

**ABSTRACTS OF PRESENTATIONS MADE AT THE
2006 CORESTA CONGRESS IN PARIS, FRANCE
AGRONOMY AND PHYTOPATHOLOGY**
(in alphabetical order of first authors)

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST01

Fertilizer N, topping height and cultivar effects on yield, grade index and chemical composition of flue-cured tobacco in Ontario.

The objective of this field study was to determine whether current topping and N fertilizer practices are optimum for yield, grade index, and chemical quality for flue-cured tobacco production in Ontario, Canada. The study was conducted at Delhi, Ontario, in a wet (2000) and two dry (2001 and 2002) years with two flue-cured tobacco cultivars (CT157 and Delfield), three fertilizer N levels (25, 50 and 75 kg ha⁻¹), and three topping heights (17, 19 and 21 leaves) except in the year 2000. In 2000, the plants were relatively small (likely due to the wetter than normal growing season) so they were topped at 15, 17 and 19 leaves. N rates optimal for yields were 75 kg ha⁻¹ in 2000 and 2001, and 50 kg ha⁻¹ in 2002; whereas N rates optimal for grade index were 75 kg ha⁻¹ in 2000 but only 25 kg ha⁻¹ in 2001, however, in 2002, grade index was unaffected by N rate. For returns, optimal N rates were 75 kg ha⁻¹ in 2000 and 50 kg ha⁻¹ in 2002, but returns were unaffected by N rate in 2001 when the increase in yield with N was counteracted by a lower grade index. Yield consistently increased with topping height; but grade index declined in two of three years. Returns were always either greatest or unaffected with high topping. Total alkaloids increased with increased nitrogen application. CT157 showed superior grade index but generally, Delfield ranked higher than CT157 in alkaloid concentrations each year. Optimum gross returns and desirable leaf quality can be achieved at 25 to 50 kg/ha N. However, the optimum topping height may be at least 19 leaves under normal conditions.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP40

Residue formation during phosphine fumigation of raw tobacco.

Phosphine is extensively used for insect control in grain or other bulk commodities such as tobacco. Occasionally a yellow dust-like residue forms during the fumigation process. It has been postulated that such residues may be composed of phosphoric acids and their polymers, the oxidative degradation products of phosphine gas.

In an attempt to elucidate structure of this yellow precipitate several chemical and physico-chemical methods were used. Measurements included studies with X-ray fluorescence, pyrolysis- GC-MS, ESCA (XPS), FT-IR, TOF-SIMS.

From these measurements it has been concluded, that in contrast to other findings, this insoluble, inorganic polymer is composed of polyphosphate and elemental (red) phosphorous segments, (P-Oa)_x- P b- (P-Ob)_y.

The structure of this polymer was further validated by a laboratory synthesis of the same polymer class. Therefore, based on the structure of the polymer, possible degradation mechanisms of the yellow residue are discussed.

In addition differences in the residue concentration on different container surfaces were analyzed. Using ECSA and TOF-SIMS, (as surface sensitive methods), no P-containing residues were detected on the inner (tobacco-side) side of the containers.

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Effect of pre-harvest ferulic acid application on TSNA levels in dark fire-cured tobacco.

Ferulic acid (C₁₀H₁₀O₄), also known as 4-hydroxy-3-methoxy cinnamic acid, is a phenolic phytochemical found in the leaves and seeds of many plants, particularly cereals such as brown rice, whole wheat, and oats. Ferulic acid has known antioxidant properties and laboratory experiments have shown that ferulic acid is a potent inhibitor of the reaction between normicotine and nitrite leading to TSNA formation.

Experiments were conducted at the University of Kentucky Research and Education Center near Princeton, KY in 2003-2005 with the objective of evaluating the effect of pre-harvest ferulic acid application on TSNA levels in dark fire-cured tobacco.

Experiments were conducted using 'Narrowleaf Madole' dark tobacco in a complete block design in the field with 3 replications of treatments. Tobacco was treated 24 hr prior to harvest with ferulic acid (FA) solution at 0.25% or 0.50% FA with a non-ionic surfactant at 0.25% by vol. using small-plot spraying equipment that was calibrated to deliver 50 gal/A (467 L/ha). A non-treated control was included for comparison. Tobacco was stalk harvested 24 hours after application and plots were completely randomized on a single tier of a tobacco barn. All tobacco received forced air from fans for the first 14 to 21 days after housing. Tobacco was then wood-fired, receiving 3 firings over a 14 to 21 day period. The experiment was conducted 6 times. Following curing, whole leaf samples were taken from each stalk position of each plot and leaf chemistry analysis was performed.

Averaged across all stalk positions, tobacco treated with pre-harvest applications of ferulic acid solutions had 25 to 60% less total TSNA than non-treated tobacco.

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Field performance of Poland's new flue-cured varieties resistant to *Chalara elegans*.

Although situated as the northern-most tobacco growing country with a high and continuously increasing pressure from the soil-borne pathogen *Chalara elegans*, until now Poland has lacked flue-cured cultivars highly resistant to that disease. The use of moderate resistance or tolerance was only a temporary expedient. In the long run rather than alleviating the problem it has made it more severe as it favoured extensive build-up of the inoculum in the soil. As a result of a breeding project started in the late 1990's an array of breeding lines was developed from the cross of the domestic cultivar Wislica with AC Gayed - a Canadian variety carrying a resistance factor from *N. debneyi*. The new lines were selected for field performance in replicated field trials and under regular farming conditions. The best performers were crossed with Wislica cms *bigelovii* to produce commercial F1 hybrids. The F1 hybrids (VRG 1, VRG 2, VRG 3) were evaluated for yield, crop quality, gross returns per unit area and per unit weight, and basic physical and chemical characteristics of the cured leaf. Under moderate to high pressure of black root rot the hybrids out yielded cv. Wislica. They were equal or superior to cv. Wislica with regard to the percentage of superior grades in the crop, some of them had improved body and texture of the cured leaf as well as improved smoking characteristics. The hybrids VRG 1 and VRG 2 are now being successfully commercialized as the first Polish-bred flue-cured cultivars highly resistant to *C. elegans*. They are also the first commercial cultivars to use the cytoplasm of *N. bigelovii* as the source of CMS.

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Management program for spotted wilt in Georgia grown flue-cured tobacco.

Spotted wilt caused by tomato spotted wilt virus was first confirmed in Georgia tobacco in 1986. Since 1995 spotted wilt has caused more loss than all other tobacco diseases combined. Research programs from 1990-

2005 have given growers a multipart program for minimizing spotted wilt loss. This program is outlined here.

TRANSPLANT DATE: Combining data from several trials shows that for any farm site the risk of highest loss is twice as great when tobacco is transplanted before 7 April.

Plant Source: There is a 90% chance of successful spotted wilt reduction with imidacloprid treated greenhouse plants as opposed to a 50% chance of success treating bare root plants.

IMIDACLOPRID: Apply imidacloprid as a spray-on/rinse-off tray drench at 7.1-14.2 grams a.i. per 1,000 transplants 1-5 days pretransplant. Imidacloprid treatment at 7.1 grams a.i. per 1000 seedlings can reduce spotted wilt ~ 30%. Increasing rate results in increased percent control.

ACIBENZOLAR -S- METHYL (ASM): Apply ASM as a foliar spray at 14.2 grams a.i. per 100,000 fully grown, field ready seedlings 5-7 days before transplanting. Combining ASM with imidacloprid can reduce spotted wilt by about 50%.

PLANT POPULATION: Decreasing in row plant spacing from a typical 55.9 cm to 40.6 cm between plants has little effect on percent of spotted wilt. Higher yields (kg/ha) can be made but plants surviving in groups suffer from crowding even at high spotted wilt incidence. Increased plant population to offset loss is not generally recommended.

IN FIELD WEEDS: In field weeds vary in importance. We suggest either treating weeds to have them dead and dry before transplant or leaving them alone.

TILLAGE: Strip till production has reduced spotted wilt in peanut. A single trial where grain stubble was left as 13-20 cm high curtain rows on the bed shoulders reduced spotted wilt in tobacco by 50%.

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Yield and nutrient uptake of flue-cured tobacco in South Africa.

Flue-cured tobacco (various cultivars), grown commercially on eight farms in South Africa, was used to collect data regarding biomass, chemical composition and nutrient uptake. Plants were sampled at 5, 7, 9, 12 and 15 weeks after planting. The highest dry mass of the aerial parts was 8126 kg ha⁻¹ and an average dry mass of 7007 kg ha⁻¹ was obtained. The highest leaf mass was 5237 kg ha⁻¹ and an average leaf mass of 4424 kg ha⁻¹ was recorded. The average uptake of nitrogen, phosphorus and potassium by the aerial parts was 145, 18 and 224 kg ha⁻¹ respectively. A root mass of as high as 3137 kg ha⁻¹ and an average root mass of 2423 kg ha⁻¹ was also recorded. This data shows that a total flue-cured tobacco biomass of roughly 10 tons ha⁻¹ has become a reality. Results show that the biomass is represented by roughly 47% leaves, 27% stems and 26% roots. The roots were found to contain 44 kg N, 5 kg P, 36 kg K, 14 kg Ca, 4 kg Mg and 7 kg Cl ha⁻¹

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Examination of blue mold-infected tobacco for the presence of oospores, 2002-2005.

During the period of 2002-2005, surveys were conducted by staff of the Canadian Tobacco Research Foundation, the Ontario Flue-Cured Tobacco Growers' Marketing Board and the Ontario Ministry of Agriculture, Food and Rural Affairs to document the occurrence and severity of Blue Mold disease (*Peronospora tabacina*, causal agent) in Canada and to determine if oospore formation had occurred within the infected tobacco tissues. Blue Mold was confirmed within the country in 2002 and 2004 but was not reported nor was it found in either of 2003 or 2005. In general, Blue Mold was of minor importance during this period and tended to occur later in the season. The first confirmed occurrence of Blue Mold in Canada was reported on July 30 in 2002 and the disease remained restricted to a single farm. The disease was reported on August 3 in 2004 and was eventually detected on five farms. These occurrences were reported to the North American Plant Disease Forecast Centre of North Carolina State University, Raleigh, NC, USA. A protocol was followed to sample farms and infected plants found on them for lesion bearing-leaves in 2002

and 2004. Blue Mold lesions were examined microscopically for the presence of oospores and the occurrence of other diseases. In total, 2,276 lesions were examined. Oospores of *P. tabacina* were not found in the sampled materials.

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A genetically determined trait in flue-cured tobacco results in low germination of *Orobanche ramosa* L. seed.

Orobanche ramosa L. is a parasitic plant that affects tobacco in Europe and Asia. In France and Germany, despite ancient presence of this parasite, significant damages occurred only recently, and tobacco production became impossible in some farms.

A screening of the ITB germplasm collection was conducted by ANITTA in heavily infested fields from Poitou in 2002 and 2003. Some flue-cured lines from Polish origin, more particularly the cultivar Wika, were consistently parasitized by a lower number of *Orobanche* shoots than other flue-cured, and these shoots appeared later in the season. Further field trials in 2004 and 2005 confirmed these results.

In an attempt to understand the underlying mechanism, a test in controlled conditions was set up at ALTADIS-Bergerac. Individual tobacco plants were grown in Petri dishes filled with a synthetic substrate and added with approximately 500 *Orobanche* seeds. Root systems of tobacco plants, as well as *Orobanche* seeds and roots, could be examined periodically in a non-destructive way under binocular lenses.

A much lower number of *Orobanche* seed germinated in Petri dishes with growing roots of the cultivar Wika than with fully susceptible checks, and this germination occurred later. However, the few germinated *Orobanche* seeds that could attain a tobacco root of Wika succeeded in parasitizing the plant.

An F₂ generation from the cross between Wika and a susceptible flue-cured line was tested in these controlled conditions. Among 120 individual plants, 25% showed the same behaviour than Wika, whereas the 75% remaining were attacked in a similar way to the susceptible parent. It was concluded that a single recessive gene is involved. Low production of germination stimulants that are required by the *Orobanche* seed to germinate might be the underlying mechanism.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP38

Good Agricultural Practices (GAP) training in Africa: the Mozambican experience.

Tobacco production in Mozambique has increased dramatically in the past ten years. However, most of the communities which are economically dependent from this crop have had insufficient technical and scientific assistance to support them in obtaining the quantity and quality of leaf which can assure better economical returns, environmental preservation and long term farming sustainability. In 2004, recognizing the importance of training and dissemination of GAP techniques throughout the region, one of the Alliance One companies (former Stancom Tabacos) idealized and established cooperation with the Agrarian Institute of Chimoio to develop training activities. In a second stage of negotiations, the major tobacco suppliers operating in Mozambique agreed to sponsor and promote the first Tobacco Leaf Technician Course (TLTC). The main objectives of this 7-month training course were to provide solid in depth training in all aspects of tobacco leaf production through the benefits of sustainable agriculture practices linked with social responsibility and adapted to the local conditions and its various challenges. TLTC activities included practical field work and extension combined with classroom lectures, assignments and workshops. Both students and instructors were challenged and encouraged to develop an understanding of all issues faced by the modern tobacco production. At the same time, they were constantly motivated to integrate acquired knowledge to identify local production issues, such as soil conservation, reforestation, food security, child labor, HIV-AIDS, and to suggest approaches to improve the quality of life of local rural communities. In addition, high quality tobacco leaf to comply with international standards can only be produced with well

trained and talented people. Therefore, part of this project was also to wake up and shape the intrinsic talent and knowledge of the students. After two course sections, two agronomists and sixty students have been successfully trained and become qualified GAP technicians. The concretization of the TLTC is an example of the comprehensive development frame-work put in action by the tobacco leaf suppliers, showing their enthusiasm with the future of the African agriculture and its society.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST08

BUTRALIN: a global product for a global business.

For more than 30 years, butralin has been supported as a tobacco sucker control agent, on a world basis to serve the tobacco market.

Today, butralin-based products are registered in five continents and 22 countries. Recently, owing to an important and a constantly updated data package comprising about 250 studies, butralin was registered in Japan (2004) and in Korea (2005). Some further registrations are underway.

Butralin is a high-quality active substance with a typical purity of 99% and a nitrosamine content controlled at less than 1 ppm. Butralin is manufactured in France in a dedicated workshop, where strict quality control prevails.

In Europe, butralin is part of the European notification programme of existing active substances, as requested by Commission regulation Nr 1490/2002 laying down the rules for the implementation of the third stage of the programme of work referred to in Article 8(2) of Council directive 91/414/EC.

Our poster will show how this performance was achieved and how this present European challenge is being coped with.

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DuPont™ SmoothTrade™ Solutions: a new tool available to tobacco agronomists.

DuPont™ SmoothTrade™ Solutions is a service which provides growers and agronomists with all necessary information around crop production using DuPont crop protection products: the best crop protection product solution for a given crop and environment, quality assets of protected yield, fit in IPM (Integrated Pest Management) programs, stewardship guidance regarding the applicator and the environment, and MRL and residue information to support technical decisions.

DuPont™ SmoothTrade™ Solutions provides technical expertise, in-depth information and data to growers and agronomists by directing questions and requests to the qualified experts in DuPont.

Two examples of what SmoothTrade™ can offer are given here:

- An educational program which addresses operator safety by describing the correct personal protective equipment (PPE) for the handling, mixing and application of agrochemicals.
- While determining the appropriate GAP for the use of the insecticide Lannate® (a.i. methomyl) in tobacco, a protocol for a residue study is proposed giving details of the procedures from the field application up to pyrolysis testing. Residue analyses were performed on fresh leaves, after flue curing, and during pyrolysis testing using the standard CORESTA procedure to analyse the smoke fraction.

Questions or requests to DuPont™ SmoothTrade™ Solutions should be sent to the following E-mail address: smoothtrade@fra.dupont.com. This e-mail, or contact to your local DuPont sales or field development representative, will be your interface with the experts available to help you in your technical decisions regarding the use of DuPont agrochemicals.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST24

Colour and aroma of tobacco: a facilitated approach for their objective evaluation in Oriental tobaccos and for cultivar classification within Virginia and Oriental tobacco types.

In the routine practice the evaluation of colour and smoke aroma, major qualitative characteristics of tobacco, is mainly based on organoleptic estimates by expert panels, with this suffering from subjectivity, high costs and health hazards. We propose a new facilitated low-cost and safe approach for objective evaluation of colour and smoke aroma in Oriental tobaccos using the chromatographic profiles of polyphenols and neutral volatiles of essential oils/valeric acids, respectively, in tobacco leaves. To this purpose the methods of high performance liquid chromatography (HPLC) and capillary gas chromatography (CGC) were applied, and formulae for a reliable numerical expression of both characteristics were developed. Moreover, chromatographic data were employed in pattern recognition method (PRM) allowing the objective classification and differentiation of cultivars within Virginia or Oriental tobacco types. The proposed approach can be beneficial for tobacco breeding and market purposes, with being a tool to aid the release of high quality tobaccos.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST13

Genetic polymorphism of tobacco varieties based on ISSR markers.

Tobacco is one of the most important non-food crops and numerous types having distinct morphological characters and chemical differences are grown commercially. At the present time, only limited information is available on the relationship between morphological variability and genetic diversity in different tobacco lines.

In this paper Inter Simple Sequence Repeat (ISSR) molecular markers were used to detect the genetic polymorphism among *N. tabacum* varieties belonging to different tobacco types.

Genomic DNA of thirty-six varieties was extracted and amplified utilizing 10 different 3' and 5' anchored primers previously selected for the reproducibility of the amplification profile. PCR products were separated on agarose gel, stained with ethidium bromide and visualized by UV transilluminator. The electrophoretic banding patterns were recorded and analyzed utilizing an image analysis system. The number of markers generated per primer ranged from 3 to 9, and the dimensions from 150 and 1700 bp.

The amount of genetic polymorphism present among *N. tabacum* lines examined was limited as evidenced by the high degree of similarity in the ISSR profiles of the different tobaccos.

These results until now obtained suggest that few genetic differences underlie the significant morphological variability observed among cultivated forms of tobacco. Further experiments of amplification with other primers are in progress.

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Characterization of *Rhizoctonia* isolates from Zimbabwe and evaluation of the pathogenicity on eight tobacco-based rotation crops.

Rhizoctonia spp. are cosmopolitan plant pathogens on a broad range of host plants. In Zimbabwe, it was previously thought that only the multinucleate group, *Rhizoctonia solani* (Kuhn) [teleomorph: *Thanatephorus cucumeris* (A.B.Frank) Donk] anastomosis groups AG3 and AG4 were important pathogens of tobacco (*Nicotiana tabacum*). However, nuclear fluorescent microscopy studies conducted on isolates originally isolated from diseased tobacco revealed that contrary to the belief, uninucleate and binucleate *Rhizoctonia* spp. were also pathogenic on tobacco. This observation necessitated a study to characterize all

Rhizoctonia spp. isolates in the TRB culture collection originally isolated from diseased tobacco, tobacco soils as well as a wide range of other crops. In addition, experiments were also set up to test the pathogenicity of binucleate isolates on tobacco and on eight potential crops in tobacco rotation systems [maize, soyabean, Katambora Rhodes grass (two selections), roundnuts, groundnuts, wheat and soyabeans]. Results of the characterization of the culture collection isolates indicate that 56% of the isolates in the culture collection are binucleates, 41% are multinucleates while 32% of the isolates from tobacco are binucleates and 65% are multinucleates. Mature cultures of the isolates on potato dextrose agar (PDA) were light brown to yellow in colour while white to yellow sclerotia were formed on the surface of the medium. The quantity, size and distribution of sclerotia on PDA differed among isolates. In pathogenicity tests on the eight crops, isolates produced varying degrees of root and stem rot as well as mortality of 14-day old plantlets. While there were significant differences ($P < 0.05$) in the pathogenicity of the different isolates on the eight crops based on a scale of 0-5, roundnuts were the least susceptible followed by maize while groundnuts and soyabean were the most susceptible.

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A comprehensive survey of the *N. tabacum* transcriptome (The European Sequencing of Tobacco Project, ESTobacco).

Tobacco genome research is expected in the next few years to improve our knowledge about the interactions between genes involved in the formation of undesirable compounds in cigarette smoke. A close link exists between gene expression in the tobacco plant, chemical composition of raw tobacco, and combustion products in cigarette smoke.

The aim of the ESTobacco project is to be complementary to other projects currently underway concerning the tobacco genome. Our strategy is to sequence only genes expressed in tobacco and not the whole genome. The size of the tobacco genome is too large to be totally sequenced (29 times more than *A. thaliana*).

This project used three "commercial" varieties of tobacco widespread throughout the world: K326 for the flue-cured type, Burley 21 and TN86 for the Burley type. In order to obtain the major genes, the organs of the plant (seeds, roots, stem, midrib, lamina and flowers) prepared at different stages of development (germination, young seedlings, before and after topping, maturity) were used as a basis for this work. A large tobacco expressed sequence tag (EST) dataset was obtained from 11 normalized cDNA libraries comprising 56,000 clones.

A DNA array designed with these sequences could allow the large-scale study of the genes expressed in tobacco. This new tool will lead to the acceleration of programs already underway concerning the origins of risks associated with tobacco and inform strategies for harm reduction. In order to encourage a wide range of initiatives on tobacco plant genetic, as with other crops, the resulting sequences obtained during the ESTobacco project are available to the worldwide scientific community through public access databases.

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Evaluation of sucker control programs to reduce or eliminate MH residues in flue-cured tobacco.

MH residues have been a major concern within the tobacco industry for many years. In 2005, a portion of the tobacco produced in North Carolina was grown under an optional contract that did not allow the use MH. Growers in return were paid a premium for delivering tobacco free of MH residues. Fatty alcohols have contact activity and flumetralin (Prime+ and Flupro) is a contact local systemic, so both fatty alcohols and flumetralin must contact the sucker to control it. Therefore, using fatty alcohols followed by flumetralin alone in some cases has resulted in large suckers late in the season due to missed leaf axils. Missed leaf axils

with flumetralin are typically in the top of the plant and may result from leaning stalks, leaves covering the leaf axil, or both preventing proper "rundown" of flumetralin into the leaf axil. Research was conducted in 2005 and similar research will be repeated in 2006 to evaluate application procedures to improve sucker control from fatty alcohols and flumetralin. Preliminary data from 2005 indicate that split applications of reduced rates of flumetralin controlled suckers better than single applications. Therefore research in 2006 will evaluate low rate, multiple applications of flumetralin to increase chances of hitting each leaf axil. In addition, new innovations available from nozzle manufacturers and different nozzle arrangements will be evaluated to improve placement and rundown of flumetralin and fatty alcohols. Multiple applications of fatty alcohols and flumetralin will also be used in 2006 to delay MH application until after first harvest so that rates can be reduced and less tobacco will be treated with MH.

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The resurgence of bacterial wilt in the southeastern USA and a re-examination of our management system.

The systems approach developed by North Carolina, experiment station and USDA (United States Department of Agriculture) scientists provided effective bacterial wilt control for nearly a half-century. Yet sudden outbreaks of bacterial wilt in previously uninfested areas as well as a general increase in disease intensity across the Carolina's since the 1980's provides evidence of a breakdown in the best management system. The introduction of *Ralstonia solanacearum*, the causal organism, into previously uninfested land is an ecological disaster that will affect production of solanaceous crops in these affected areas across generations. Disease losses in some locations are disturbingly reminiscent of the losses in the earlier part of the last century where entire fields were lost to bacterial wilt and farm families financially ruined. This paper will review investigations on how our management system failed and how this might be prevented in the future. The rapid increase in disease occurred concurrently with drastic changes in the flue-cured tobacco support program. A rapid decline in the number of allotment holders and average support price changed patterns of production. Farmers mechanized production and expanded production acreage across counties to form economically viable production units. Farm machinery was moved from farm to farm-violating principals of pathogen quarantine. Evidence suggests that mechanical flower removal and a reliance on mechanical leaf harvesting has selected for populations of *R. solanacearum* that can be moved mechanically. The scientific response to these challenges will be discussed along with the formation of a revised management system.

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Effects of sulphur and Thiobacillus inoculants on quality and quantity yields in Virginia tobacco (var. Coker 347).

This study was performed in RCBD with ten treatments and three replications for two years (2004-2005) in Tirtash Research and Education Center, Iran, to evaluate the effects of different amounts of sulphur and Thiobacillus inoculants with banding and broadcast methods on quality and quantity yields in Virginia Tobacco.

Treatments were:

T1: Check treatment - no applied sulphur (S) and Thiobacillus Inoculants (Thio).

T2: No applied S and 5 kg Thio/ha (Banding). T3: 250 kg S/ha + 5 kg Thio/ha (Banding).

T4: 250 kg S/ha + 5 kg Thio/ha (Broadcast). T5: 500 kg S/ha + 10 kg Thio/ha (Banding).

T6: 500 kg S/ha + 10 kg Thio/ha (Broadcast). T7: 750 kg S/ha + 15 kg Thio/ha (Banding).

T8: 750 kg S/ha + 15 kg Thio/ha (Broadcast). T9: 1000 kg S/ha + 20 kg Thio/ha (Banding).

T10: 1000 kg S/ha + 20 kg Thio/ha (Broadcast).

The amount of Thiobacillus inoculants applied equal to 500 gr. per 25 kg of sulphur. In T2 was supposed that 250 kg sulphur is in one hectare of soil.

The analysis of variance results in 2004 showed that none of the treatments had significant effects in %5 level on the aforesaid factors. But, in 2005 results showed that the average price per kg in %1 level and income per hectare in %5 level was significant between treatments. The highest average price per kg and income per hectare was obtained for T7.

The results of combined analysis of variance for two years showed that none of the treatments had significant effects in %5 level on the measured factors. But, green and dry yields, the average price per kg and income factors in T7 than T1 were increased %4.8, %4.7, %6 and %10.3 respectively. Also, banding method in all measured factors was better than broadcast method. Therefore, it was recommended to apply 750 kg S/ha + 15 kg Thio/ha with banding method.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP28

A method for rapid determination of black shank races of tobacco.

Race determination of black shank, caused by *Phytophthora parasitica* var *nicotianae*, has become very important for farmers, to manage the disease, and breeders, to develop new resistant varieties. This determination is time consuming and requires the use of large amount of space in the greenhouse or in the field. A method for black shank race determination that significantly reduces the time and space used was developed. Six tobacco cultivars with different levels of resistance to black shank races were grown on a 12-cells falcon tissue culture (TC) plates. Each cell was filled with 3.5 cc of perlite (<2 mm), 1.5 ml deionized water, and 5 to 10 seeds (1 cultivar per cell). Plates were then incubated under 12-h dark/light alternation at room temperature. Seedlings were fertilized with a 200 ppm N solution 10 days after seeding and every week after it. After 25 days, seedlings were inoculated with a piece of *Catharanthus roseus* (syn. *Vinca rosea*) stem colonized with *P. parasitica* per cell. After inoculation, TC plates were incubated in a growth chamber at 27.5 °C /13 h light and 23 °C/11 h dark. The method was compared to the standard root inoculation method in the greenhouse used to determine black shank races. Disease incidence was evaluated at 7 and 14 days after inoculation in the TP plates and the greenhouse. The TC method was as consistent as the greenhouse method in determining different races. Also it required less time (approximately 40 days comparing to 60 days) and space than the greenhouse method.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. PPOST08

Host plant resistance management strategies for the control of black root rot disease.

Black Root Rot (BRR) caused by *Chalara elegans* Nag Raj and Kendrick [*Thielaviopsis basicola* (Berk. & Broome) Ferraris], can be a serious root disease problem in the flue-cured tobacco growing areas of Ontario. The disease, characterized by black lesions on the root, causes stunting, late maturation, and an uneven stand, all of which reduce the yield and quality of tobacco. Yield losses in southwestern Ontario from BRR damage and a lack of widely adapted alternatives have increased the dependency of growers upon soil fumigation as a control procedure.

Two flue-cured tobacco varieties (AC Gayed and CT681) with a high level of monogenic BRR resistance derived from *N. debneyi* have been used in the past for the control of BRR disease in Ontario. However, these inbred lines are not widely accepted by the growers because of the association of negative leaf quality traits with this type of resistance. This could be the result of linked deleterious genes carried over with the resistance gene during introgression. Data from 2005 trials comparing AC Gayed and CT681 with a newly developed F1 hybrid, CTH2, showed that all three strains are immune to BRR. Yield of CTH2 was similar to CT681, however the grade index and leaf quality traits of CTH2 were superior to AC Gayed and CT681.

This clearly suggests that when the gene conferring resistance to BRR disease is in heterozygous condition, as in the case of CTH2, its impact on leaf quality traits is less significant.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST20

Investigation of genetic diversity and classification of different Oriental tobacco varieties.

In order to study genetic diversity and classification of different Oriental tobacco varieties, 36 genotypes were investigated with a 6×6 simple lattice design in Tirtash Education and Research Center in 2004. In this study 19 chemical, morphological and agronomic traits were evaluated. The results showed that there are significant differences among genotypes for all traits. The highest and the lowest genotypic and phenotypic variation coefficient was related to the percent nicotine and percent protein nitrogen respectively. Broad sense heritability was the highest for days to flowering and plant height and the lowest for percent phosphorus. Cluster analysis based on Ward's minimum variance showed that genotypes were classified into five groups. 8, 12, 2, 7 and 7 varieties were located in clusters 1, 2, 3, 4, and 5 respectively.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST06

New varieties of tobacco of the famous origin "Krumovgrad".

Bulgaria is a country with traditions in the production of high-quality tobacco of Oriental type. The increased interest to the tobacco of origin "Krumovgrad" gives grounds for the purpose of this study - creating new varieties with complex improved characteristics.

This purpose was achieved with the new varieties - "Krumovgrad 17" and "Krumovgrad 944".

METHODS USED - inter-variety hybridization and continuous individual selection.

RESULTS - two new varieties of Oriental tobacco were created - "Krumovgrad 17", "Krumovgrad 944" with the following advantages:

1. High quality first grade according to the Bulgarian State Standard 30-40%, with highly reduced share of third-low grade - 4-7%.
2. High productivity - 210-220 kg/decare average yield.
3. Resistance against premature flowering, overripening and burning the leaves at the conditions of soil and air dryness.
4. Strong, resistible to lodging stems in case of rain and wind, which is favourable for rhythmical of leaves harvesting and not worsening the production quality.
5. Small leaves 38-40 in number, with sizes lower belt 19-21 cm / 11-13 cm; middle belt 22-24 cm / 13-15 cm, upper belt 13-15 cm / 9-10 cm.
6. High quality of drying without damages of the leaves, with even colouring of the whole leaf blade, from golden-yellow to orange.
7. Full of matter and elastic tissue, aroma-typical for origin Krumovgrad
8. Well-balanced chemical composition - nicotine - 1.4-1.6%; soluble carbohydrates - 12-15%; albumen nitrogen - 6-8%.

The new varieties are accepted very well by growers and tobacco trade companies and are quickly introduced into wide production.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP35

Temperature conditions affecting winter survival of the cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae).

The cigarette beetle is a cosmopolitan pest of tropical origin and is not fully adapted to temperate climates. Several studies have described winter extinction of this pest under constant laboratory conditions, but studies under natural winter conditions have been limited. In this study, we examined winter survival of the last instar larvae in three tobacco warehouses and three sheds at five locations in Japan to determine critical temperature conditions for eradication under natural situations in locations where temperatures fluctuate seasonally and diurnally. In the tobacco warehouses, where mean diurnal temperature variations were less than 1 °C, the combined exposure to temperatures lower than 7 °C for 9-10 weeks and lower than 6 °C for 1-2 weeks inside tobacco cases was found to be required for eradication. Temperatures inside tobacco cases approximated the 7-day moving averages of outside temperatures but were about 1 °C higher. To disinfest the larvae living inside, tobacco cases should be exposed to outside temperatures lower than 6 °C for 7-8 weeks and 5 °C for 3-4 weeks. Near the critical conditions, larger diurnal temperature ranges resulted in higher winter survival rates. This result suggests that the larvae may survive even in areas cold enough to normally cause eradication provided that daytime temperatures rise sufficiently. The information obtained has been practically considered when locating new tobacco warehouses so as to reduce the infestation of this pest without chemical control.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP19

The role of chimeric plants in the stability of nicotine to nornicotine conversion in Burley tobacco.

The presence of converter plants that demethylate nicotine to nornicotine in tobacco (*Nicotiana tabacum* L.) is undesirable. With the current state of knowledge, this trait is very unpredictable in Burley tobacco. This study evaluated the genetic effect of unstable plants on subsequent generations, and investigated possible reasons for this instability.

Progeny of selected parental plants were grown in the field and one leaf per plant was sampled, treated with ethylene to maximize conversion, cured and analyzed for nicotine and nornicotine. These parental plants were chosen from four cultivars; L8, TN90, KY9, and VA509, and included non-converters as well as low, intermediate and high converters. On the basis of the initial data, further sampling was done on selected lines and plants. Eight leaves per plant, at evenly spaced intervals, were sampled from plants in lines which were very variable or very uniform, and from atypical plants in lines which were otherwise relatively uniform, both converters and non-converters.

In lines that were uniform, at either high or low levels, the multiple leaves were generally similar in conversion level. In lines that were highly variable, and in plants that were atypical for their line, the leaves on any one plant were often very different. Such plants would often be classified as converters on the basis of one leaf, and as non-converters on the basis of another. This variation in conversion across leaf position suggests the presence of chimeras in some plants, as proposed by E.A. Wernsman.

There is considerable variation between varieties in their propensity for instability and for chimeric plants. Those varieties that are most unstable tend to produce more chimeric plants, while those that are most stable produce few, if any, chimeric plants. This variation is apparent in subsequent generations; conversion is lower and more uniform in L8 (stable, non-chimeric) than in VA509 (unstable, chimeric).

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP36

Effect of arbuscular mycorrhizae on cadmium uptake by tobacco (*Nicotiana tabacum*).

Cadmium (Cd) uptake by tobacco (*Nicotiana tabacum* L.) varieties Basma BEK, K326 and TN90 inoculated with five arbuscular mycorrhizae (AM) isolates was studied in a pot experiment. Despite low levels of root colonisation, AM symbiosis affected Cd accumulation in tobacco. Cadmium concentration in leaves was increased by inoculation with most AM isolates in the Basma BEK variety grown in a soil with low Cd

availability. In contrast, Cd concentration in leaves was decreased by inoculation with selected AM isolates in K326 and TN90 grown in soils with higher Cd availability. Furthermore, leaves had significantly higher concentrations of phosphorus, nitrogen and several other essential elements in treatments where Cd was most affected. Leaf biomass was not increased. One AM isolate was identified as the most efficient in improving mineral nutrition and influencing Cd concentrations in all the three combinations of variety and soil. AM symbiosis probably affected mineral nutrition and Cd uptake of tobacco by indirect mechanisms such as stimulation of root growth or mycorrhizal plant mediated changes in chemical or biological soil properties. To evidence potential inhibitory effects of the nutrient solution on mycorrhization, the applied fertilizer was tested against a slow-release fertilizer in K326 and TN90 with selected AM strains. Again, low levels of mycorrhization were found. When compared to the corresponding controls, only some AM strains had an impact on Cd concentrations. Significant differences in Cd concentrations were found between the two fertilization treatments, especially for the K326 × soil combination.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. PPOST06

Potential of *Pseudomonas putida* JB-1 producing heat-stable protenaceous materials in controlling tobacco mosaic virus.

Out of various fungi and bacteria tested for inhibition of tobacco mosaic virus (TMV) infection using *Nicotiana tabacum* cv. Xanthi-nc, a local lesion host, a bacterial isolate JB-1, identified as *Pseudomonas putida* based on Biolog and 16S rDNA sequencing analyses, had a strong inhibitory activity of TMV. Almost no lesion was formed by the direct application of the culture of *P. putida* JB-1 to the tobacco leaf prior to TMV inoculation, and also its systemic activity by its applying to the opposite side of the inoculating leaf or to rhizosphere was noted at around a half level of control efficacy. It reduced disease severity in the susceptible tobacco, *N. tabacum* cv. NC 82 possibly by reducing TMV infection on the inoculated leaf (resulting in movement of few TMV particles to other non-inoculated leaves). More than 70% control efficacy of TMV infection was obtained when the bacterial culture was treated after 3 days before TMV inoculation. TMV-inhibitory material(s) from JB-1 were revealed to be water-soluble, protenaceous, and especially heat-stable in a series of biological tests. These materials seemed not to directly combine with virus particles to hinder their infection processes. However, electron microscopy revealed unknown granule-like materials from the culture supernatant attached to TMV particles. All of these aspects suggest that *P. putida* JB-1 may be used as a potential agent for the control of TMV especially when transplanting tobacco seedlings to main fields.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP15

The role of integrated nematode management in developing best management practices for tobacco.

Plant parasitic nematodes remain important tobacco pathogens, despite significant progress in developing integrated systems for their management. Cultural practices have been identified that reduce nematode population densities at transplanting and the Rk gene for resistance to races 1 and 3 of *Meloidogyne incognita* has been incorporated into many flue-cured tobacco cultivars grown throughout the world. Cultivars resistant to cyst nematodes (*Globodera tabacum*) are also available. Entries combining the Rk gene with resistance to *M. arenaria* and *M. javanica* (and sometimes *G. tabacum* as well) are currently being evaluated for improved yield, quality, and other traits of interest. In regions where the environment is only marginally favorable for nematode reproduction, resistant cultivars can be used with cultural practices such

as crop rotation to limit nematode damage without using a nematicide. However, nematicide use remains common where plant parasitic nematode populations are widely distributed, cultural practices for nematode management are generally ineffective, impractical, or not economically feasible, and nematode-resistant cultivars possessing other desirable traits are not available. The declining number of effective nematicides available threatens the productivity of tobacco culture in these areas. Meaningful progress in improving use of nematode assay and advisory services use has been difficult, but new molecular approaches, especially to rapidly identify and differentiate root-knot nematode species, may significantly improve their use. Identification of reliably effective and practical biocontrol alternatives for tobacco nematode control has also been slow, but new approaches are being developed, many in other crops, that may yet prove effective. Induced resistance has proven a valuable tool for control of other tobacco diseases, but not, as yet, for tobacco nematodes. Research to improve nematode management systems for tobacco is continuing so that best management practices can be implemented as quickly and widely as possible.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP03

Detection of QTLs linked to leaf and smoke properties in *Nicotiana tabacum*.

Improving tobacco agronomic and chemical properties is a complex goal for breeders, as development of genetic markers and mapping strategy are hampered by the lack of polymorphism within *Nicotiana tabacum*. A starting point for discovering Quantitative Trait Loci (QTLs) linked to leaf and smoke properties is presented. A Recombinant Inbred Line population was developed from an intraspecific cross between two flue-cured *Nicotiana tabacum* L. inbred lines with unrelated breeding origins. A total of 59 traits, related to diverse agronomic, leaf quality, chemical composition and smoke properties were assessed. Near Infrared Reflectance Spectroscopy (NIRS) analyses of leaf lamina powders and mechanical smoking under a standard ISO regime were used to estimate chemical traits and smoke mutagenicity of the main smoke stream. The 114 RILs were screened for 184 AFLP (Amplified Fragment Length Polymorphism), ISSR (Inter Simple Sequence Repeat), SSAP (Sequence Specific Amplified Polymorphism), and SCAR (Sequence Characterized Amplified Region) markers. Loci involved in resistance to PVY^N (Potato Virus Y necrotic strain) and black root rot (*Chalara elegans*) were also mapped. Out of all tested markers, 75% were mapped on a partial genetic map including 18 linkage groups. Substantial segregation distortion (47%) was observed in linkage groups throughout the genome. A total of 75 significant QTLs were detected for 52 traits by simple and composite interval mapping, with 8 to 41.5% of the variation in the examined traits. Nineteen QTLs had opposite effects to those expected from the ranking of parental means.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST09

Growth, maturation and senescence of Oriental tobacco leaves as affected by ethephon and inhibitors of ethylene biosynthesis.

Ethylene is a gaseous plant growth regulator that it has long been known to promote fruit ripening and senescence of plant tissues. In contrast, aminoethoxyvinylglycine (AVG) and aminoxyacetic acid (AOA) both inhibitors of ethylene biosynthesis are responsible for delaying maturity and ripeness in several fruits. Ethephon (2-chloroethylphosphonic acid) an ethylene releasing agent can be applied as a spray field treatment to plants and plant parts. A field experiment was established during 2002 growing season in order to determine the effect of ethephon, AVG and AOA on leaf growth, ethylene evolution, respiratory rate, maturation and senescence processes of leaves of Oriental tobacco type (Basma, cv. BZ 7). Immature leaves of tobacco plants before the completion of their expansion were sprayed with 100 ppm ethephon, 50 ppm AVG, 0.5 mM AOA or water (control). Six sequential samplings of leaves at the accurately same age and position on the stalk were carried out at 2-day intervals after the application. All samplings were replicated 4

times. Leaf samples were analyzed for chlorophyll, total carotenoids and total proteins. Ethephon application caused inhibition in dry matter accumulation of immature leaves and significant reduction in the relative growth rate (RGR), in the ratio fresh/dry weight and in the relative water content (RWC), but increased the ethylene and CO₂ evolution, just 2 days after its application and throughout the whole period of samplings. In contrast to ethephon, AVG had the opposite effects on most of the characteristics recorded, but in a much weaker way, whereas the effect of AOA was not significant. In addition, chlorophyll degradation was more rapid in ethephon treated leaves than in AVG, AOA and control ones during the whole period of samplings. Total proteins and total carotenoids were significantly decreased in ethephon treated leaves, but were increased in AVG treatments in comparison to those of AOA and control. We conclude that ethephon promoted whereas AVG delayed the maturation and senescence processes and both caused significant modifications in chemical characteristics of leaves.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP16

Reduction of TSNA in Burley tobacco leaves using bacteria.

In air-cured tobacco, it is believed that the nitrite formed by microbial reduction of nitrate during curing reacts with tobacco alkaloids to form TSNA. It was considered that TSNA formation could be suppressed by decreasing nitrite in the leaves using denitrifying bacteria, which can reduce nitrite to nitrogen, and that TSNA existing in the cured leaves could be decreased using TSNA-decomposing bacteria. Using two types of bacteria obtained from tobacco plants, the effect of these bacteria on TSNA content was investigated.

Burley tobacco leaves were sprayed with an isolate of *Agrobacterium radiobacter* LG77, which was one of the denitrifying bacteria, three times, immediately after harvest, 3 and 8 days after harvest, and cured. As a control, the leaves without bacterial treatment were cured, too. In comparison with the nitrite nitrogen and TSNA (3.30 and 2.33 µg/g respectively) in the cured leaves of the control, these formations in the LG77-treated leaves were more suppressed (2.09 and 1.71 µg/g respectively).

Next, Burley cured leaves were sprayed with an isolate of *Pseudomonas fluorescens* LG38, which could decompose 4 types of TSNA (NNN, NAT, NAB and NNK), and stored at 30 °C, 70%RH and 80%RH. After 3 months' storage, TSNA increased by approximately 1.8 and 1.3 times in the non-bacterial treated lamina stored at 70 and 80%RH, compared with that at the beginning of the storage. The TSNA levels in the LG38-treated lamina were almost equal to and 0.9 times those at the beginning of the storage.

It was considered that using denitrifying and TSNA-decomposing bacteria were effective to decrease TSNA.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. PPOST09

Early diagnosis of *Peronospora tabacina* Adam in tobacco plants with systemic infection.

A disease characterised by the absence of sporulation on leaves and evident necrosis underneath the bark tissues, affected adult tobacco plants during 2005 in South Italy. In the field, diagnosis is based mainly on recognition of symptoms, often non-specific, that could result in the application of useless control strategy. Two current diagnostic methods based on microscopic observations were compared with polymerase chain reaction (PCR) technique. DNA extraction protocol was modified by replacing sonication with a further lysis step at 60 °C and PCR was performed using primers that amplified a specific DNA fragment (230 bp). Microscopy methods were able to detect the pathogen in 50% and 6% of cases, respectively, while PCR was able to detect *P. tabacina* in all diseased plants. Elimination of sonication in DNA extraction rendered the method easier to perform and avoided the possibility of DNA fragmentation. The results demonstrated that PCR makes the diagnosis fast and reliable while microscopy gives discontinuous results, is time consuming and not applicable for monitoring.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST04

Estimation of nitrous oxide emissions from the tobacco soils in Cuba in the harvests 2002-2003, 2003-2004, 2004-2005.

The preliminary estimate of the direct and indirect emissions of nitrous oxide (N₂O) from the agricultural soils dedicated to the tobacco crop in Cuba are presented in the harvests 2002-2003, 2003-2004, 2004-2005, for the main Cuban tobacco zones, as well as the analysis of quality and quantifying uncertainties. The methodology used was that proposed by the IPCC for the estimation of direct and indirect N₂O emissions from agricultural soils, excluding those non-representative terms of the agricultural practices of the tobacco crop in Cuba. The uncertainty analysis of the estimate preliminary obtained was carried out using the qualitative methods EMEP/CORINAIR 1996, Oliver 2002, the experts' opinions and the error propagation equation (level method 1).

A work tool was obtained that allowed the estimation of direct and indirect N₂O emissions from the Cuban tobacco soils in the study harvests.

The major estimate belongs to the emissions induced by the application of synthetic fertilizers with a value average among the three campaigns of 0.0768 Gg, followed by leaching with an average of 0.05119 Gg, atmospheric deposition with 0.0068 Gg and lastly those induced by incorporation of residuals of the tobacco crops with 0.0007 Gg.

The estimation of direct N₂O emissions was higher than those obtained for the indirect emissions.

The estimates obtained were compared with those reported in the Cuban National Greenhouse Gas Inventories (INGEI) of the year 2000. The result is that the four studied source categories do not constitute an important source in the national emissions of this gas from the agricultural soils.

It was noted that within the main tobacco zones of Cuba, Pinar del Río was the major contributor to the emissions of this gas in the four studied source categories.

A relationship was observed between the N₂O emissions induced by the application of synthetic fertilizers and the amount of fertilizer applied and the total sowed area.

The value of uncertainty for the harvests 2003-2004 was 50.2% and for the harvest 2004-2005 it was 50.3%, taking in both cases the harvest 2002-2003 as base year. The quality and uncertainty of the estimate obtained is within the established parameters at the international level for this gas.

With this preliminary study we can conclude that the tobacco crops in Cuba do not generate important negative impacts to the environmental deterioration from the N₂O rates, one of the greenhouse gases contributing to climatic change.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP04

Analysis of an introgressed *N. tomentosae* QTL affecting leaf number and correlated traits in *N. tabacum* L.

Utilization of germplasm from certain relatives of *N. tabacum* could be of value for improving quantitative traits in cultivated tobacco. Also, introgression of germplasm from closely related species (those from sections *Tomentosae* and *Sylvestres*) might facilitate mapping of genes underlying quantitative traits. To investigate these possibilities, we examined near isogenic tobacco lines (NILs) differing for an introgressed chromosome segment derived from *N. tomentosae* which carries a quantitative trait locus (QTL) that exhibits a large influence on leaf number and correlated traits. Within a 'Red Russian' genetic background, the QTL was found to act in an additive to partially dominant fashion with respect to leaf number, flowering time, plant height, and green leaf yield. The effect of the QTL in diverse genetic backgrounds was investigated through field experiments that evaluated F₁ hybrids between the two Red Russian NILs and each of six tobacco lines varying widely for plant type, leaf number, flowering time, and yield. The introgressed QTL affected all of these traits, but to varying degrees in the different backgrounds. The degree of recombination within the introgressed *N. tomentosae* segment was then investigated. Fifteen AFLP markers were identified that differentiated the two NILs. These were used to genotype 207 field-grown BC₁F₁ individuals

segregating for the introgressed QTL. All markers were found to be located within a single linkage group. All BC₁F₁ plants were phenotyped for leaf number, flowering time, plant height, green leaf yield, leaf length, and leaf width. Significant associations between all markers and measured traits were found. Sufficient recombination within the region allowed for mapping the chromosome position of the alien gene(s) influencing the measured traits. Introgression of germplasm from diploid species closely related to *N. tabacum* may contribute useful genetic variation and facilitate mapping of genes controlling quantitative traits in *Nicotiana*.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP20

Reducing TSNA in air-cured tobaccos by controlled environment curing.

Tobacco specific nitrosamine (TSNA) concentration in air-cured tobaccos varies from year-to-year, largely due to differences in the curing environments. High nitrate concentrations in the leaf may be reduced to nitrite by microbial activity under certain air-curing conditions (particularly high humidity) beginning at the time the leaf turns from a yellow to a brown color. The nitrite and secondary alkaloids then may combine to form TSNA during the coloring phase (Wahlberg, 1999). Because ambient curing conditions change from day-to-day and year-to-year, there is reason to believe that relatively consistent chemistry levels can only be achieved by air-curing in controlled environments. A computer-controlled curing schedule that permits precise control of temperature and RH during a 25 - 28 day air-curing period produced dark tobaccos with TSNA levels significantly lower than the shed-cured check, and acceptable physical quality. Controlling only RH during curing resulted in higher TSNA levels, but acceptable physical quality. Burley air-cured under similar conditions resulted in TSNA levels similar to the shed cured check, but the physical quality was slightly less desirable. Because Burley possesses a thinner leaf than dark tobacco, an adjustment in the RH schedule is probably warranted.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP21

The protection of tobacco varieties with the UPOV system in the EU.

In 1961, an International Convention for the Protection of New Varieties of Plant was adopted in Paris in order to protect them by an adapted intellectual property right. This text was gradually adopted in EU for some plant species, but tobacco was not included in the list then and the protection of new tobacco varieties was still based on plant patents.

Since 1996, all plant species are concerned by the International Convention and tobacco varieties can be protected by a system adapted to the plant kingdom, called "New plant variety certificate" (COV). Therefore, the use of patents is no longer possible in EU to protect tobacco varieties.

The COV gives the breeder an exclusive right of exploitation for his variety during a limited time, which allows him to recover his research investment. However, it remains possible for other breeders to use the protected variety for experimental or breeding purposes, which permits to keep a free access to genetic variability. The COV is delivered after a technical examination, following guidelines established by International Union for the Protection of New Varieties of Plant (UPOV) in 2002. If the variety is new, distinct, uniform, and stable and if it has an approved denomination, then it can be protected by a COV. The Certificate may only concern France or the protection may be extended to the whole European Community. The first COVs for commercial tobacco varieties have just been obtained in 2006, for two Burley varieties with low nicotine content, created at the Bergerac Tobacco Institute (Altadis).

Because they permit the identification of tobacco varieties, COV will entail a better traceability for the whole European tobacco industry.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. PPOST07

Evaluation of isolates of *Pseudomonas syringae pv tabaci* Tox+ for race determination.

Wildfire is a major disease affecting tobacco production in Malawi. It is caused by *Pseudomonas syringae pv tabaci*, a pathogen that is known to have several races throughout the world. However, it is not clear which races are predominant in Malawi, a factor that makes it difficult to have meaningful breeding programmes for wildfire resistant tobacco varieties. A trial was therefore conducted in 2002/2003 to 2004/2005 seasons at Kandiya Research station in Malawi to determine the races of wildfire (*Pseudomonas syringae pv tabaci* Tox+) available in the country. Wildfire isolates were collected from tobacco farmers throughout the country and inoculated on eight-week-old plants of four standard indicator cultivars grown in a screen house. The four cultivars used were: KE-1 (susceptible to all races), K35 (resistance to races 0 and 1) KM10 (resistance to race 0) and WZ (resistance to races 0 and 1). The trial was laid out in a Completely Randomized Design. A suspension of the wildfire isolate was inoculated on each indicator plant by spraying 25 mls on leaves of each plant that was bruised by clipping. The incidence and severity of wildfire was assessed at 7 and 14 days from inoculation. The results show that wildfire Race 1 is prevalent in Malawi while Races 0 and 2 are not. This means our wildfire chemical control programmes and breeding for resistance to wildfire in Malawi should target race 1.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. PPOST11

Impact of early-season thrips exclusion and suppression on the incidence of spotted wilt in flue-cured tobacco.

Replicated field trials were conducted in 2001-2005 to assess tomato spotted wilt tospovirus (TSW) incidence in flue-cured tobacco protected in early-season with thrips exclusion (cages) or thrips suppression (weekly acephate insecticide applications with or without an imidacloprid tray trench treatment). TSW is a serious economic threat to tobacco production in Georgia, USA, and is spread by tiny insects in the order Thysanoptera (thrips). There were fewer TSW symptomatic plants observed when plants were covered with thrips exclusion cages for 6 weeks following transplanting. Plant height, leaves per plant and total leaf weight per plant were similar for TSW symptomatic plants compared to non-symptomatic plants when the plants were caged for 6 weeks. This lack of plant growth response was primarily due to the late-season initiation of TSW symptoms (10-12 weeks after transplanting) once the cages were removed. Height, leaves and weight per plant were lower in the plants expressing TSW symptoms when the plants were either uncaged or caged 2 or 4 weeks after transplanting, due to the early onset of TSW symptoms (4-8 weeks after transplanting). Weekly acephate insecticide sprays for 2 or 4 weeks after transplanting reduced thrips populations for up to 5 weeks after transplanting while the 6 weekly sprays had lower thrips populations for up to 8 weeks. TSW was lower in both the 4 and 6 weekly acephate treatments than in the untreated. A tray drench application of imidacloprid also reduced thrips populations in early season plus lowered the percentage of TSW compared to no imidacloprid. The tobacco thrips, *Frankliniella fusca* (Hinds), was the predominate thrips species on tobacco foliage, although low numbers of other thrips were observed. The imidacloprid tray drench treatment and 6 weekly acephate foliar sprays also had lower densities of the tobacco-adapted form of *Myzus persicae* (Sulzer) aphids throughout the season.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST19

The effect of drought stress in different growth stages on quantitative and qualitative yields on Virginia tobacco.

To evaluate the effect of drought stress in different growth stages on quantitative and qualitative yields, of Virginia tobacco, the experiment was carried-out in CRD with 8 treatments and 3 replications in potty conditions for two years (2004-5). Three stages were: 1- until 30 days after transplanting, 2- from 31 days after transplanting to flowering and 3- from flowering to end of growth stage. Treatments were: (1) stress in three stages; (2) stress in stages 1 and 3; (3) stress in stages 2 and 3; (4) stress in stage 2; (5) non -stress in three stages; (6) stress in stages 1 and 2; (7) stress in stage 3, and (8) stress in stage 1. The results showed that there were significant differences on 1% level for yield, income, plant height and on the 5% level for length leaf, price of 1 kilo and sugar percent. The greatest and lowest yields belong to the 8th treatment with 92.55 g/plant and the 3rd treatment with 51.5 g/plant respectively. Second treatment with 1.51 g/litre had more water use efficiency. The results of combined analysis for two years showed that all factors affected by year except water use efficiency, income and price of 1 kilo. But the interaction effect between year and treatments showed that factors had same process in two years. According to results, irrigation during the first 30 days after transplanting when irrigation is not possible in other stages is not useful because irrigation in this period causes more growth of stem and less growth in roots. But non-irrigation in first 30 days after transplanting and irrigation after this time gives higher yield. As water deficiency at the first stage increases root growth yet limits stem growth, the plant can use water in other stages more efficiently.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST14

Tobacco float system transplant production and transplanting with new growing media and trays.

Tobacco float system transplant production (TFS-TP) is widely used for its clear advantages. However, handling and disposal of polystyrene foam trays after service have become major problems in all the producing countries, and therefore also a major research topic. In previous papers, a patented new growing medium (NGM), made of biodegradable polymer-agglomerated peat, was introduced and tested. On a weight basis, it consists of water (80%), peat (12-15%), and aggregating polymers (5-8%). Plug units of the NGM, lying on a plastic film, floating on a reusable polystyrene foam flat panel, were managed in the greenhouse in the same way as the standard trays of a TFS-TP system.

This paper shows the results of the tests carried out on this NGM at Fattoria Autonoma Tabacchi of Città di Castello (Perugia, Italy). A key-point, to make this TFS-TP process practically feasible, was the development of an innovative transplanter. In fact, root binding among adjacent plugs was found to be a major problem and this required to design *ex-novo* the conveying system of the transplanter. A mechanical prototype was developed, in which the plug units are set manually by an operator on the conveyor. The plastic films underneath are recovered for reuse. However, an electronic, fully automatic, transplanter is under development. The proposed TFS-TP system and transplanting process has an economic advantage over the traditional one.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. PPOST10

Evaluating phytotoxicity and efficacy of azoxystrobin applications on burley and flue-cured tobacco diseases in North Carolina.

Currently the most important foliar diseases of tobacco in North Carolina (NC) are target spot, caused by *Rhizoctonia solani*, and blue mold, caused by *Peronospora tabacina*. Reported problems caused by target spot have gradually increased in frequency; significant yield losses recorded in flue-cured tobacco during the 2004 and 2005 growing seasons. Blue mold is a continual problem every year in this region, and most burley tobacco varieties are highly susceptible to this disease. Availability of effective chemical treatments for preventing and managing these two diseases is crucial for production of tobacco in North Carolina. An

emergency exemption for the use of Quadris flowable fungicide, an azoxystrobin compound, was granted in NC for 2 weeks in 2005. Previous trials have shown that this product, when applied at 6, 9, or 12 fl oz/A, provided superior control of target spot on flue-cured tobacco, and moderate control of blue mold on burley tobacco. However, weather flecking has been reported with the use of this product on burley tobacco grown in the mountains of NC. In 2005 application of Quadris in flue cured tobacco in 2 locations did not demonstrate any weather flecking. Field trials will be conducted again during the 2006 growing season in multiple locations across the state with Quadris applications on both burley and flue-cured tobacco to document any phytotoxicity, as well as its efficacy against these two important foliar diseases.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST17

Comparison of quantitative and qualitative characteristics of Virginia flue-cured tobacco using new boxes and Iranian racks in bulk curing.

In Iran two methods are used for flue-curing, bulk curing barns using racks and conventional barns using strings to hang the leaves. The Iranian racks are most widely used in bulk curing. A survey has been done in order to compare Iranian racks with new boxes, for what regards the number of operators and labour cost, time of loading and unloading tobacco leaves, quantity, quality and grades, average market price, chemical compositions and organoleptic properties (flavour, taste, combustibility, impact and irritation) of tobacco cured with the two methods. This research was conducted for the first time at the Tirtash Tobacco Research and Education Center in Iran during 2003-2005. Tobacco leaves were harvested in four or five primings from the various stalk positions of cultivar Coker 347. Then new boxes and racks were filled by hand. 14 boxes (length 183 cm, width 60 cm, height 60 cm, weight 30 kg) and 46 racks (length 183 cm, width 20 cm, weight 5 kg) were placed in two bulk curing barns (length 475 cm, width 183 cm, height 195 cm), and leaves were cured with the same conditions during yellowing, colour fixing, leaf and stem drying stages. The results showed that new boxes reduced number of operators needed and labour cost, loading and unloading time of tobacco leaves by about one-half. But differences were not significant in nicotine, reducing sugars, protein, total N, average market price, quality and organoleptic properties. Boxes and racks were almost similar on the 1, 2 and 3 grades and percentage of variegated tobaccos. Average of wet and cured tobacco were 53 and 11 kg each new box and 17.3 and 4 kg each Iranian rack.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST05

Concentration ranges in typical nutrient deficiency for dark tobacco seedlings.

For some plant species the range of known visual symptoms due to deficiency, toxicity or imbalance of plant nutrients or other elements is very large, but for other is not so (Martin-Prével and Gagnard, 1987). The deficiency ranges for black tobacco is not defined in the literature, as far as to our knowledge. Cuba, the land of the international famous "Habanos" cigars, produces mainly black tobacco. Thus, the present experiment was done, in order to establish the adequate nutritional status for the diagnosis of deficiencies, using our own critical levels and images. Nutrient solution experiments were carried out under controlled conditions at the Institute of Plant Nutrition in the University of Hohenheim, Germany. Six treatments for individual nutrient deficiencies were established for the following elements: Nitrogen, Phosphorus, Potassium, Magnesium, Zinc and Boron. After the first visual symptoms appeared, two old (bottom) and two young (top) leaves were sampled. The ranges of critical values for various nutrients, in dark tobacco are different to the reported for the rest of tobacco types in literature. The appearances of visual symptoms in middle lower leaves instead of older, could be due to senescence process which had already started in oldest leaves. The use of old and young leaves from the same plant, for analysis is a better approach than a single leaf, to get information of the nutritional status of the plants.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP05

Plasma membrane microdomains and tobacco defense responses.

Plants are able to defend themselves against pathogens by triggering a wide range of mechanisms. We have been studying the molecular basis of these defense responses using tobacco, and a protein secreted by the oomycete *Phytophthora cryptogea*, able to trigger hypersensitive reaction and systemic acquired resistance on tobacco plants. Many of the results previously obtained on this model indicated that the plant plasma membrane plays a decisive role in the perception of the elicitor and in the set up of the signalling cascade leading to plant defense. A large body of evidence from the past decade supports the existence, in membranes of animal and yeast cells, of functional microdomains (also called "lipid rafts") that play important roles in protein sorting, signal transduction or infection by pathogens.

We showed that similar microdomains also exist in plant plasma membrane isolated from both tobacco leaves and BY2 cells. These microdomains were found to be greatly enriched in sphingolipids and sterols, as previously observed for animal lipid rafts. Membrane proteins were separated by gel electrophoresis and the profiles obtained clearly indicate that tobacco microdomains are able to recruit a specific set of membrane proteins and exclude others. We used mass spectrometry to give an extensive description of tobacco microdomains proteic composition and this led to the identification of 145 proteins whose functional characteristics were analysed in silico. This analysis indicated that if a primary function of the plasma membrane, such as transport, seems underrepresented in the microdomains, others undergo a significant increase of their relative importance. Among these are: signalling and response to biotic and abiotic stress, cellular trafficking and cell wall metabolism. This suggests that these domains are likely to constitute, as in animal cells, signalling platforms involved in these physiological functions and particularly in the set up of plant defense responses.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP06

Evaluation of alternative growth media for raising tobacco seedlings in the float tray system.

The use of Methyl Bromide in the production of tobacco seedlings has been replaced in Malawi by Basamid, Herbifume and hydroponics system using floating trays. The hydroponics system is the most environmentally acceptable but the cost of the growth media and floating trays is prohibitive as these are imported. In order to reduce the cost of media, a nursery experiment was initiated in 2002/2003 season at Kandiya Research Station in Malawi to evaluate the suitability of locally available materials as alternative growth media to the imported medium for raising tobacco seedlings in floating trays. Composted rice husks, groundnut shells, macadamia husks and pine bark were the growth media evaluated as pure treatments and in combination with sand in the ratio of 50:50 by volume. The eight treatments were evaluated in a randomized complete block design (RCBD) with four replications. Individual plots consisted of 3 trays of 200 cells per tray. The basal fertilizer of 20:10:20 was split applied at 10, 21 and 35 days after sowing. Top dressing with Calcium nitrate followed 42 days after sowing. All other cultural practices were similar to standard seedling production procedures. The various treatments had significant effects ($P=0.05$) on seedling height. Seedlings from pure groundnut shells and pine bark were comparable in performance and gave the tallest seedlings among all the treatments tested with average seedling height of 13.7 and 14.4 cm, respectively. The satisfactory performance of groundnut shells offers an opportunity for use in the floating tray system by many farmers as the shells are readily available in most of the tobacco growing areas of Malawi.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP27

Characterization of new sources of resistance to black shank (*Phytophthora parasitica* var. *nicotianae*).

Two main sources for black shank resistance exist within commercial U.S. flue-cured and Burley varieties. A partial non race-specific resistance from the cigar cultivar Florida 301 has been used extensively, and varying levels of this resistance exist. Complete resistance to race 0 has been transferred into tobacco from *Nicotiana plumbaginifolia* and *N. longiflora*, and is used in hybrid form in many varieties. Race 0 was the predominant race present in the U.S., but with the widespread deployment of the *Ph* gene from *N. plumbaginifolia*, the population of black shank has shifted so that race 1 is much more common. As a result, there is substantial interest in new sources of resistance against race 1. One possible source is a spontaneous form of black shank resistance arising from anther culture of black shank susceptible breeding lines (Nichols and Rufty, 1992). Although the level of resistance was moderately high, this resistance was never incorporated into a tobacco breeding program since the existing resistances were thought to be sufficient for black shank control. In greenhouse and field tests this resistance has been found to be a non race-specific, partially dominant resistance that is similar to K346 in the level of resistance, and the genetic control of this resistance is currently being characterized. In addition, an examination of the resistance available in a large *N. rustica* collection is being characterized in field and greenhouse tests. These tests have indicated that almost all of the *N. rustica* collection is highly resistant to both race 0 and race 1. Several accessions are currently being crossed to tobacco in order to determine if the resistance can be transferred. These materials may be useful in future breeding for black shank resistance.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST07

Genetic improvement of *Nicotiana tabacum* by applying remote hybridization and biotechnological methods.

Wild *Nicotiana* species possess a high potential of useful features. Interspecific hybridization is a very effective method for the introducing of desired characters into tobacco and achieving crop improvement. Its successful realization is limited due to various incompatibilities. Those barriers of incompatibility could be overcome by applying *in vitro* techniques (embryocultures for embryo rescue and tissue cultures in order to produce viable F₁ hybrid plants and to improve their fertility). We realized six interspecific crosses through routine hybridization technique. Wild species *N. sylvestris*, *N. plumbaginifolia*, *N. benthamiana*, *N. cavicola* and *N. gossei* were used as female and *N. tabacum* as male parents. Tobacco was used as a mother parent only in the combination with *N. sanderae*. Embryos from the crosses *N. plumbaginifolia* × *N. tabacum* and *N. tabacum* × *N. sanderae* were grown *in vitro* in order to produce viable F₁ plants. Morphological, cytological and biochemical examinations confirmed true hybridity of the obtained interspecific combinations. Male and female sterility and meiotic abnormalities were characteristic for all of them. It was overcome through *in vitro* growing of stem pit parenchyma for callus induction and organ formation. The regenerants (R) obtained after longer callus cultivation (3th-9th passages) were with 31.4%- 62.5% viable pollen and formed seeded capsules via self-pollination. They were tested for resistance to some diseases and pests. R₁ from the combinations *N. benthamiana* × *N. tabacum* and *N. gossei* × *N. tabacum* were shown to be resistant to aphids. These of *N. gossei* × *N. tabacum* were tolerant to powdery mildew and tobacco strain of TMV. F₁ regenerants of *N. cavicola* × *N. tabacum* were resistant to ToMV and these of *N. tabacum* × *N. sanderae* showed resistance toward TSWV. These plants are included in our breeding program.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST11

Analysis of the microbial community of fermenting cigar tobacco by culture-independent approaches.

Italian-style cigar production includes a step during which dark-fired tobacco leaves are moistened and fermented. The fermentation process is carried out by a wild microflora that deeply modifies the substrate. These modifications mainly consists in: 1) tobacco temperature increase, 2) pH shift from acidic to alkaline values, 3) decrease in reducing sugars and organic acids, 4) increase in nitrite and TSNA.

As previously reported, tobacco microbial ecology was studied by culture dependent methods and the occurrence of microbial groups such as yeasts, lactic acid bacteria, coryneforms and low G+C-content endospore-forming gram-positive bacilli was recorded.

Yet, for many years our understanding of microorganisms in the environment has been based primarily on those microorganisms that we could culture on media, and which represent between only 0.1 and 10% of the total microbial flora within any given environment.

Recent years have seen a revolution in microbial ecology, catalyzed by the application of molecular approaches to enable the previously uncultured and numerically dominant microbial groups to be studied. The use of these molecular tools allowed us to develop methods to isolate total DNA from tobacco fermentation environment and, by the subsequent application of polymerase chain reaction (PCR) approaches, to amplify microbial ribosomal genes. In particular, Single Strand Conformation Polymorphism (SSCP) and Denaturing Gradient Gel Electrophoresis (DGGE) were set up and utilized to study fermenting tobacco microflora.

Several microorganisms were identified, most of them already isolated in previously culture-dependant approach, while others represented uncultivable or not previously isolated new species.

These molecular techniques have proven to be very powerful and could be used to enlarge our knowledge of tobacco associated microflora.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP10

Changes in flue-cured tobacco fertilization practices in North Carolina.

Traditionally flue-cured tobacco growers have used blended fertilizers that consisted of N, P, and K in ratios of 1-3-3 to 1-1-3 as a base for their fertilizer program. Historically, sodium nitrate or calcium nitrate is applied one to two weeks later to supply the rest of the nitrogen needed. Years of using base fertilizers with high levels of phosphorous has led to fields with very high residual amounts of phosphorous in the soil. Because of the high amounts of phosphorous already in place growers can now use other fertilizer materials to produce their tobacco crop at a reduced cost.

Tests have been conducted in North Carolina since 2003 to determine: methods to reduce phosphorous application while maintaining early season growth, methods for applying potassium, and which sources and rates of nitrogen can a flue-cured producer use while reducing input costs, increasing application efficiency, and maintaining yield and leaf quality.

Test results prove that on a high P soil a grower can apply 8 kg/ha of P in the transplant water; increase early season growth, decrease days to flowering, and reduce P inputs from 45 to 8 kg/ha (83%). Tests have evaluated different blends of potassium sources applied by broadcasting prior to transplanting or banding at or just after transplanting. Results from these tests show no difference between methods of potassium application for yield or quality. However, it should be noted that potassium could be leached out of the root zone if broadcast prior to transplanting. Tests have also been conducted to evaluate different sources and rates of nitrogen. By using a liquid nitrogen source such as 30% UAN or a higher analysis granular fertilizer, growers can be more accurate and/or efficient in their application while saving money.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP12

Bacterial wilt in the southeastern USA and the scientific response during the early 20th century.

When bacterial wilt, caused by the soilborne pathogen *Ralstonia solanacearum*, first appeared in 1881 on several farms in Granville County, North Carolina, little did people realize the enormous and devastating consequences the disease would have on tobacco production and people's lives in the increasingly important bright leaf tobacco belt of central North Carolina. Over the next several decades, however, the ramifications of this dreadful disease became all too apparent as losses from bacterial wilt repeatedly reached 25 to 100%. By the 1920s, substantial economic hardship in Granville and nearby counties forced farmers to abandon tobacco and, in many cases, to abandon their farms and even their way of life. Land prices plummeted and standards of living collapsed. Because of the great importance of the disease to the agricultural economy of North Carolina, experiment station and USDA (United States Department of Agriculture) scientists rallied during the first half of the 20th century to find successful control measures. This paper will investigate the scientific response to bacterial wilt, focusing specifically on the control work of some of the leading scientists during the era including T.E. Smith, E.E. Clayton and A. Kelman. By exploring their research and the work of others, it will be shown how practices of crop rotation, chemical control and host resistance were developed successfully for tobacco growers by mid-century. In addition, this paper will examine how these practices were combined in a unique way and introduced to growers as the "systems control" concept, a best management strategy that proved highly effective for bacterial wilt control for almost half a century.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP02

Application of Near Infrared Reflectance Spectroscopy to tobacco plant breeding.

In order to select new tobacco varieties, ability of candidate lines to produce cured leaves with the desired chemical characteristics has to be assessed as early as possible in the breeding process. The high number of candidate lines renders necessary to use fast prediction tools instead of chemical analyses. For this purpose, Near Infrared Reflectance Spectroscopy (NIRS) allows estimating a number of chemical parameters, as well as more complex traits, directly from powder obtained by grinding cured tobacco leaves and this technique is now used routinely at the Bergerac Tobacco Institute.

For each tobacco type, *i.e.* dark air-cured, Burley and flue-cured, Modified PLS (Partial Least Squares) predictive models, connecting NIR spectral data to the alkaloid, nitrogen, reducing sugar, and ash content were first developed.

Models were set up from more than 1600 powder samples (crop years 1995 to 2000), including an important variability as far as spectral data and levels of chemical components are concerned. Calibration equations are based on a given population mainly at the Bergerac location. They show a high degree of performance for predicting classical chemical analyses results.

Validation was performed with samples from the same crop years and external validation is also performed each year.

A PLS discriminant equation allows the classification of each sample into a tobacco type according to its NIR spectrum and the predictions with the appropriate models.

More recently, other criteria were investigated and predictive models are now available for chemical compounds such as NH₃, chlorine, polyphenols and smoke parameters such as tar yield from ISO 4387 smoking test and the number of revertants / mg TPM determined by the Ames test.

Prospects for applications to field using a portable NIR spectrometer and for predictions from a whole tobacco leaf without grinding are also discussed.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP09

Effect of natural zeolite application and K₂O on yield and quality of tobacco (*Nicotiana tabacum* Var. Coker 347).

Many reports have described that natural zeolite have many useful properties, such as high Cation Exchange Capacity (CEC=1-4 meq / 100 gr), ability of absorption and emission of water, ability of ammonium absorption and inhibiting of nitrate leaching which is useful to soil amendment. In order to study the effects of zeolite and potassium fertilizer application on yield and quality of tobacco (Coker 347), a farm experiment was conducted in factorial with two factors and the basis of completely randomized block design (RCBD) with 15 factorial treatments and 3 replications for 2 years (2005, 2006). The A factor was 4 level of zeolite (1000, 2000, 3000, 4000 kg) and B factor was 3 level of K₂O (100, 200, 300 kg). Important plant characteristics including, plant height, number of leaf, stem diameter, flowering date, fresh and dry weight of leaf, leaf chlorophyll content, specific leaf area (SLA), yield price, reducing sugar and nicotine content, total nitrogen and potassium were measured. Factor analysis of variance for each year and analysis of variance for two factors combined data based on two years in same place with same randomization in each year was done on the basis of RCBD. Results showed that the main effects of zeolite on dry weight of leaf and yield price was significant and main effect of K₂O on reducing sugar and yield price was significant. Contradiction of Zeolite and K₂O on plant height, dry weight of leaf, yield price was significant. Contradiction of Zeolite and K₂O and year on dry weight of leaf and yield was significant. Mean comparison of main effect A on dry weight of leaf based on Duncans multiple range test showed that combined treatment number 7 (Z₃,K₁) with 2310 kg/h is better than other treatment. Investigation correlation between dry weight of leaf and other parts of yield showed that dry weight of leaf has strong relation ($r=0/77$) with (SLA).

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST23

Determination of maximum allowable depletion fraction in different stages of growth for Oriental tobacco, variety B.S.31.

Irrigation is an important component for the production of quality tobacco in unreliable rainfall condition of the West Azerbaijan, Iran. Different crops respond in a specific manner to the deficit of water depending on the crop development stage. The portion of the available soil water that is easily absorbed by plant and usually leads to maximum yield is defined as "Maximum Allowable Depletion" fraction and is shown as MAD. This is an important parameter for determination the irrigation depth and frequency in irrigation scheduling that generally is more creditable than other parameter (such as associative evaporation from flat and leaf temperature) in irrigation scheduling. In order to determine MAD in two growth stages of Oriental tobacco (variety B.S.31), an experiment was conducted with a split plot design and four replication in Urmia Tobacco Research Center for two growing season (2004 and 2005). The main plots and subplots allocated to two levels of MAD fraction in the harvest stage (B1=0.6 and B2=0.8) and three levels of MAD fraction in the rapid stage (A1=0.4, A2=0.6 and A3=0.8), respectively. The soil moisture content was determined from starting rapid growth stage with gypsum blocks that had been buried in soil at 25 cm depth. When the soil moisture was reached to specific levels in different treatments, the irrigation started immediately.

The results showed that MAD levels in rapid growth stage had significant effects on fresh leaf and usable dry leaf yield that were maximum in A1. Also, this levels of MAD in rapid growth stage had significant effects on leaf quality and gross income of tobacco farming that was maximum in A2, mainly because of high quality. In harvest stage, MAD levels had significant effect on total, usable dry leaf yield and water use efficiency that were maximum in B1, but the quality of dry leaf in B1 was lower than that in B2. Heavy applications tended to affect quality adversely. MAD fraction for rapid growth and harvest stages were determined as 0.6 and 0.8, respectively. Crop water requirement content was measured 400 mm. Irrigation frequency for rapid growth and harvest stage was measured on the average 9 (8 to 10 days) and 11 days (10 to 12 days), respectively.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP17

Role of nitrogen oxides in tobacco-specific nitrosamine formation in Burley tobacco.

It was reported that tobacco specific nitrosamines (TSNA) were formed when neat alkaloids were exposed to high-level (500 or 1000-ppm) nitrogen oxides (NOx) for one hour^[1].

On the other hand, it was reported that the concentration of NOx generated from Burley tobacco was low-level (0.7 to 20-ppm^[2] or 1.8 to 8.3-ppm^[3]) when it was stored in a sealed container.

To determine the influence of low-level NOx generated from cured leaves on TSNA formation, we exposed neat alkaloids and Burley tobacco to low-level nitric oxide (NO) gas.

NNN and NNK or only NNN were formed when nicotine or nornicotine was exposed to 23-ppm NO gas at 30 °C for three days.

The ground or cut lamina of a nornicotine converter and a non-converter was exposed to 3 to 23-ppm NO gas at 10, 30 or 60 °C for one week. TSNA formation in the converter was less than 1 µg/g at 10 °C, 0.4 to 5.5 µg/g at 30 °C and 5 to 28 µg/g at 60 °C. The TSNA formation in the non-converter was less than that in the converter.

These results made it clear that TSNA were formed when Burley tobacco was exposed to the low-level NOx generated from itself stored in sealed conditions.

It was also found that the increase of TSNA during NO treatment could be expressed by a function formula with NO concentration and temperature as parameters.

[1] T.B. Nestor *et al.*, Beitr. Tabakforsch. Int. 20, 467-475, 2003

[2] H. Saito *et al.*, 57th TSRC, 2003

[3] S. Katsuya *et al.*, CORESTA AP Meeting, 2005

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. PPOST05

Efficiency of a pheromone trap "GACHON" for the tobacco moth.

The tobacco moth, *Ephestia elutella* (Lepidoptera: Pyralidae) and the tobacco beetle, *Lasioderma serricorne* (Coleoptera: Anobiidae) are the most serious pests infesting stored tobacco leaves. In tobacco companies, therefore, monitoring of the both insects is very important to find out their early occurrences, their source of infestation and seasonal prevalence under Integrated Pest Management (IPM). Pheromone traps for the both insects have been used to monitor the insects in tobacco factories and warehouses.

When the pheromone traps are used in factories and warehouses, their efficacy is necessary to determine distance among the traps, census interval and their replacement intervals. Furthermore, the trap efficacy is affected by period after their production and/or conditions during their storages. Detail efficacy of the pheromone trap of "NEW SERRICO" for the tobacco beetle was presented at 2002 CORESTA CONGRESS in New Orleans by Shinoda *et al.*

In the present study, therefore, we show the detail efficacy of a pheromone trap, "GACHON" for the tobacco moth. The detail efficacy includes capturing efficiency, shelf life, available distance and storage conditions and duration. On the basis of the efficacy, we show some recommendations to get useful information for IPM by monitoring.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP29

Identification and quantification of high-risk site characteristics associated with the occurrence of tobacco black shank and establishment of *Phytophthora nicotianae* race 1.

Races 0 and 1 of the black shank pathogen, *Phytophthora nicotianae*, occur throughout the flue-cured and Burley tobacco production areas of North Carolina (NC). Recent studies documented the rapid occurrence of pathogen race shifts from race 0 to race 1 in response to deployment of cultivars that contain the highly effective Ph gene from *Nicotiana glauca*. Although race 1 has been reported from most areas in NC, the establishment of this race occurred much more rapidly in some growing areas than others. Severity

of black shank is greatest in soils that have pH values >6.0 and are high in basic cations. The objective of this study is to further quantify the factors that are most conducive to black shank development and the establishment of race 1 by conducting an intensive survey of the Burley and flue-cured tobacco growing regions in NC. Starting in 2006, isolates of *P. nicotianae* will be collected to characterize the race structure of field populations in >100 fields across the state for a three year period. Pathogen population race profiles will be correlated with supplementary crop and field-site information. Based on results obtained, strategies for more effective disease management will be tailored to the various regions in the state.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP13

Monitoring *Peronospora tabacina* populations for sensitivity to mefenoxam and dimethomorph in North Carolina, a review through 2005.

Availability of effective chemical treatments for preventing and managing blue mold, caused by *Peronospora tabacina*, is crucial for production of tobacco in North Carolina. Mefenoxam was used effectively to manage blue mold between 1980 and the mid 1990's. Studies with the new chemistry fungicide dimethomorph began in 1988 and provided data on efficacy and mode of action against the pathogen. Isolates of *P. tabacina* have been collected from tobacco fields in North Carolina each year blue mold has occurred and tested for sensitivity to mefenoxam at 1.0, 10, and 100 µg/ml and to dimethomorph at 1.0 and 10 µg/ml. Over the years, isolate sensitivity to mefenoxam has shifted from sensitive at 1.0 µg/ml prior to 1994 to insensitive at 100 µg/ml between 1995 and 2005. However, isolates have remained sensitive to dimethomorph at 1.0 µg/ml from the time of its introduction through 2005. Implications of these results will be discussed in relation to future blue mold management.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST18

Study of growing medium to replace peat in float system for seedling production.

In order to study growing medium to replace peat in float system seedling production resources that are available in Guilan province were used in an experiment and compared with peat. Results of this experiment can have much benefits to farmers and country in general. The experiment was conducted in randomized complete design with 12 treatments and 3 replications. Treatments were: (1) peat; (2) peat 50% + vermiculite 25% + field soil 25%; (3) Manure 50% + field soil 25% + sand 25%; (4) manure 50% + field soil 25% + vermiculite 25%; (5) Manure 75% + field soil 25%; (6) tree cortex compost 50% + field soil 25% + Manure 25%; (7) tree cortex compost 50% + vermiculite 25% + manure 25%; (8) tree cortex compost and tea residue 50% + vermiculite 25% + field soil 25%; (9) Manure 50%, Perlite 50%; (10) Perlite 50%, Azola 25%, manure 25%; (11) manure 50%, Azola 25%, tree cortex Compost 25%; (12) manure 50%, Azola 25%, tree cortex compost and tea residue 50%. Following properties were evaluated: percent and speed of germination, stem and root length, dry weight of stem and root, crown diameter. The best treatments that can replace peat, are 2, 4 and 9. Also, if seed has high vigour treatment, No. 9 is the best.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST12

Growth and yield response of furrow-irrigated Burley tobacco to deficit irrigation.

Irrigation plays a key role in determining yield and quality of Burley tobacco and it is often indispensable for commercial cultivation of this crop. In semi-arid tobacco growing areas of Southern Italy, the priority of irrigation is to save water since it is one of the major limiting factors for growth and yield. In 2005 a field experiment was conducted using furrow-irrigated Burley tobacco (cv. C104) to compare two deficit irrigation treatments (50% ET_c), imposed either using conventional deficit irrigation (DI50) or alternate row irrigation (ARI50), with a full irrigation treatment (100% ET_c, control). In the DI50 treatment plants received half amount of water uniformly applied to both sides of the row whereas in the ARI50 treatment one-half of the root zone was wetted while the other half was maintained dry, thereby the half amount of irrigation water was applied; in particular, the wetted and dry root zone were interchanged in two subsequent irrigations. Seedlings were transplanted at a 1.0 × 0.5 m distance on May 30 and fertilized with 120 kg ha⁻¹ of nitrogen. All plants were topped at flowering (first week of August), harvested on September 13 and air-cured in ventilated rooms. DI50 and ARI50 decreased yield by 31% and 10%, respectively, compared to the control. DI50 and ARI50 increased the irrigation water use efficiency (IWUE, kg cured leaves ha⁻¹ mm⁻¹ of irrigation water applied) by 39 and 79%, respectively, compared to the control treatment. The dry matter at commercial harvest of DI50 and ARI50 was 46 and 24% less, respectively, than that of the control. Considering both the maintenance of yield and dry matter and the high water use efficiency of ARI50 treatment, results of the present experiment indicated that ARI50 could be a feasible irrigation strategy.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP08

Algae and Pythium root rot control in float bed production system in Zimbabwe.

The float bed tobacco seedling production system has been widely adopted in Zimbabwe due to increasing international pressure to phase out the use of ozone depleting methyl bromide. However, algae and root rot caused by *Pythium* spp. are a major challenge in this production system in Zimbabwe. These two problems are attributed to the constantly moist environment providing extended periods of leaf wetness and promoting succulent plant tissues, which favour the growth of algae as well as the development and spread of fungal diseases. Reports indicate that various oxidising agents and fungicides applied into the float water can be used to manage these two problems. Therefore, an experiment was set up to evaluate a Quaternary Ammonium Compound (QAC), an oxidizing agent [potassium permanganate (KMnO₄)], copper oxychloride and two systemic fungicides [metalaxyl and probamocarb-HCl] for the management of the two problems. The systemic fungicides were also tested in combination with QAC, KMnO₄ and copper oxychloride. The QAC, KMnO₄ and copper oxychloride were applied in the float water at sowing while the systemic fungicides were applied at 5 - 6 weeks after sowing as a float water treatment. The trial was first done in a greenhouse and repeated in the seedbed. In both trials, none of the treatments had an adverse effect on seedling germination and survival. At the early stages of seedling growth, before the systemic fungicides could be applied, algae were controlled best by QAC and copper oxychloride. Results also indicate that QAC in combination with metalaxyl provides the best (P<0.05) algae control throughout the seedling growth period, while metalaxyl on its own and in all three combinations gives excellent (p<0.05) control of *Pythium* root rot.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP32

Research and education efforts to support expansion of Burley tobacco production into non-traditional areas in North Carolina.

From 1938 through 2004, the production of Burley, flue-cured and other tobacco types was limited to specific regions in the U.S.A. However, as a result of deregulation legislation adopted in October 2004, tobacco types can now be produced in any region of the country with a suitable production environment and purchasing infrastructure. In 2005, approximately 250 flue-cured producers in North Carolina produced

Burley for the first time. Based on successful production in 2005, it is expected that the number of producers will increase to 300 or more, and production will double to 2.7 to 3 million kg in 2006. The objectives of this research and extension effort are to: 1) Develop the research base needed to provide production recommendations, and 2) Mechanize harvest and stripping operations primarily through the adaptation of existing technology. Studies were initiated in 2005 and continued in 2006 to determine: optimum planting and harvest dates to maximize yield and quality, and minimize labor conflicts between flue-cured and Burley operations; proper nitrogen rates; impact of fatty alcohols on phytotoxicity; and the performance of automated and semi-automated cutting-notching harvest systems. In 2005, yield was nearly 25% less for Burley planted in late May or early June compared to that planted the first week in May. In addition, Burley harvest occurred before the end of flue-cured harvest at all planting dates. Burley yield was higher at 280 Kg N/ha than at 200 Kg N/ha and the plants tolerated two applications of C-10 fatty alcohol without injury or leaf drop. Cutting and notching at harvest with an adaptation of the University of Kentucky machine was much less consistent than cutting and notching in separate operations. Conveyor type stripping aids reduced labor by 40%. In 2006, the Kirpy harvester from France will be evaluated.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP33

Use of an ethylene binding inhibitor to delay ripening in flue-cured tobacco.

Research was conducted in 2005 at the Central Crops Research Station near Clayton NC and the Border Belt Tobacco Research Station near Whiteville NC. Ethylene inhibitor studies evaluated the effect of 1-methylcyclopropene (1-MCP) on the holdability of flue-cured tobacco in both locations. Ethylene is a natural plant hormone synthesized in the roots that is responsible for senescence in aging tissue. Ethylene is also produced in response to environmental stresses and physical damage, mainly wind and water damage. 1-MCP is an ethylene binding inhibitor that has 10 times the affinity for the ethylene-binding site than does ethylene. Once 1-MCP successfully occupies the ethylene-binding site, 1-MCP causes inhibition of the active complex and the effects of ethylene are then lost. 1-MCP was applied at 14 and 7 days pre-harvest and at normal harvest time to determine effects on holdability. Harvest treatments were imposed at normal and normal plus 10, 20, and 30 days. A specially designed crop oil used with 1-MCP was also tested at 14 and 7 days pre-harvest and at normal harvest. According to data received in 2005, no differences in holdability or grade of flue-cured tobacco were achieved when 1-MCP was compared to the control. Treatment time applications were giving as suggestions from the manufacturer of 1-MCP, Agro Fresh Inc. Applications of 1-MCP at 14 and 7 day pre-harvest were not successful in binding to the ethylene-binding site. Field observations suggest that the natural ethylene supply of the plant had already bound to the receptor site and elicited action. Based on these observations, 1-MCP must be applied at earlier dates in order for the natural ethylene supply of the plant to be inhibited. Insufficient rain accumulation also caused the crop to start the senescence process earlier than expected. In the 2006 growing season, in addition to field experiments, greenhouse applications of 1-MCP will be applied to flue-cured varieties to determine correct timing of 1-MCP application. After application, leaves will be sampled and then analyzed by gas chromatograph to determine exogenous ethylene levels.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. PPOST03

Development of double haploid lines of black root rot (*Chalara elegans* Nag Raj and Kendrick) resistant tobacco.

Black root rot, caused by *Chalara elegans* syn. *Thielaviopsis basicola*, is a widespread and severe disease in many tobacco (*Nicotiana tabacum* L.) growing regions of the world. In Poland, the disease is favoured by cool, wet soil conditions. Yield losses may be severe when growing conditions are not conducive for tobacco growth.

We attempted to obtain double haploid lines derived from F1 plants of two flue-cured cultivars: K 326 - highly susceptible to black root rot and L 121 -with resistance from AC Gayed, by the use of *in vitro* culture. From the total number of 132 anthers cultured on Nitch medium, 179 mature plants were obtained. Haploid plants processed spontaneous diploidization in a stem pith tissue culture. Regenerated mature plants were evaluated in respect to morphological and cytological characters and fertility. The majority of regenerated plants (87.6%) were haploids ($2n=2x=24$). The remaining plants (9.88%) were double haploids ($2n=4x=48$) or (2.47%) mixoploids containing haploid and diploid cells. Double haploid regenerants showed fairly regular meiosis with 23-24 bivalents in pollen mother cells and set relatively well filled seed capsules under open pollination. Chimeras were sterile and showed flower aberrations revealed with stamens and petals malformations.

Artificial black root rot greenhouse inoculation was applied that provided efficient means of selecting plants with resistance. One-week-old seedlings were set into polystyrene trays filled with peat medium and exposed to *Chalara elegans* after transplanting to the float system. After four weeks the roots were scored microscopically for damage. A total of twenty double haploid derivatives of F1 hybrids cv. K 326 × L 121 were tested. Some DH lines were resistant to *Chalara elegans* while one of them was morphologically very close to the parental cultivar K 326. The DH line carrying black root rot resistance trait will be used in the development of flue-cured cultivars combining good quality with *Ch. elegans* resistance.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST03

Effect of eighteen years of tobacco production in monoculture and crop rotation upon the yield, quality and NO_3N leaching on semigley soil in Northern Croatia.

Reduced nitrogen fertilization is a major characteristic of Virginia tobacco culture. Compared to wheat and maize fertilization, the difference is considerable. As tobacco is grown on 6000 ha in Northern Croatia, it can be assumed that soil and water pollution by nitrates is substantially reduced on this areas. Pedoclimatic conditions, lighter soils and lower precipitation intensity influenced higher surface, runoff, as well as translocation of water into lower soil horizons and leaching of nitrates, caused by low absorption. Since mainly small farms have been found in this region certain problems occurred in tobacco production due to its frequent growing in a very narrow crop rotation or even in monoculture. Negative effects of such growing include spreading of diseases and pests, partial exhaustion of nutrients and reduction of tobacco yield and quality.

These investigations will have a long-term character and are carried out on the experimental field of the Tobacco Institute Zagreb.

Several types of crop rotation were tested: tobacco monoculture, two-crop (winter wheat, tobacco), three-crop (winter wheat, tobacco, maize), four-crop (winter wheat, tobacco, maize, soybean), five-crop (maize, soybean, winter wheat, oil-seed rape and tobacco) and six-crop (red clover, red clover, maize, soybean, wheat, tobacco) rotation.

Tobacco is the key crop in these investigations while winter wheat and oil-seed rape were the preceding crops in trials.

Investigations started in 1987 and were carried out on semigley soil (sandy loam) characteristics of the area where flue-cured tobacco is grown in Northern Croatia. The soil is of poor porosity in the eluvial (E/Bt) and illuvial (Bt) horizons.

Tobacco growing in monoculture reduced the yield from 2.63 t/ha in the first year (1987) to 1.56 t/ha in the eighteenth year (2005). A significantly lower yield and quality were obtained after the fourth year of tobacco monoculture compared to tobacco growing in crop rotation. Tobacco grown in crop rotation had a higher percent of a better quality.

The highest nitrate content in plough and sub-plough layers was recorded at lysimeter where the crop rotation involved maize and wheat, which was succeeded by tobacco. The lowest nitrate content (8.7 kg $\text{NO}_3\text{N}/\text{ha}$) was leached in unfertilized tobacco monoculture, which served as check treatment.

The highest content of leached nitrogen (46.3 kg/ha NO_3N) was recorded after the harvest of maize, which was fertilized with 180 kg nitrogen, wheat fertilized with 160 kg of nitrogen, was shown.

More intensive agricultural practices (deeper ploughing, subsoiling, frequent cultivation) will enable a more rapid percolation of water through the soil profile as well as leaching of larger nitrogen quantities.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP39

Evaluation of resistance to the tobacco cyst nematode complex (*Globodera tabacum*) in the genus *Nicotiana* and the species *Nicotiana tabacum* L.

Cyst nematodes from the complex species *Globodera tabacum* (GT) affect tobacco in Europe and U.S.A. In France, damages have been recorded in the Garonne valley and adjacent areas (south west), since at least 1985.

Several *Nicotiana* species and *N. tabacum* accessions were challenged with 15 *Globodera* strains from U.S.A., Mexico or France, in controlled conditions. These 15 strains had been previously characterized with RAPD and AFLP (Mugniéry *et al.*, 1992, Marché *et al.* 2001) and assigned to *Globodera tabacum tabacum* (GTT), *G. tabacum solanacearum* (GTS), *G. tabacum virginiae* (GTV) or *Globodera mexicana* (GM). Strains from France had been identified as GTT. Resistance was assessed based on the number of cysts formed on root systems.

GT strains formed many cysts on *N. sylvestris*. In contrast, *N. repanda* and the inter-specific hybrid *N. repanda* × *sylvestris* showed complete resistance, indicating dominant resistance factors in *N. repanda*.

N. longiflora and *N. benthamiana* also showed some resistance to GT, but none of the *N. tabacum* accessions were found to be fully resistant. With respect to virulence patterns, GTT from the Garonne valley differed from American GTT or GTS. In particular, PBD6, a line close to the ancient Paraguay type, traditionally grown in the southwest of France, showed high susceptibility to the Garonne valley GTT only.

Various *N. tabacum* accessions were also evaluated in infested fields from the Garonne valley. Based on counting cysts by visual examination of root systems, none showed complete resistance. Coker 371 displayed one of the lowest cyst numbers, which is similar to previous findings in U.S.A with GTS (Johnson, 1994). Twenty-six F3 families derived from the cross between Coker 371 and a susceptible line were assessed. Data fit with the hypothesis of a single dominant factor inherited from Coker 371, conferring partial resistance to GT.

In a longer term approach, efforts to find other resistance factors to GTT within *N. tabacum* are being pursued.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP23

Overview of tobacco variety evolution in France 1989-2005 and impact to the raw matter characteristics.

From 1989 to 2005, the French tobacco crop evolved dramatically. A near complete disappearance of dark air-cured types was accompanied by a substantial increase in flue-cured and Burley. Varieties from foreign origin were lacking appropriate adaptation and disease resistances, and an intensive breeding work, initiated in the 70s, was continued up to present. With the coordination of ARREAT¹, then ANITTA², a system for assessing candidate varieties was set up in collaboration between growers' co-operatives, the leaf transformer UCAPT³ and the manufacturer ALTADIS. Field tests to compare candidate varieties were organised each year in several locations. The raw matter was assessed by tobacco experts. Tar and nicotine yield potentials were evaluated by standardized smoking tests.

Using these data, least square estimates of the genetic potential of cultivars for agronomic as well as quality traits have been obtained. Combining them with the acreage devoted to each cultivar allowed us to estimate the evolution of the genetic potential of the tobacco crop, and the resulting raw matter produced in France.

In both types, a substantial increase in quality parameters occurred.

In flue-cured, stability for alkaloid contents (1989-1999) was followed by a slight increase. At the same time, the average leaf harvest date decreased sharply, indicating a renewal towards earlier maturing cultivars. A constant decrease in reducing sugar content could be achieved, and paralleled a 15% decrease in tar delivery.

In Burley, the alkaloid content, tar delivery and total nitrogen remained constant. Nicotine delivery per cigarette increased slightly. New Burley varieties tend to flower later, despite the fact that this trait had not been used for selection. Roguing of converter plants is performed during production of foundation seeds of these varieties, ensuring a low nicotine content.

These trends have been obtained concurrently with generalization of disease resistances that facilitates implementation of GAP principles.

1. Association pour la Rationalisation des Recherches Agronomiques sur le Tabac
2. Association Nationale Interprofessionnelle Technique du Tabac
3. Union des Coopératives Agricoles de Planteurs de Tabac

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. AP34

Evaluation of two fully-mechanical Burley harvesting systems.

Development of a fully-mechanical harvesting system for Burley tobacco began at the University of Kentucky in the early 1980's with a major research grant from Philip Morris USA. By the mid-1990s a prototype system had been fabricated with a design capacity of approximately 1.5 ha per day for two workers. The system utilizes a self-propelled harvester that cuts and inverts mature Burley plants, cuts notches near the stalk base and hangs plants in portable steel curing frames. Filled curing frames holding 450 plants each are unloaded by the harvester, left for 1 week and then covered with waterproof material. Approximately 37 portable curing frames are required to harvest 1 ha. Thorough evaluation of the system was compromised by the having only 25 portable curing frames for testing. Until 2004, the availability of inexpensive migrant laborers, production quotas and adequate curing barns limited grower interest in the system, which would require substantial capital investment. In 2005, Philip Morris USA purchased 400 curing frames and arranged for the system to be evaluated by one of their Production Expansion Program (PEP) growers in Larue County, KY. Approximately 10.5 ha were harvested. Some functional problems were identified requiring design modifications. GCH International, Inc. will manufacture three prototype systems that will be evaluated during 2006 by Phillip Morris USA PEP growers. Approximately 100 ha will be harvested and we will report on operational performance, including: average harvest rate (ha/hr), average field loss (plants/ha), field operating efficiency (time harvesting/total field time), etc. A second fully mechanical harvesting system was designed and fabricated in 2005. This system features a tractor-drawn harvester that employs the same methods of cutting, inverting and notching plants as the original system, except that notched plants are placed in formed steel rails approximately 3 m long. Ten filled rails, holding approximately 40 plants each, are unloaded simultaneously. The unloaded filled rails are retrieved by a separate tractor-drawn transporter and placed on a field curing structure. This system was designed as a lower cost alternative to the system described above. A prototype system will be evaluated by Phillip Morris USA PEP growers during 2006. Approximately 6 ha will be harvested and we will report on system performance, including: average harvest rate (ha/hr), average field loss (plants/ha), field operating efficiency (time harvesting/total field time), etc. Philip Morris USA has provided ongoing support for this work for three decades.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. PPOST04

Seed transmission of tobacco mosaic virus in tobacco.

Tobacco mosaic virus (TMV: Genus Tobamovirus) occurs worldwide. TMV is seedborne but traditionally has not been considered seed transmitted due to the lack of visual symptoms on seedlings from grow-out tests conducted with seed collected from TMV infected plants. Seed transmission studies were conducted both in greenhouse and growth chamber environments. Eighteen growth chamber experiments were conducted each in a randomized complete block design with four or five replications. Seed were germinated at 22 °C with continuous light. Additionally, thirteen greenhouse tests were conducted each in a randomized complete block design with four replications. Seed for all experiments were collected from TMV infected and non-infected field grown 'K 326' flue-cured tobacco plants. Seed collected from the TMV infected plants were either pelleted with a split-coat or melt away-coat or evaluated as non-pelleted (raw). Seedlings were separated from seed coats 14 d after seeding and both were tested for TMV using an enzyme linked immunosorbent assay (ELISA) test. Seed coat and seedling samples from non-infected K 326 seed from all tests were negative for TMV. Seed coat samples from the three treatments using seed from a TMV infected plant ranged from 71% to 100% positive for TMV in growth chamber tests. Seedling samples from these same treatments ranged from 2% to 77% positive for TMV. The highest rate of seed transmission was observed in the nonpelleted treatment followed by the melt-away and then split-coat treatments. Similar results were observed in greenhouse tests although transmission rates were lower when compared to growth chamber tests. Infectivity assays verified biological activity of TMV. Results of these tests successfully demonstrated seed transmission of TMV to 14-day-old flue-cured tobacco seedlings germinated from seed produced on TMV-infected plants.

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CORESTA Congress, Paris, 2006, Agronomy/Phytopathology Groups, abstr. APOST02

Physiological and ecological effects of different soil temperatures on flue-cured tobacco root system.

Accordingly, it is of importance to study the action mechanisms of soil temperature on the growth of flue-cured tobacco in order to avoid disease incidences. The mulching methods with different covering and mulching materials were adopted to adjust root system temperature of flue-cured tobacco in the experiment, which consisted of six treatments, greenhouse film mulching, rice straw + greenhouse film mulching, soil-mulching plastic film mulching, rice straw mulching, rice straw mulching + sun-shading net covering and sun-shading net covering, and hence to study the physiological and ecological effects of different root system temperatures on flue-cured tobacco. The results indicated that the accumulative temperature of 400 °C in early growth of tobacco seedlings was critical for nurserying vigorous tobacco seedlings. The accumulative temperature of more than 400 °C could result in the rank growth characterized by deteriorated growth and physiological traits and low dry-matter accumulation although it was capable of increasing the plant heights and root lengths of tobacco seedlings; as the soil accumulative temperature increased with the accumulative temperature for tobacco seedlings below than 400 °C, various growth and physiological traits became favourable and vigorous growth resulted, which were in favour of acclimatizing the seedling for nurserying vigorous seedlings. The study also revealed that mulching materials had a close relation to the climate. Different mulching materials showed a strong temperature-increasing effect at a high air temperature. The combined mulching of plastic film and rice straw was a mulching method that combined organic and inorganic materials, and complemented each other in temperature-increasing effect, and thus it would be of certain value in preventing the cold damage often occurring after seedling transplanting of flue-cured tobacco.

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Specific molecular markers of biocontrol fungus *Pochonia chlamydospora* ZK7 for tobacco root-knot nematode.

Biocontrol fungus *Pochonia chlamydospora* ZK7 are highly effective in the biological control against root-knot nematodes infecting tobacco. To obtain the specific molecular marker of *P. chlamydospora* ZK7, distinct and reproducible molecular fingerprints of 97 isolates of different fungi, including biocontrol fungus *P. chlamydospora* ZK7, and some related and common soil fungi were amplified using the primers of random amplified polymorphic DNA (RAPD), intergenic spacers (IGS), enterobacterial repetitive intergenic consensus (ERIC), repetitive extragenic palindromic (REP) and a subunit of the Box element (BOX). Two specific DNA fragments of biocontrol fungus ZK7 were obtained upon amplification of genomic DNA of them with the random primers OPL-02 and OPD-05. Two amplified DNA fragments of 1200 (Vc1200) and 2000 (Vc2000)bp were diagnostic for *P. chlamydospora* ZK7, which clearly distinguishing them from 96 related isolates of control strains respectively. These two RAPD DNA fragments were isolated from agarose gel and purified, and the extracted DNA was ligated into a pGEM®-T Easy Vector, *E. coli* DH5 α competent cells were transformed with ligated DNA. The transformants were selected with blue-white dot and their plasmid DNA was extracted to verify the presence of inserts by electrophoresis. The cloned fragments were sequenced, their GenBank Accession No. were AY265801 and AY265802 respectively. Two pairs of oligonucleotide SCAR-PCR primers for each cloned fragment were designed. Use of the primers in the PCR assay resulted in the amplification of DNA fragments of the same size as the cloned RAPD fragments from genomic DNA of *P. chlamydospora* ZK7 respectively, but not control strains. These two amplified fragment specifically were labelled with digoxigenin and recognized the corresponding fragment present in the genomic DNA of *P. chlamydospora* ZK7 in dot blotting. In classical polymerase chain reaction, with a series of dilution of *P. chlamydospora* ZK7 DNA, the limit of detection of two pairs of oligonucleotide SCAR-PCR primers was 10 and 1000 pg/ μ l respectively, and the limit detection of dot blotting were 0.1 μ g/ μ l.

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