resistance, among others. Taking into account this evidence three years ago the effectiveness of *A. vera* over tobacco was evaluated. This paper critically describes the potential molecular mode of action of *A. vera* extracts chemical compounds that justified the achieved results. *A. vera* extract application was done according Izquierdo, 2008 protocol, using the respective statistical tools in open field evaluations. The use of *A. vera* extract increased root development, diminished the latency period for root emergence and varied the needed fertilizer amounts for good plant development. The potential chemical compounds responsible of these results are putatively the same compounds responsible for *A. vera* anti-inflammatory activity in diabetes patients. This work describes also the current direction of this research to achieve a real applicable productive solution for the tobacco industry demonstrating the valuable function of universities in knowledge generation.

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Chlorophyll meter reading - a reliable tool for the estimation of tobacco leaf ripeness for harvesting.

Field experiments were conducted to determine the reliability and efficiency of non-destructive, rapid, instrumental tools in tobacco production for predicting the optimum leaf ripeness for harvest. In this investigation tobacco leaf ripeness was assessed by an CCM-200 portable chlorophyll meter. The experiments were carried out during 2007 and 2008. The treatments were the manner of harvest (7, 4 and 3 primings) and the degree of ripeness at the time of harvest (unripe, ripe and over-ripe). In unripe, ripe and over-ripe stages, tobacco was harvested on the base of chlorophyll readings: in 2007 in the ranges of 20-25, 15-20 and 10-15; in 2008, in the ranges of 15-20, 10-15 and 5-10, respectively. The dependability and efficacy of the estimation of the ripeness of tobacco leaf for harvest was evaluated on the basis of agronomic traits observed in the light of previous knowledge and the results of other authors when in similar investigations the harvesting was carried out pursuant to a visual

estimation of the ripeness of leaf for harvest. The greatest yield, price and total value in 2007 were achieved with the harvest of over-ripe tobacco and in 2008 with the harvest of ripe tobacco. In 2007 there was no fall in the value of the agronomic traits investigated with harvesting done in the phase of over-ripeness, which was on the whole the case in the research of other authors, and which did in fact happen in the following year. In both years of research, the best results were achieved when tobacco was harvested in a chlorophyll reading range of 10-15. Results of this investigation undoubtedly suggest the conclusion that chlorophyll reading is a reliable and effective tool for the estimation of the ripeness of tobacco leaf for harvesting.

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Heavy metal content in soil and dry tobacco leaves in Croatia.

Systematic field and laboratory studies on soil and plants were conducted in the tobacco growing regions of Croatia from 2006 to 2008. The aim was to get better insight into the soil status and its influence on tobacco quality. In this paper contents of some heavy metals (Cd, Cr, Cu, Mn, Mo, Ni, Pb and Zn) found in different soil types and their accumulation in the tobacco leaf are presented. Soil and plant samples were taken from 72 locations, i.e., 72 family farms. Concentration of particular metals was determined by inductively coupled plasma optical emission spectrometry (Varian Vista MPX). Because of different physical and chemical soil characteristics and different parent material, the results are presented for six pedosystematic units. Comparison of concentrations of the investigated metal with allowable threshold values showed that all pedosystematic units of soils used for tobacco productions were not contaminated by heavy metals. Their concentrations were below allowable threshold values for soils in conventional and ecological agriculture. Content of particular metals in dry tobacco leaves is in the reference interval for this plant. Also, the low content of toxic metals, i.e. cadmium and lead, has to be emphasised.

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The insecticidal compounds isolated from the herbal medicines Saishin and Sekishōkon against the cigarette beetle.

Botanical pesticides can be recommended as an eco-chemical and sustainable strategy in the management of agricultural pests. To find candidate plant sources, the author has screened insecticidal plants from herbal medicines, which are well-known sources of various biologically active compounds, against the cigarette beetle. In the previous CORESTA Agronomy/Phytopathology Joint Study Groups meeting, the author described the insecticidal activity of 11 herbal medicines and the structure identification of the principal insecticidal compound from Inchinko (Yinchenhao), which exhibited the highest activity of them. In the present study, the author describes the isolation and identification of the active components from other active herbal medicines Saishin (xi xin), the root of *Asiasarum sieboldii* (Aristolochiaceae), and Sekishōkon (shi chang pu), the rhizome of *Acorus gramineus* (Araceae). The active components were isolated through successive silica-gel column chromatography and HPLC and characterized as methyl eugenol (4-allyl-1,2-dimethoxybenzene) and b-asarone (1,2,4-trimethoxy-5-[(*Z*)-prop-1-enyl]benzene) by GC and GC/MS analyses. As these two chemicals share the same partial structure, a benzene ring with adjacent methoxy groups and a propenyl group at the opposite position, the insecticidal activity of 20 related compounds were

checked to find the structure-activity relationship. Insecticidal activity of myristicin (4-methoxy-6-allyl-1,3-benzodioxole) was found additionally, although the structure-activity relationship was not clarified. The possible carcinogenic or psychopharmacological properties of these compounds will make the practical use of these chemicals or plant extracts difficult.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP02

The effect of topping on yield, quality and TSNA accumulation in Burley tobacco.

It is known that lower alkaloids result in lower TSNAs, and that untopped tobacco has lower alkaloids than topped tobacco. The objective of this study was to quantify the effect of not topping on TSNAs, other nitrogenous components, physical characteristics, yield and quality. Experimental design was a split plot, with varieties as main plots and topping treatments as subplots. Varieties were a high converter selection of TN 90 (TN 90H) and commercial low converter TN 90 (TN 90LC). Topping treatments were topped (10% bloom) and untopped (topped one day before harvest). Suckers were controlled with contact suckerides. NNN, NAT, total TSNAs, total alkaloids, nitrite and total nitrogen were lower in the untopped treatment. Marginal means for total TSNAs were 7.1 ppm in the topped treatment and 4.2 ppm in the untopped. The untopped treatment reduced TSNAs from 2.5 ppm to 1.4 ppm in TN 90LC, and from 10.5 ppm to 6.2 ppm in TN 90H. Nicotine to nornicotine conversion was higher in the untopped treatment. There were no differences between topping treatments for nitrate, leaf length and area, leaf number and stalk height. Yield (2515 vs. 2691 kg/ha), grade index (49 vs. 65), crop index and % F grades were lower in the untopped treatment. The low grade index in the untopped tobacco was largely a function of the preponderance of K grades (40% vs. 13%). There were no differences between topping treatments for % tip grades, price, value per ha and quality grades. Not topping almost halved TSNAs, but had a very detrimental effect on the quality of the tobacco, changing it from quality flavor leaf to filler.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP07

Isolation of protein from *Spinacia oleracea* against tobacco mosaic virus and research on the antiviral mechanism.

Tobacco mosaic virus (TMV) is a worldwide-occurring tobacco viral disease and usually results in big losses in tobacco production. How to inhibit TMV infection and replication has become a hotspot in the field of plant virus disease control. Antiviral protein isolated from *Spinacia oleracea* were examined with bioassay-guide, and the mechanism against TMV was studied. The results indicated that crude extracts and fractions of 0%-40% ammonium sulfate precipitation were shown to have a high inhibitory activity on TMV infection. One active fraction was further purified with cation-exchange chromatography (SP-sepharose HP) and activity screening. By local lesion assay, the inhibitory rate was up to 94.35% with purified faction of concentration 50 μg/ml. The inhibition rate can be up to 81.82% when 50 μg/ml concentration of purified faction was used nine hours before inoculation. The results showed that the fraction could inactivate TMV and had high antiviral activity preventing infection against TMV, but the inhibitory activity on TMV replication was not significant. The results presented in this paper provide a solid foundation for further isolation of antiviral protein from plants, which is also helpful for designing and creating new types of biological agents for the prevention and cure of tobacco viral disease.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP29

Tobacco response to simulated drift of glufosinate, dicamba, and 2,4-D.

In addition to crops already tolerant to glyphosate and glufosinate, prospective advances in agricultural technology have resulted in cultivars that will be tolerant to topical applications of dicamba and 2,4-D. However, these herbicides can cause injuries in non-target, sensitive crops if spray or vapor drift were to occur. Research was conducted to simulate drift rates of glufosinate, dicamba and 2,4-D to evaluate injury and effects on tobacco yield and quality.

Experiments were conducted at two locations during the 2009 growing season. Tobacco cultivar NC 71 was grown under recommended cultural practices until early to mid-June when treatments were applied. Dicamba and 2,4-D rates were 1/2, 1/8, 1/32, 1/128, and 1/512 the recommended rate of 0.25 lbs and 0.48 lbs a.i. per acre, respectively. Glufosinate rates were 1/2, 1/4, 1/8, 1/16, and 1/32 the recommended rate of 0.54 lbs a.i. per acre. Treatments were arranged in a randomized complete block design with four replications. Plots were two rows by 40 feet with treatments applied to the right hand row of each plot. Visual injury was recorded 7 and 14 days after treatment on a scale of 0 (no injury) to 100 (plant death). Photographs were also taken 7 and 14 days after treatment to document injury. Yield and quality data were collected along with cured leaf samples for chemical residue analyses.

All treatments resulted in visual injury which increased with rate. The 1/2 rate of dicamba reduced yield at both locations and the 1/8 rate reduced yield at one location. Crop value was reduced with the 1/2 rate of dicamba at one location. The 1/2 rate of 2, 4-D and glufosinate both resulted in significant yield loss at one location each. Analysis of cured leaves showed all treatment residues were below the detection limit. Detection limits were 0.5 ppm for glufosinate and 0.05 ppm for dicamba and 2,4-D. Trials will be repeated in 2010 and in-season residue samples will be collected to correlate injury and predict recovery.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP19

Impact of resistance associated with the *Ph_p* gene on management of tobacco black shank and tobacco cyst nematode in Virginia.

A gene from *Nicotiana plumbaginifolia* (*Ph_p*) has been incorporated into a large number of flue-cured tobacco cultivars since 1996, and an increasing number of Burley and dark fire-cured tobacco cultivars. This gene provides resistance against races 0 and 3 of the black shank pathogen (*Phytophthora nicotianae*) and also suppresses reproduction of a tobacco cyst nematode (*Globodera tabacum solanacearum* - *Gts*), often to levels where nematicide use becomes unnecessary. Laboratory (RAPD) analyses have been conducted to identify tobacco entries possessing *Ph_p* since 2005. Annual field evaluations of *Gts* reproduction on flue-cured tobacco germplasm have also characterized suppression of *Gts* reproduction linked with *Ph_p*. However, widespread planting of cultivars possessing *Ph_p* has been linked with a dramatic shift in the *P. nicotianae* population in US tobacco fields from predominantly race 0 to mostly race 1. Due to the lower levels of race 1 resistance in available flue-cured tobacco cultivars, this race shift has increased crop loss and significantly increased soil fungicide use. Since 2007, annually-collected percent survival data generated by the US Regional Flue-Cured Tobacco Variety Evaluation Committee has been subdivided into separate categories for race 0 or 1 of the black shank pathogen. The "resistance level" of each cultivar to each race of *P. nicotianae* is then described by average cultivar survival in fields within each category over multiple years and locations. These "resistance levels" are also used to weight the average relative yield of each entry from annual variety tests to estimate relative yield when black shank is either present (a "Black Shank Yield Index") or absent. "Resistance level" and "Black Shank Yield Index"

results from these annual analyses are being extended to Virginia tobacco producers to assist them in optimizing tobacco yield and quality, as well as their use of crop protection agents for nematode and black shank control.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. APPOST07

Reducing fuel wood energy requirement and improving leaf quality through partial sun air-curing of dark-fired tobacco in Malawi.

Dark-fired tobacco has recently regained demands by tobacco processors in Malawi. The curing of the tobacco is done in three sequential stages: yellowing, lamina drying and midrib drying. Currently, all the three stages are done in dark-fired barns with the last two stages requiring 3.5 kg wood per kg of cured tobacco leaf. The demand for wood energy for curing tobacco and domestic use has resulted in increased deforestation in Malawi. The Agricultural Research and Extension Trust is making efforts to develop technologies that will reduce wood requirement for tobacco curing in order to curb deforestation. During the 2008/09 and 2009/10 seasons, a trial was conducted at Kandiya Research Station in Lilongwe to evaluate the effect of partial sun air-curing of dark-fired tobacco on leaf quality, wood requirements and curing duration. Three curing treatments: complete curing in dark-fired barns (control); partial curing of midrib drying stage under transparent plastic sheet in the sun following yellowing, and; lamina drying in barns and partial curing of midrib drying stage in a thatched open shed following yellowing and lamina drying in barns, were evaluated in a completely randomized design with five replications. Results showed that specific fuel consumption for wood was significantly reduced by partial sun air curing of dark-fired tobacco. Wood savings of 40 to 45% were obtained both under plastic sheet and in the shed. Nicotine, sugar and nitrogen levels in the cured leaf were not significantly different. Curing duration was significantly different with partial curing under plastic sheet roof and in the shed taking 23 to 28 days and 29 to 32 days respectively, compared to 11 days in dark-fired barns. Partial curing has proven to be an effective method of curing tobacco and results in substantial wood savings and no quality losses.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. APPOST01

Effect of the degree of leaf ripeness at harvest on the yield, quality and chemical characteristics of Oriental (sun-cured) tobacco.

Harvest date is one of the most important crop management practices affecting tobacco yield, quality and usability after curing. In a two-year field experiment, investigations were made into the effect of the degree of leaf ripeness at harvest on several agronomic, physical, and chemical characteristics of Oriental (sun-cured) tobacco (cv. Basma Z/7) grown in Greece. Even though there was no objective and standard method to assess leaf ripeness of Oriental tobacco, a general model of changes of some leaf characteristics (agronomic, physical, and chemical) was constructed to determine the optimal harvest date. The experiment was set up with twelve harvesting treatments at two-day intervals, before (under-ripe), during (ripe) and after (over-ripe) visually empirically judging the full physiological maturity, the stage of maximum accumulation of dry matter. Harvestings were carried out by picking three fully expanded lower leaves from the accurately same stalk position, which were in uniform size and mature stage. Quality, physical and chemical characteristics were calculated at each harvesting time to determine the optimum harvest date. The results showed that yield and quality parameters of leaves both at harvest and after curing were influenced by the degree of leaf ripeness. Leaves harvested after the full physiological maturity stage had the higher quality index (as measured

by the grade index) and more desirable chemical composition in comparison with leaves harvested too early or too late. Based on the changes in leaf yield, quality and chemical characteristics after sun-curing, the optimal harvest time for Oriental tobacco leaves was found to be the stage of early senescence, just after the full physiological maturity stage. Preliminary results suggested that changes in nitrate concentration in fresh leaves may provide a simple test for the initiation of harvest. Sap nitrate concentration in the fully expanded bottom leaves should be less than 0.9-1% at the commencement of harvesting for optimal yield and quality. The sap nitrate test, determined by a commercially available field test, can be used as an indicator of fresh leaf ripeness.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. APPOST12

Haploid isolation in *Nicotiana tabacum* using transgenic over-expression of the *Arabidopsis thaliana PAP1 MYB* transcription factor.

Haploid plants of maternal or paternal origin have utility in tobacco breeding for the development of doubled haploid lines. In addition, paternal haploids derived from seed can be used to rapidly convert inbred lines to cytoplasmic male sterility (*Cms*). To date, haploid plants have been generated using anther culture or isolated from seed using interspecific hybridization with *N. africana*. The latter method causes F_1 hybrid seedling lethality and has been reported to permit identification of *N. tabacum* haploids produced via parthenogenesis. Neither of these methods permit rapid conversion of tobacco lines to *Cms*, however. This approach requires a dominant seedling marker to be expressed within a *N. tabacum* female parental line. We investigated the use of a purple seedling trait conferred through over-expression of the *Arabidopsis thaliana PAP1* gene for identifying spontaneous maternally-derived tobacco plants produced from seed. *PAP1* over-expression activates genes in the anthocyanin pathway and results in purple-colored plants. A line of 'Xanthi' homozygous for two *35S:PAP1* transgene inserts was crossed as a pollen parent with two Burley tobacco cultivars to produce two large F_1 hybrid seed lots. For comparison, two seed lots were also produced by pollinating these cultivars with *N. africana*. Spontaneous haploid plants in the *PAP1*-based system were recognized as green seedlings amongst purple F_1 hybrid seedlings. The frequency of maternal haploids using the *PAP1*-based system was 0.00026, while the frequency using the *N. africana*-based system was approximately seven times greater (0.00188). The large difference in haploid frequency between the two systems may suggest differences in the mechanisms of haploid production. Alternatively, the pollen parent may influence the rate of parthenogenesis. The frequency of paternal haploidy using the *PAP1*-based system was not investigated, but the method may be useful for converting inbred lines to *Cms*.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP17

Patenting plants new challenges.

Current and future agricultural challenges are creating an urgent demand and constantly increasing the need to improve the performance of crop plants. This has led to an increase in agro-biotech research by academic institutions and industry. New technologies have been and will be developed for the purpose of improving plants, for example genetic transformation of plants. The patent landscape reflects the development of these technologies, as the number of patent applications for plant related inventions has significantly increased in the last 20 to 30 years. In light of the high costs involved in agro-biotech research, obtaining patents for the technologies developed is a major goal of the companies working in the area.

Although the law and the case law of the Patent Offices has and still is co-evolving with these technologies, a number of patents have been granted by various Patent Offices. Illustrative examples of patents directed to plants and decisions from Patent Offices will be provided. There are discussions on potential negative effects of patents on innovation. At this time it does not appear that these discussions will result in a change in policy that will cause major Patent Offices - like the US Patent and Trademark Office or the European Patent Office - to deviate from their current practice to grant patents on plants.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP05

Abundance of tomato spotted wilt thrips vectors on alternative plant hosts associated with the tobacco farmscape in Georgia, U.S.A.

Certain species of insect pests called thrips (Family: Thripidae) are known to transmit, or vector, tomato spotted wilt (TSW), a serious virus of flue-cured tobacco produced in the USA. The weed species present in the tobacco farmscape were sampled during January through May in 2007-2009, to determine thrips abundance and species composition. Nearly 10,000 adult thrips were collected and identified from plant hosts during this study, with the objective to determine if vector species were present, and if so, on what host plants. The tobacco thrips, *Frankliniella fusca*, was the most common TSW vector present in the tobacco farmscape, and was collected from 19 of the 24 host plants sampled. *F. fusca* were most numerous on wild radish, nutsedge, primrose, rye cover crop, and volunteer soybeans. The western flower thrips, *F. occidentalis*, another TSW vector, also was commonly collected from 16 of the host plants in the farmscape. Western flower thrips were most common on blooming wild radish, vetch, clover, nutsedge, and rye cover crop. Other non-vector thrips species were observed on all 24 of the host plants surveyed in the farmscape. The tobacco thrips was the predominant thrips species present on tobacco foliage (85%) during the growing season, while the non-vector flower thrips, *F. tritici*, was the predominant thrips present on tobacco blooms (93%). Thrips vectors of TSW were collected every month of the survey, and were most abundant during March through May. Most host plants also were suitable for thrips reproduction and development as evidenced by the large numbers of immature thrips observed. These data indicate that numerous plant hosts are available in the tobacco farmscape to maintain TSW innoculum and TSW vectors throughout the winter and early spring months, prior to transplanting the tobacco crop.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP27

Natural and biological treatments to enhance tobacco phosphate nutrition in the field.

Research is in progress at Fattoria Autonoma Tabacchi of Città di Castello (Perugia, Central Italy), to investigate the possibility to use natural and biological treatments, instead of the ordinary chemical ones, to enhance tobacco early growth in the field, by improving early plant phosphate uptake. Growers of Central Italy, where the tobacco growing season is relatively short, are very concerned about low soil phosphate availability in their sub-alkaline soils. This problem is increased by progressively reduced phosphate applications, due to higher P fertilizer costs, and the so-called agro-environmental measures, which affect P allowed application rate. Natural acidifying sweet chestnut-tannin, applied as in-the-row placed treatments or by microirrigation, proved to increase P availability in sub-alkaline soils. These applications have also an interesting nemastat side effect, which is under study mostly on vegetable crops and tobacco in Northern Italy. Some tests have been carried out for the last two years also on VA mycorrhizae, *Trichoderma* spp. and rhizosphere bacteria based

formulations, applied soon before field transplanting to the trays of transplant seedlings, and at field tobacco transplanting, to enhance early plant nutrition and resistance to abiotic stresses and root diseases. These investigations have indicated that these natural and biological treatments offer potential benefits to tobacco growers, especially where the agro-environmental measures are applicable. A decreased dependency upon fertilizer agrochemicals improves tobacco sustainability, and helps reduce crop environmental impact.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP11

Chemical induction of premature flowering in tobacco.

There is often a need to reduce flowering time in tobacco (*Nicotiana tabacum L.*) to facilitate development of new cultivars. In our tobacco breeding program, premature flowering has been occasionally observed in tobacco transplants treated with Terramaster, a fungicide labeled for use in hydroponic tobacco transplant production in the United States. Research studies were conducted to determine if high rates of Terramaster could be utilized to routinely induce premature flowering in tobacco. Approximately 21 days after seeding, seedlings of flue-cured, Burley, and dark tobacco were transferred into Styrofoam float trays, which were subsequently placed on water containing high concentrations of Terramaster (1, 2, 4, 6, 10, 15, 20, 50 or 100X the labeled rate) approximately one week later. The seedlings remained on the Terramaster until the first indication of flowering. Premature flowering was initially observed approximately 60-70 days after seeding, depending on tobacco type. Premature floral induction was much more prevalent in flue-cured and dark tobacco in comparison to Burley tobacco. At 77 days after seeding, percent flowering was 62, 56, and 28% for flue-cured, dark, and Burley, respectively; the percentages increased to 84, 84, and 50%, respectively, at 87 days after seeding. The optimum concentration of Terramaster for premature floral induction ranged from 10X to 20X the labeled rate, with higher rates more effective for Burley tobacco. Concentrations of Terramaster greater than 20X the labeled rate resulted in extreme stunting of the plants. Subsequent studies indicated that premature flowering is easier to achieve during summer versus winter months, and in early maturing versus late maturing tobacco varieties.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP28

Evaluation of the partial or complete substitution of inorganic calcium ammonium nitrate with organic TwinN as top dressing fertilizer in Burley tobacco production.

Inorganic fertilizers are a major cost of tobacco production in Malawi. Cheaper and effective sources of nutrients would, therefore, reduce the cost and improve the profit margins for tobacco. TwinN, a freeze dried biological natural soil improver has proved effective on wheat and maize but no work has been reported on tobacco. Experiments were, therefore, conducted for two seasons to evaluate the effect of TwinN on the growth, yield and quality of Burley tobacco at Kandiya Research Station in Lilongwe. Tobacco seedlings were raised by either applying the standard nursery fertilizers ("S Mixture + Nitrate of Soda") or standard plus TwinN. Seedlings from each nursery treatment were then transplanted and grown under three different top dressing fertilizer combinations of Calcium Ammonium Nitrate (CAN) + CAN; CAN + TwinN and TwinN + TwinN. The experimental design was a 2 x 3 factorial, arranged in a randomized complete block design with four replications. The

highest average yield of 2054 kg/ha was obtained with the top dressing application of CAN + CAN and this was comparable to the CAN + TwinN treatment at 2005 kg/ha. The plant height, root volume and Leaf Area Index Values (LAI) were also similar for the two treatments. Partial substitution of CAN also produced the highest proportion of red leaf (22%) and reduced the input costs by 14%. The complete substitution of CAN with TwinN resulted in the lowest average yield of 1467 kg/ha. The same treatment had the shortest plants, lowest root volume and LAI values but produced the highest proportion of the lemon leaf (50%). The results from this experiment suggest that TwinN would be beneficial in tobacco production and may substitute the second top dressing CAN in Burley tobacco.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. APPOST13

Ensuring product integrity of Malawi tobacco.

Tobacco is Malawi's leading foreign exchange earner. Grown by more than 50,000 farmers and offering direct employment to thousands more, tobacco is a crop of significant national economic importance. Noting this, the Government and the growers jointly established the Agricultural Research and Extension Trust (ARET) in 1995 to scientifically develop and transfer tobacco technologies that aim at maintaining the integrity of the country's tobacco leaf styles. ARET achieves this by assisting farmers technically throughout the production chain. ARET implements strong variety breeding programmes, develops appropriate agronomic practices, integrated pest management technologies and innovative leaf curing technologies. The Trust also runs a tobacco seed multiplication programme for all locally bred varieties and operates fully equipped seed storage facilities capable of holding enough certified tobacco seed for three years. It also acts as a clearing house for all importation of seed for foreign tobacco varieties released in Malawi. This ensures a high degree of traceability for the tobacco grown in the country. Additionally, ARET operates a wide network of certified tobacco seed outlets in collaboration with trading companies and tobacco grower organizations. ARET also implements demonstration programs to show and teach farmers how they can grow the tobacco using approved cultural practices, integrated pest management (IPM) and correct use of recommended crop protection agents (CPAs) at all times. Annually ARET releases a list of recommended CPAs for use on tobacco and updates a list of prohibited chemicals in tobacco. Additionally, during the growing season ARET samples tobacco leaf and analyses for pesticides residues and nicotine levels to ensure farmers' compliance to recommendations. Farmers are also given support with environmental management techniques. All this ensures that the Malawi tobacco comes from certified seed that is traceable, free from banned chemicals and produced in a manner that is environmentally friendly using legal labour.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP20

Distribution of race and mating type of the tobacco black shank pathogen, *Phytophthora nicotianae*, in Virginia in 2006-2009.

Black shank, caused by *Phytophthora nicotianae*, is a significant problem in tobacco-growing regions throughout the world, including Virginia. A total of 217 isolates of *P. nicotianae* were collected from stem pith samples from flue-cured, Burley, and dark fire-cured tobacco sampled from fields in 12 Virginia counties in 2006-09. Isolate race identities were determined using host differential assays employing three differential cultivars, Hicks, L8 and NC1071, under greenhouse conditions. Seventy-six percent of the isolates were race 1, 21% race 0, and 3% race 3. This race structure is comparable to the other tobacco producing states in the USA. The mating type of all 217 isolates was determined based on a conventional pairing assay using known *P. meadii* and/or *P. nicotianae* testers. This study

showed that most of the isolates (94 percent) were, in fact, of the A^2 mating type, and only 6 percent of the isolates belonged to the A^1 mating type. The proportion of A^2 to A^1 in *Ph* gene containing cultivars and non-*Ph* gene containing cultivars was very similar, 93:7 and 94:6, respectively. All isolates recovered from Burley cultivars were A^2 mating type. Isolates from flue-cured and dark fire-cured tobaccos represented both mating types. Only a single mating type was recovered from 88 percent of the fields sampled, with A^2 mating type accounting for 84 percent of the fields, both mating types were recovered from 12 percent of the fields. A single mating type in most of Virginia's tobacco fields may indicate a lower possibility for sexual recombination, possibly creating a biological bottleneck to adaptation by the pathogen population. A detailed genetic diversity study is underway employing simple sequence repeats and random amplified polymorphic DNA markers to evaluate the diversity among populations within flue-cured, Burley and dark tobacco fields in Virginia. Results from this study will be discussed.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP34

The influence of growing and curing environment on TSNA content and cured leaf quality of Burley tobacco.

The quality of Burley tobacco (*Nicotiana tabacum* L.) is determined by field growing conditions and curing conditions (temperature, humidity, and air flow). Research has shown that TSNA formation occurs in Burley tobacco during the curing process. The objective of this study was to evaluate the effects of growing and curing environments on TSNA accumulation and cured leaf quality. Two Burley tobacco cultivars, KT 204LC and a selection of TN 90, selected for high conversion of nicotine to nornicotine (TN 90HC) were used at all locations for both years. Burley tobacco was grown at 3 locations in 2008 and 2009 (Glade Spring, VA, Blackstone, VA, and Greeneville, TN). At each location 224 kg ha^{-1} nitrogen fertilizer was applied, other fertilization was according to soil test. Both years, a portion of tobacco from each growing location was harvested, transported and air-cured at 3 curing locations (Glade Spring, VA, Blackstone, VA, and Greeneville, TN). Curing environment for each location was monitored and evaluated using the U14 hobo data logger with the external temperature and relative humidity sensor. At grading the 4th leaf from the top of the stalk was combined across the plants for each replication, midrib removed and lamina prepared for chemical analysis. In 2008, both growing and curing locations had a significant effect of TSNA accumulation in TN 90HC. Tobacco grown at Glade Spring, VA and tobacco cured at Glade Spring, VA resulted in lower TSNA accumulation, compared to other growing and curing locations. In 2009, both growing and curing locations had a significant effect on TSNA accumulation in both KT 204 LC and TN 90HC. Tobacco cured at Greeneville, TN resulted in higher TSNA accumulation and tobacco grown at Glade Spring, VA resulted in higher TSNA accumulation compared to other growing and curing locations. In 2009 tobacco from each growing and curing location were evaluated closely by USDA and tobacco manufactures for physical quality. This data is still being compiled but will be reported.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP22

Maleic hydrazide application can alter bacterial wilt severity based on application timing.

Bacterial wilt caused by *Ralstonia solanacearum* is an extremely damaging disease of flue-cured tobacco in the southeastern USA. Mechanical transmission of *R. solanacearum* during flower (topping) and leaf (multipass harvester) removal has contributed significantly to the spread and severity of bacterial wilt and also coincides with the time period when maleic hydrazide (MH) is applied to arrest axillary shoot growth (suckers). Field observations suggest MH application can retard mechanical transmission of bacterial wilt during mechanical topping and leaf removal. The present study evaluated the effect of MH application on *R. solanacearum* establishment and disease development following mechanical transmission of the bacterium. Controlled environment studies where MH was applied 4 days prior to inoculation with *R. solanacearum*, simultaneous application with *R. solanacearum* inoculation and 4 days post inoculation demonstrated significant differences in the amount of stem necrosis and numbers of stem lesions during simulated mechanical transmission. MH application 4 days prior to inoculation with *R. solanacearum* reduced stem necrosis 62.5% (P=0.05) over the untreated inoculated control and was the most effective treatment for a reduction in numbers of stem lesions and stem necrosis. The role of MH on bacterial wilt development and its implications for MH leaf residues will be discussed.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP12

Molecular characterization of a new trait associated with the PVY^{ntn} resistance in *Nicotiana tabacum*.

The PVY^{ntn} is a very severe variant of the Potato Virus Y (PVY), Tobacco Vein Banding, and has been reported causing losses greater than 30% in yield and serious damages to the quality in many tobacco growing areas around world. The first genetic resistance source to the PVY^{ntn} variant in tobacco was described by Pulcinelli *et al.* (2009) that identified a new induced mutant immune to this variant. To use this mutant as a source of resistance to the PVY^{ntn} variant it is very important to know the genetic behaviour and inheritance of this new trait and also its molecular characterization, which are the basis for the development of new markers for MAS strategy. With this purpose the following generations were obtained: mutant line (P_1), original variety K326 (P_2), F_2, BC_1, and BC_2. From these generations seeds were sown and the seedlings were transplanted to pots and grown in a greenhouse. A population as large as 20 plants of each parental line and 200 plants for the F_2 and BC's generations were grown and approximately 40 days after transplanting were inoculated with the PVY^{ntn} variant. On the 21st day after inoculation all the plants were evaluated and the expected and observed frequencies were compared through a Chi-square test, confirming the genetic control of this characteristic as recessive. The DNA of each plant was extracted and analyzed through AFLP method using the AFLP Analysis System I (Invitrogen Life Technologies. 2003). The total DNA was digested with a combination of restriction *Eco*R I and *Mse* I endonucleases. The generated fragments were ligated to *Eco*R I and *Mse* I to obtain the template DNA for amplification. Two consecutive PCR reactions were performed for generating the desired fingerprinting. For the selective AFLP amplification, 28 different pairs of primers combinations between *Eco*R I and *Mse* I primers were used. From these 28 combinations it was possible to identify seven that showed very clear polymorphism between the mutant and the original variety. The combination of E-AGG / M-CAA primers showed to be the best combination to detect the existing polymorphism between the mutant and the original variety. The next step will be to compare the molecular segregation (genotyping) against the phenotypic segregation (results from the inoculated plants) to assure the detected polymorphism is associated with the new trait. Once confirmed the amplified region will be sequenced for design specific primers (SCAR) for Marker Assisted Selection strategy linked to the tobacco breeding program.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP30

Preliminary evaluation of a floating plastic tray for greenhouse tobacco transplant production.

The expanded polystyrene (EPS) tray is the basis of the current float production for greenhouse tobacco transplants. Although the EPS float tray is the standard of the industry, the lack of recycling, proper disposal, and effective sanitation procedures are significant drawbacks of the tray. The REKA plastic float tray addresses each of these problems. The tray is actually a two-piece tray with the plug tray resting on a floatation tray. Buoyancy is provided by inverted, closed cells that traps air under the tray. The tray can be constructed with varying amounts of recycled plastic and can be disposed as recyclable material. The amount of buoyancy can be regulated by the number openings in the inverted cells in the floatation tray. Trials have been conducted to calibrate the depth of tray floatation with different brands of commercial soilless growing media. Trays were filled and dibbled by hand in these calibration trials and data were collected on the wetting rates of the media. Subsequent work was conducted to adapt the REKA plant tray to an automatic tray filling and seeding line used for EPS float trays. The advantages and disadvantages of the REKA tray compared to the EPS float tray will be discussed.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. APPOST08

The attractiveness of a tobacco moth lure for three pyralid moth species.

The tobacco moth, *Ephestia elutella* (Lepidoptera: Pyralidae), is a serious pest of stored tobacco. The species has the female-produced sex pheromone. The pheromone components were identified as (Z,E)-9,12-tetradecadien-1-ol acetate (ZETA), and (Z,E)-9,12-tetradecadien-1-ol (ZETOH) by Brady and Nordlund (1971) and Kuwahara *et al.* (1973). Based on their findings, pheromone traps with these components have been developed and used to monitor the species.

The Indian meal moth, *Plodia interpunctella*, the almond moth, *Cadra* (*Ephestia*) *cautella*, and the Mediterranean flour moth, *Ephestia* (*Anagasta*) *kuehniella*, belong to the pyralidae family like the tobacco moth. The former three species are pests of mills and food-processing plants and do not feed on cured tobacco. They also have the female sex pheromones and the four species share two components, ZETA and ZETOH. Consequently, when the four species inhabit the same place, they are caught by a pheromone trap with the same pheromone components.

In the present study, we clarified the attractiveness of a tobacco moth lure (GACHON) with ZETA and ZETHO for three pyralid moth species in a laboratory. Attractiveness was the highest for the tobacco moth. Ratios of attractiveness for the other three species to attractiveness for the tobacco moth were 0.32 (*P. interpunctella*), 0.15 (*C. cautella*) and 0.24 (*E. kuehniella*). In conclusion, the tobacco moth lure attracted four pyralid moths. However, most of the pyralid moths caught by the pheromone trap were the tobacco moths.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. APPOST16

Insecticide resistance in the orange morph of the green peach aphid on tobacco in Virginia.

An orange-colored morph of the tobacco-adapted form of the green peach aphid, *Myzus persicae* (Sulzer), has become increasingly important on tobacco in Virginia since 2006. This orange morph appears to be more difficult to control with some insecticides than the common red and green morphs. Research was conducted at the Virginia Tech Southern Piedmont AREC in 2007-2009, to compare the seasonal abundance of the three morphs, to assess the field performance of six aphicides, and to compare the levels of resistance in the three morphs in laboratory bioassays with two commonly used insecticides. In 2008 and 2009, field treatments included imidacloprid (Admire Pro) applied as a tray drench, aldicarb (Temik) applied to the soil at bedding, and acephate (Orthene), and lambda-cyhalothrin (Warrior) (2008) or bifenthrin (Capture) (2009) applied as foliar sprays. Laboratory bioassays assessed resistance to acephate and imidacloprid in the three morphs collected from various locations in Virginia. The orange morph increased from 1% of the population on tobacco in 2007 to over 70% in 2009. In 2008, >50% (70% on one date) of the aphids in acephate plots were orange compared with <5% in lambda-cyhalothrin plots. Acephate was the most effective treatment in 2008 when orange morphs made up 10% of the population, but it was much less effective in 2009 when >70% of the aphids were orange. Bifenthrin gave excellent control of aphids in 2009, but only 35% of the aphids were orange. In 2008, aldicarb and imidacloprid provided excellent early-season control, but they were much less effective in 2009 when 70% of the aphids were orange. In bioassay tests, the orange morphs averaged 2- to 4-fold higher LC_{50} values for acephate and methomyl than the red and green morphs with maximum resistance factors of 20 for acephate. More recent information will also be discussed.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP03

Improvement on the trait of nicotine to nornicotine conversion for Chinese Burley hybrids and the effectiveness of quality increase and harm reduction.

The problem of nicotine to nornicotine conversion is severe in Burley production in Sichuan, which is a fast growing Burley producing area in China. The improvement on the trait of nicotine conversion for a series of Burley hybrids was conducted through early identification, selection and hybridation of non-converter parent plants, and the effects of harm reduction and quality enhancing were evaluated for the improved hybrids Dabai1NN, Dabai2NN, Dabai3NN, compared with the regular hybrids. The selection was conducted by using early ethryl stimulation and identification methods for the parent population. Through 3 generation systemic selections, the proportion of converters in the population of Dasuo26 (male parent of Dabai 1 and Dabai 2) and Dasuo27 (male parent of Dabai 3), both of which were main contributors of converting genes to the relevant hybrids, reduced to less than 5% and less than 9%, respectively, from 53.2% and 96.0% in the original population. The proportions of total converters in the 3 improved hybrids were 4.65%, 4.76% and 6.52%, respectively, 80.1%, 61.9% and 91.7% lower than the regular hybrids, and the newly emerged converters were mostly low converters. The nicotine conversion rates of the 3 improved hybrids in the bulk samples were 3.0%, 2.2% and 2.63%, respectively, 72.5%, 71.1% and 94.9% lower than the regular hybrids, respectively. The total TSNA contents in the 3 improved hybrids decreased 38.5%, 29.2% and 50.8% compared with regular hybrids, and the difference was mainly caused by NNN reduction, with the decreasing rate of 61.3%, 49.6% and 71.0%, respectively. Improved hybrids had better tobacco flavour quality than the regular hybrids, with increased Burley style, increased aroma quality and quantity, and decreased offensive odour. The blended cigarettes made from improved Burley tobacco had more reasonable alkaloid composition, higher flavour quality and lower TSNA delivery in the smoke. In addition to the

selection of non-converters in the selfing lines of non-converter plants, we also selected newly emerged low converters and planted their selfing lines in the next generation, and high converters were found produced from low converter lines; this result proved that converters can come from non-converter line and the degree of conversion level can accumulate and increase through generations.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. APPOST15

Current status of field testing program for pesticide residues in North Carolina flue-cured tobacco.

Research was conducted from 2007 to 2009 at two locations in North Carolina to evaluate cured leaf residues resulting from the application of maleic hydrazide, flumetralin, butralin, cypermethrin, imidacloprid, or azoxystrobin to flue-cured tobacco. All pesticides were applied at maximum labeled rates and following all label recommendations so that maximum expected residues could be determined. Cured, whole-leaf, samples were collected from the bottom, middle, and upper stalk positions and were analyzed by Global Laboratories in Wilson, NC. Residue levels for maleic hydrazide ranged from 77 to 151 ppm, for flumetralin from <0.05 to 0.62 ppm, for butralin from <0.05 to 0.82 ppm, for cypermethrin from 4.0 to 26.5 ppm, for imidacloprid from <0.25 to <0.70 ppm, and for azoxystrobin from 1.45 to 13.39 ppm. Beginning in 2010, four pesticides will be tested in the program following the protocol and procedures used previously. These will be bifenthrin, chlorantraniliprole, clothianidin, and flubendiamide.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. APPOST05

Screening and identification of antagonistic bacteria against tobacco wildfire.

As one of the most common and destructive diseases, tobacco wildfire disease, caused by *Pseudomonas syringae* pv. Tabaci, results in significant losses every year in Chinese tobacco growing areas. In order to develop biocontrol agents against tobacco wildfire, 14 strains that have antagonism against tobacco wildfire were screened out of 278 isolates separated from tobacco leaves and identified as *Bacillus subtilis* based on 16S rDNA sequence. The inhibition zone of the most antagonistic strains against tobacco wildfire were 9.3 mm. After spraying of the antagonistic bacteria, the activity of phenylalanine ammonialyase (PAL), peroxide enzyme (POD) and the polyphenoloxidase (PPO) activeness in the tobacco leaf were increased significantly. The results of the efficiency test confirmed that when the plants were covered with antagonistic bacteria before inoculation of pathogen bacteria, the control efficacy to tobacco wildfire reached 97.54%, which is significantly higher than that of agro-streptomycin. And the control efficacy was 51.40% when the plants were sprayed with antagonistic bacteria after inoculation of the pathogen bacteria.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP04

Decrease in contents of TSNAs and NOx in smoke by reduced expression of nitrate transporter gene (*NtNRT1*).

We previously reported (CORESTA 2004) that two members of a low-affinity nitrate transporter gene family *NtNRT1* isolated from *Nicotiana tabacum*, *NtNRT1.1* and *NtNRT1.2*, showed the highest expression in roots, and that overexpression of *NtNRT1.1* enhanced the accumulation of nitrate in transgenic tobacco BY2 cells. For the present study, we prepared transgenic tobacco plants having reduced expression of *NtNRT1*. The RNAi-mediated gene silencing of *NtNRT1* in tobacco did not affect the morphology. The nitrate contents in both green leaves and air-cured leaves were lower in transgenic tobacco than in wild type. The nitrate contents of air-cured leaves were 30% lower at the lower leaf position of the transgenic tobacco. The TSNAs (NNN, NNK, NAT, and NAB) and total nitrogen contents were also lower in air-cured leaves. Moreover, the contents of free amino acids and cell wall components (cellulose, hemi-cellulose, lignin, and pectin) were higher. We next prepared hand-rolled cigarettes from the transgenic and wild-type laminas for ISO standard analysis of smoke constituents. The TSNAs and NOx contents in mainstream smoke were lower by 8% and 18%, respectively, in cigarettes prepared from the transgenic laminas. These data suggest that a genetic approach to regulating the expression of *NtNRT1* might be useful to decrease TSNAs and nitrate contents in air-cured leaves and to decrease TSNAs and NOx contents in mainstream smoke.

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Evaluation of host suitability for *Meloidogyne* species of rotational crops used in tobacco cultivation in South Africa.

Crop rotation is the single most useful practice that can realistically be applied to annual and short-term perennial crops. It is also not uncommon for a single field to have several nematode species present that could have a limiting effect on a specific crop. The root-knot nematodes *Meloidogyne incognita* races 2 and 4 and *M. javanica* are the key nematode problems in South Africa. Depending on the time and population density, root-knot nematodes can cause considerable damage to a tobacco crop. Crop-rotation schemes are complicated by the presence of different root-knot nematode species and races because the reproduction of different species and of *Meloidogyne* varies with crop and cultivar. It is therefore necessary to know the host-status of the cultivars of crops to the major root-knot nematode species. A project was initiated to evaluate the resistance of the various cultivars of the rotational crops to the principal root-knot nematode species and races. Until 1999 extensive greenhouse screenings have been conducted by ARC-IIC. Evaluations were done on a cultivar level in the greenhouse and information on 310 entries, including cash crops, pasture grasses, cover crops and lesser known crops with a specific niche market, is available. The project was resumed in 2007 and to date cultivars of various crops such as millet, sunn hemp, oats, maize, wheat, pea and rye grass cultivars were evaluated. From these crops the rye grass cultivar Caversham, oat cultivars Witteberg and Kompasberg and wheat cultivars Olifants, Baviaans, Kariega and Steenbras, were resistant to the 3 major root-knot nematodes in South Africa, whilst the pea cultivars Raxel and Bolero were susceptible and the other cultivars of crops evaluated, varied between susceptible, tolerant and resistant.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP31

Evaluation of potassium application methods and rates on the yield and quality of flue-cured tobacco.

With rising input costs, growing environmental concerns, and new higher yielding cultivars, potassium fertilizer recommendations must be accurate. Research is being conducted to evaluate the effects various potassium rates and application methods have on the yield and quality of flue-cured tobacco. Research was conducted at the Upper Coastal Plain Research Station in Rocky Mount, NC and the Oxford Tobacco Research Station in Oxford, NC in 2009.

One trial evaluated the effects of nine potassium (K-Mag) rates from 0 to 225 lbs/A, all applied at transplanting, on flue-cured tobacco. Potassium-magnesium sulfate was used to supply fertilizer potassium. Nitrogen fertilizer was sidedressed after transplant in the form of 30% UAN at Rocky Mount and 15.5-0-0 at Oxford. Control plots received a base fertilizer and a sidedressed application of nitrogen. Soil samples were collected at transplant from control plots, and tissue samples were collected throughout the season. Cured leaf yield as well as chemical and physical quality data was also collected.

The second trial evaluated the effects of four potassium rates; 75, 125, 175, 222 lbs/A, and four application timings; broadcast one month before planting, broadcast one week before planting, at planting, and at planting and layby, on flue-cured tobacco. Potassium-magnesium sulfate was used to supply fertilizer potassium. Nitrogen fertilizer was sidedressed after transplant in the form of 30% UAN at Rocky Mount and 15.5-0-0 at Oxford. Control plots were sidedressed with nitrogen, and did not receive any potassium amendments. Soil and tissue samples were collected throughout the season. Cured leaf yield as well as chemical and physical quality data was also collected.

There was no significant difference between any fertilizer treatments and control plots receiving no additional potassium. Lower rates of K-Mag were acceptable under these conditions, along with broadcast application.

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Modification of trichome gum chemistry in tobaccos.

Tobacco trichomes produce tobacco gum, which mainly consists of diterpenoids and sugar esters having organoleptic potential. We have utilized RNAi to modify the chemistry of both of these groups of gum compounds in the experimental tobacco T.I. 1068. The degree of knockdown of specific diterpenoids observed varied with all the genes manipulated from low to near complete in T0 populations, and was found to be stable in further generations, where studied. This suggests that levels/mixtures of various diterpenoids may be subject to manipulation by design. However, while in some knockdown studies results were consistent with working models that predict diterpenoid metabolic pathways in tobacco trichome glands, in other cases results appear to reveal unexpected impacts that suggest complex regulation of carbon flow in glands. The possibility that knockdown of endogenous diterpenoid metabolism may provide substrate for enzymes encoded by introduced foreign genes (e.g., diterpene synthases) is being studied.

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CORESTA Congress, Edinburgh, 2010, Agronomy/Phytopathology Groups, abstr. AP32

Effects of different ridge height and cover pattern on nitrification and denitrification in field soil, and growth in Burley tobacco.

This experiment was carried out to study the effects of different ridge heights and cover patterns on nitrification, denitrification and root development on field soil, nicotine concentration and leaf yield in Burley tobacco. The results showed that a ridge measurement of 10 cm and film-covering could remarkably enhance the intensity of nitrification during rosette stage, and reduce denitrification before

rosette stage and after the fast growing period This is useful for promoting nitrogen supply during early stage and reducing denitrificational loss of soil nitrogen. With regard to root development, film-covering was better than non-film-covering. Among the four treatments of ridge height, the 10 cm ridge was more propitious to generation of the adventitious roots and ramification of the superior root, which form more a harmonious distribution of the root in the soil. Nicotine concentration in leaf increased as the ridge height increased, and the concentration under the film-covering treatment was higher than that under the non-film-covering treatment. On the leaf yield and its factors, compared with the non-film-covering treatment, the film-covering treatment could get higher leaf yield, production value, leaf price, and the percent of the classy and middling rank of leaf; and among the four ridge heights under the film-covering condition, the 10 cm ridge obtained the best leaf yield and production value. Considering altogether on the nicotine concentration and leaf yield, a 10 cm ridge and film-covering can be recommended as the optimum integrated production measure to obtain a high level of leaf yield and quality.

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