

**ABSTRACTS OF PRESENTATIONS MADE AT THE  
2013 CORESTA JOINT MEETING OF THE  
AGRONOMY & LEAF INTEGRITY AND PHYTOPATHOLOGY & GENETICS  
STUDY GROUPS**

**BRUFA DI TORGIANO, ITALY**

*(by alphabetical order of first authors)*

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. APOST 11

**Di@noPlant® Tobacco: a new mobile tool to identify tobacco diseases in the field**

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

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

This development has two aims:

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

Developed in French by INRA, Di@noPlant® Tobacco has been translated into English by CORESTA to make this tool accessible worldwide.

The application will be demonstrated during the poster session.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 06

**Preliminary evaluation of cured leaf residues of common pesticides used in dark air-cured and dark fire-cured tobacco**

Pesticide residue data for dark tobacco is limited compared to data available for Burley and flue-cured tobacco. It is also unknown if the curing process (air-curing or fire-curing) affects pesticide residues that remain in the cured leaf. Pesticide residue trials were conducted in 2011 to evaluate cured leaf residues of azoxystrobin, acephate, lambda-cyhalothrin, and maleic hydrazide in dark air-cured and dark fire-cured tobacco. All pesticides were applied at the maximum use rates per application, maximum number of applications, and minimal preharvest intervals allowed on product labels. Two adjacent field experiments were established with the same dark tobacco variety (PD 7309LC), transplanting date, and harvest date. One experiment was air-cured while the other was fire-cured. Average residues across three stalk positions in air-cured tobacco was 12.6 ppm for azoxystrobin, 4.1 ppm for acephate (methamidiphos), 0.56 ppm for lambda-cyhalothrin, and 67 ppm for maleic hydrazide. Residues in fire-cured tobacco were lower than those in the air-cured trial for all pesticides evaluated. Fire-cured residues were 2.2 ppm for azoxystrobin, 1.0 ppm for acephate (methamidiphos), 0.46 ppm for lambda-cyhalothrin, and 52 ppm for maleic hydrazide.

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**BERBEC A.**

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. APPOST 07

**Experiences with multiple-cross hybrids in flue-cured tobacco**

Multiple-cross hybrids are routinely used in several outcrossing crops mainly to bypass difficulties in commercial hybrid seed production. An advantage of using a multiple-cross hybrid in tobacco, a self-pollinating crop, may lie in adding some variation to a cultivar thus making it more flexible in its response to environmental variability – e.g. soil- or weather-related. Even though self-fertile cultivars may show some residual variation left deliberately by the breeder, single-cross hybrids, now prevalent, are most often based on homozygous lines rather than cultivars and lack any inherent genetical variability. Another possible benefit may consist in more flexibility in constructing useful genotypes from available parental inbred lines or varieties. The main issue is the choice of the original inbred lines so that the introduced variation does not compromise the homogeneity of the agronomic traits required of a cultivar. Several three line hybrids of flue-cured tobacco were produced, tested and compared against corresponding single-cross (F<sub>1</sub>) hybrids based on the same true-breeding lines. One of these three line hybrids, dubbed VRG 5 TL, was found to out-yield two F<sub>1</sub> hybrid combinations which involved the true-breeding parent and either of the homozygous lines used to produce the F<sub>1</sub> parent. VRG 5TL has been recently successfully released to tobacco growers in Poland. It shows acceptable agronomic performance which is in many ways superior to that of formerly released and widely grown F<sub>1</sub> hybrids.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. APPOST 04

### **Influence of plant density and leaf priming collection on seed and seed oil yield in tobacco**

In the EU the Common Agricultural Policy (CAP) reform of 2004 has established a gradual reduction in incentives for the production of tobacco. In Italy there was a reduction of about 30% of the area under tobacco cultivation from 2000 to 2010. Value-added products, such as tobacco seed oil (TSO), could enhance economic returns to tobacco farmers and avoid further reduction of the cultivated area. It is known that tobacco seed is rich in oil, free of nicotine, and is classified as semi-drying oil which is used as a raw material in the soap, varnish and coating industry and might be an appropriate substitute for diesel fuel, and as a non-food renewable source of energy.

In order to evaluate the feasibility of diversifying the use pattern of the tobacco crop, a study of the effect of leaf priming on seed oil yield was performed in 2011 and replicated in 2012.

Two tobacco genotypes (I917 and Pr81), with high seed yield, were evaluated in a trial performed in the experimental field of COTIR, at two planting densities (S1 = 4 plants m<sup>-2</sup> and S2 = 8 plants m<sup>-2</sup> respectively) under unprimed and primed conditions (M1 and M2 respectively), for assessment of seed and oil yield. Results showed an increase of 38.5%, on average, of tobacco leaf dry matter yield at S2 plant density; a leaf dry matter yield of 5.94 t ha<sup>-1</sup> for I917 and of 5.13 t ha<sup>-1</sup> for Pr81. At plant density S2, an increase of 32.5%, on average, of oil yield was also observed. No significant difference in tobacco seed oil yield between unprimed and primed conditions (499 l ha<sup>-1</sup> at M1 and 516 l ha<sup>-1</sup> at M2) was observed.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 10 (Poster)

### **Flue-cured tobacco curing cost in relation to curing fuel**

In Croatia, natural gas has been used for curing flue-cured tobacco. The price of gas has increased over 100% during last five years. However, the price of cured tobacco leaf has not followed the curing cost increase and flue-cured tobacco is becoming less interesting profit wise to farmers. Use of a cheaper fuel for curing could make flue-cured tobacco again attractive to farmers as a cash crop. In 2011 and 2012 on-farm tests were performed to assess the curing costs using various forms of biomass, i.e. firewood, wooden chips and pellets instead of the natural gas. For that purpose, thermo generators with and without the possibility of pyrolytic biomass burning (dry distillation) were installed into the existing curing barns. Reduction of curing cost with biomass, compared to the cost of natural gas, ranged from 52% (pellets, no pyrolysis) to 84% (fire wood, pyrolysis). The investment into curing barn adaption for burning biomass without dry distillation could be paid back within two years due to the lower curing cost and within three years with dry distillation, assuming the present fuel and cured tobacco prices.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 08

**Evolution in agrochemical use on tobacco over the last 30 years to reduce leaf residues and environmental impact**

This presentation describes the evolution in active ingredients (a.i.), and their use on the tobacco crop, since the beginning of 1980s. Progress in reducing application rates has been achieved not only by the use of more efficient and target-oriented a.i.s and their formulations, but also by the introduction of improved spraying equipment, and switching, e.g. in the case of herbicides, to band instead of broadcast, applications. Since 2010 most of the tobacco crop in Central Italy has been treated according to the Agro-Environmental Measures of Regione Umbria, which have posed even stricter limits to the use of some agrochemicals belonging to the higher hazard risk classes, and in general to agrochemical rates. Scouting and treating when risk thresholds are exceeded, and attention to maintain the correct interval before harvest, have been improved with time. With reference to the previous technical improvements, leaf residues have been reduced accordingly, and this has improved the quality of the product, reduced the risks for growers, and lessened the environmental impact of the tobacco crop. Data on past and present residues in the leaf are presented to exemplify the results achieved in monitoring crop quality.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 33

**A non-invasive optical method for quality control of maturing and cured tobacco leaves**

The fluorescence-based sensor used in the present study represents a rapid and non-invasive tool, already used for assessing grape phenolic maturity and quality, and recently tested also on plums (for maturity assessing) and turf (for nitrogen concentration). It may represent in perspective an alternative to destructive laboratory analyses, which are time-consuming and require an accurate sampling approach. Based on our knowledge, this is the first experience of its use on tobacco to test flavonols and nicotine derivatives in fresh and cured leaves. The activity carried out in 2012 on tobacco of Fattoria Autonoma Tabacchi of Città di Castello compared HPLC/DAD/MS analyses of the hydroalcoholic extracts of tobacco leaves for flavonols and nicotine derivatives with those collected by a portable fluorimeter sensor UV-Vos (Multiplex®, Force-A). Various parts of leaves, belonging to different crowns, and tobacco varieties were tested. This work aimed at establishing any correlation between the sets of data under study. Presently, the activity has been transferred to field grown tobacco at Fattoria Autonoma Tabacchi of Città di Castello, to find any correlation between actual measurements and leaf maturity parameters. Preliminary data are presented on the activity in progress. In a short perspective, the use of this sensor is expected to become a tool to drive agronomic treatments and best crop harvest time.

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## **CARDINALI D.**

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 01

### **Overview on Italian tobacco production**

Deltafina - on behalf of the entire Italian tobacco chain - wish to thank all the agronomists, researchers and scientists taking part in the operating groups of CORESTA for the efforts in their professional activities and for having chosen the Umbria region for their conference.

We will be presenting a brief introduction on Universal Leaf Tobacco Company in Europe, with particular reference to Deltafina SpA's participation.

This will be followed by the subject of the presentation, which will be to present the reality of Italian tobacco, within the context of the other producing countries in the EU, both in terms of productive social and political aspects. This analysis will be carried out taking into account the last three crops, so as to give a clear idea of the evolution of tobacco cultivation in Italy.

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## **CHANG Dong; LIU Guoshun**

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 38 (Poster)

### **Delineation of site-specific management zones using fuzzy clustering analysis for a tobacco field based hilly land**

Recent precision agriculture research has focused on the use of management zones (MZs) as a method for variable-rate fertiliser application. Topography factors have a profound influence on MZs. Delineating management zones by soil properties and elevations is therefore a practical approach. To achieve this objective, we selected a 3.1-ha typical mountain slope tobacco field in Sichuan Province in China, and collected 88 soil samples from the top 20 cm on an approximately 25-m grid of the study area, location and elevation were obtained by GPS. Geostatistics and the application of geographic information system (GIS) were necessary to combine soil properties and elevation for management zones delineation. Geostatistics analysis indicated that soil pH, OM, AK, TK, Fe and Zn were best represented through an exponential model; AN, AP, TP, Mn and Cu were spherical model. Principal component analysis (PCA) and fuzzy cluster algorithm were performed to delineate MZs; fuzzy performance index (FPI) and normalised classification entropy (NCE) were used to determine the optimum cluster number. Results showed that the optimum number of MZs for this study area was four and the elevation of each zone was relatively homogeneous. Analysis of variance indicated the heterogeneity of soil properties among the four MZs. We conclude that mountain tobacco fields can be managed in a site-specific manner by these methods.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 31

**Somatic hybrids between *N. stocktonii* and *N. tabacum*, as a source of black shank (*Phytophthora parasitica* var *nicotianae*) resistance in *N. tabacum***

Black shank (*Phytophthora parasitica* var *nicotianae*) is one of the most serious diseases of tobacco in the major tobacco leaf production area of China. Complete resistance to race 0 from *N. plumbaginifolia* has been transferred into cultivated tobacco (Ph gene) and will be released widely. With widespread deployment of the Ph gene, rapid shifts in pathogen populations from race 0 to race 1 could be observed. The wild species *N. stocktonii* is resistant to black shank (race 0 and race 1). No report of a direct hybridisation between *N. stocktonii* and *N. tabacum* (cv. Yunyan 85) exist. In this paper, somatic hybrids were obtained by protoplast fusion between *N. stocktonii* and *N. tabacum*. F1 plants were completely sterile. The hybrids sterility has been overcome by 3 passages on midvein tissue culture techniques. More than 23.1% viable pollen was produced and seeded capsules were formed in the regenerants (R). GISH, morphological and ISSR examinations confirmed the true hybrid state of the obtained inter-specific combination. Tests of resistance to black shank were conducted on R1 plants, which were shown to be resistant. The material may be useful in future breeding for black shank resistance.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 20

**Molecular analysis of the complete genomic sequence of tobacco etch virus isolated from China**

The tobacco etch virus (TEV) is a species of the largest plant virus genus *Potyvirus*. TEV-Shaanxi induced systemic symptoms including mosaic and necrotic lines or etching in *N. tabacum*. The efficiency of TEV-Shaanxi transmission by *M. persicae* was higher than *A. gossypii*. The purpose of this study was to determine the complete genomic sequence of TEV for a better understanding of the genomic organization, genetic diversity and evolutionary origin of the virus. Seven pairs of primers were sequentially used in PCR to amplify most of the viral genomic sequence. The primers were designed according to sequences of other TEV isolates. For the 5'-terminal sequence, 5'RACE reaction was conducted using the 5'RACE system. The complete genome sequence of TEV consisted of 9,494 nucleotides, excluding the 3' poly (A) tail. The genome contains a single large open reading frame (ORF) encoding a polyprotein of 3,054 amino acids, with an AUG start codon and a UGA stop codon. The sequences of the separate genes (except the small 6K1 and 6K2) were compared with 32 other species of the genus *Potyvirus*. Results showed differing distributions of values reflecting the different degrees of conservation of the different genes. Comparative analyses show that the P3 and particularly the P1 genes displayed the greatest variability. The amino acid sequences of NIa-Pro, NIb and CI, however, are highly conserved among species of the genus *Potyvirus*. The sequence homologies of the ORF of TEV range from 45.5% to 55.5% and from 37.8% to 49.5% compared to other members of the genus *Potyvirus*, at the nucleotide and amino acid level, respectively. Phylogenetic analysis of the polyprotein sequences of TEV and other species in the genus *Potyvirus* imported from the GenBank indicates that TEV is a member of a subgroup, which includes potato virus A (PVA), TMVMV, tobacco vein banding mosaic virus (TVBMV), wild tomato mosaic virus (WTMV), chilli veinal mottle virus (ChiVMV) and pepper veinal mottle virus (PVMV). TEV isolates can be clustered into four genetic groups that are linked with their geographical origin.

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**COZZOLINO E.; LEONE V.; LOMBARDI P.**

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. APPOST 01

**Performance of fire-cured tobacco with compost from urban sources**

A two-year trial of Kentucky fire-cured tobacco fertilisation with compost from urban organic waste was conducted with three cultivars (Foiano, Riccio beneventano and SKL) on two farms in the province of Benevento (Italy). A commercial compost, produced by Gesenu, was applied each year on the same plots at levels of 10 and 20 t/ha, supplemented or not with 75 kg/ha N, and was compared with a pure mineral fertilisation with 135 kg/ha N. Such levels of compost were chosen more on account of waste recycling aims than on account of Kentucky tobacco needs. In the first year cultivars SLK and Riccio performed better than Foiano with the mineral N supplemented compost treatments. Chemical and visual quality of tobacco was not influenced by the compost or its application level and the range of responses was rather narrow. In the second year the 20 t/ha rate of compost compared well with the whole mineral treatment, with yields higher than the 10 t/ha rate, but was inferior to the latter supplemented by mineral N. Chemical and visual quality was better with the lower level of compost supplemented with mineral N. Cultivar SKL was the best for both yield and quality.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. APPOST 05

**New cultivars for the Italian Burley industry**

Lack of information about cultivar adaptation to specific areas is a hindrance to the progress of the tobacco industry. To provide such information for Italian Burley areas, an on-farm variety trial is underway at Vitulazio, in the province of Caserta, where fifteen lines are being tested in two replicates with cultural practices customary for the type. We report here the first year results. Cured tobacco was visually graded on a decimal scale and chemical and physical traits were determined on plot composites of three leaf positions. An equivalent yield of maximum value was obtained by multiplying yield with a summary quality index, synthesised by geometrically averaging, after normalising to 0:1 scale, the ratio of nicotine to total and nitric N, the proportion of leaf lamina, the combustibility index and the visual assessment score. The lines could be grouped in terms of earliness and yield, which were negatively correlated: late flowering lines (PM34, START AR, 737AR, FB82) yielded considerably more than early flowering ones (FB3117 e FB3119) and intermediate ones (all the others). Compared in terms of equivalent yield the late flowering lines START AR and PM34 were clearly superior, with yields around 65 t ha<sup>-1</sup>, while the two early flowering ones were markedly inferior, with yields around 3.6 t ha<sup>-1</sup>.

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**CZUBACKA A.; DOROSZEWSKA T.**

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. APPOST 10

**Effectiveness of transgenes in protection against potato virus Y in tobacco breeding lines**

Progress in genetic transformation techniques has allowed successful production of transgenic plants with improved traits. However, it is important that introduced genes are stably inherited and expressed.

Three tobacco transgenic lines coming from transformation of cultivars MN 944 and AC Gayed were the material of the study. They were previously transformed using *Agrobacterium tumefaciens* and leaf disk culture in order to obtain plants resistant to potato virus Y. The tested breeding lines contained three constructs: LMV CP - containing gene of Lettuce mosaic virus coat protein, ROKY1 - containing sense gene of PVY replicase, and ROKY2 - containing antisense gene of PVY replicase.

The subsequent generations of transgenic plants obtained by self-pollination were studied to determine the rate of plants containing transgenes. They were also tested for resistance to PVY by means of artificial inoculation with the virus under greenhouse conditions.

Within subsequent generations of the tested lines we observed segregation of individuals in respect of presence of the transgenes and increasing rate of transgenic plants. However, the increased frequency of transgenes not always correlated with increased resistance of plants. Line MN 944 LMV showed an increasing level of resistance up to generation T<sub>4</sub> but in T<sub>5</sub> many plants were susceptible in spite of the confirmed presence of the LMV CP transgene. Furthermore, resistance of line AC Gayed ROKY1 was falling through generations in spite of the increasing percentage of plants with the transgene. Within line AC Gayed ROKY2 the resistance of plants was generally high but unstably inherited.

The results showed unstable effectiveness of transgenes and indicated the need for further analyses including studies on expression of transgenes and their presumable modification.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. APPOST 08

**SSR analysis of genetic polymorphism in a *Nicotiana* Germplasm Collection**

Tobacco (*Nicotiana tabacum* L.) is an important agricultural crop plant that plays a significant role in the economies of many countries. Tobacco breeding aims to develop varieties with wide adaptability, higher potential and suitable chemical constituents for the cigarette industry. To explore the genetic potential and select suitable parents it is necessary to assess genetic diversity of tobacco germplasm resources. The aim of this study was to investigate the genetic variability in the CRA-CAT germplasm collection of *Nicotiana* genus, as revealed by SSR molecular markers. 70 accessions of *N. tabacum*, including unrelated and related lines and 10 wild *Nicotiana* species, were examined. Tobacco genotypes were analysed with 35 SSR primer pairs that produced from 1 to 7 bands each with sizes ranging from 70 to 350 bp. A total of 112 bands were generated of which 45 were polymorphic. Among the tobaccos and the wild *Nicotiana* species, the 35 SSR primer pairs produced from 2 to 16 bands each with sizes ranging from 70 to 350 bp. A total of 237 bands were generated of which 125 were polymorphic. The analysis carried out by UPGMA clustering, based on Jaccard genetic distances, revealed a high rate of genetic similarity among the analysed tobacco genotypes. Genetic variability revealed by SSR analysis at inter and intra-specific level will be discussed.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 16

**Quantification of spatial light distribution of a field-grown tobacco canopy based on three-dimensional modelling**

Quantification of the interaction of a plant with its light-environment is pivotal in agricultural research. Three-dimensional (3-D) digitising and laser-scanning of field-grown tobacco plants were conducted and a realistic 3-D architectural model of a tobacco canopy was reconstructed. Spatial light distribution in the tobacco canopy was measured and this dataset was used to validate a 3-D canopy light distribution model based on Monte Carlo ray tracing. It was shown that the simulation results matched the measurement satisfactorily. High correlation between the field measurements and the simulation of light distribution in the tobacco canopy was reached ( $R^2=0.99$ ). The diurnal course of spatial light distribution in the tobacco canopy was simulated using the model. This work provides a foundation for quantifying 3-D radiation distribution and photosynthesis in the tobacco canopy as well as assessing tobacco ideotype using functional-structural plant models.

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**DIJKSTRA F.E.**

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**Conservation agriculture in Brazil: The transformation to environmentally sustainable, socially responsive, and profitable production systems**

Dairy was the first economically successful agricultural production model for early Brazilian immigrants with limited capital who settled on acidic, infertile tropical soils. The settlers were quick to recognise the positive response of soils to application of manures and later chemical fertilisers and lime. Mechanisation in the 1940's made possible a larger economy of scale and diversification from not only dairy but also grain and other commodities. However, up to nine tillage operations per year led to rapid soil degradation from erosion and loss of soil organic matter. This resulted in loss of productivity and environmental harm from sedimentation and nutrient loss to surface waters. Over the years many possible solutions were tried and failed, including contour farming and expensive terracing. In the early 1970's a few pioneers were receptive to research on No-Tillage Agriculture in the USA. They rapidly evolved this model to a *Systems Approach to Conservation Agriculture*; elimination of soil tillage, inclusion of cover crops to provide mulch for soil coverage, and intensive crop rotations were key elements. *Conservation Agriculture* reduced erosion and runoff dramatically, increased soil water infiltration and conservation, conserved soil nutrients, reduced fuel consumption, and reduced the need for pesticides. Further integration of these cropping systems with animal production made agriculture even more profitable. On our farm, wheat, corn, soybean, and edible bean yields have dramatically increased. Intensive forage production in these rotations results in three cuttings per year, providing 50-60 thousand litres of milk/hectare/year. Animal wastes are recycled to the soil, composted and used for biogas energy in an environmentally benign model. Brazilians have developed their own applied research and Technology Transfer Centers and formed partnerships with government agencies, agribusiness interests, and NGOs to rapidly disseminate the developing technologies. Today *Conservation Agriculture* has expanded to more than 60% of arable land in Brazil.

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**DIMITRIESKI M.; MICESKA G.; KORUBIN-ALEKSOSKA A.**

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. APPOST 14

**Production characteristics of four Oriental tobacco lines resistant to black shank (*Phytophthora parasitica* var. *nicotianae*)**

The aim of the investigation was to evaluate morphological, production and qualitative traits of four newly created fertile inbred lines and their resistance to black shank disease (*Phytophthora parasitica* var. *nicotianae*), compared to the susceptible standard variety YV 125/3 (Ø).

The experimental lines were obtained by intraspecific hybridisation. The breeding process started with crossing introduced Oriental tobacco varieties with stable domestic lines resistant to black shank (AA) which were used as maternal parents and susceptible lines and varieties (aa), used as paternal parents. Selection of hybrid progeny was made using the pedigree method. The experimental lines were stable in plant height and in number, shape and size of the leaves.

According to the results of field experiments, the newly created resistant lines had a higher leaf number per plant (45-53 leaves) as compared to the variety YV 125/3 Ø (37 leaves). They also achieved higher yields (33.66% - 68.80%), higher purchase price (14.3% - 27.7%) and greater crop value / Euros/ha (41.01% to 81.29%). Black shank is an economically important disease of Oriental tobacco throughout the world. In conditions favourable for its occurrence it can also cause severe damage in some microregions with mass production of Yaka tobacco. The tobacco varieties and lines were investigated for their resistance to the causal agent of *Phytophthora parasitica* var. *nicotianae* during 2010, with artificial inoculation in glasshouse conditions at the Tobacco Institute - Prilep.

Of the four lines investigated, three were evaluated as highly resistant to black shank and they can be included not only in commercial production but also as sources of resistance in breeding programs.

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**DOROSZEWSKA T.; DEPTA A.**

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. APPOST 09

**Sources of resistance to viral diseases within the genus *Nicotiana***

Viral diseases cause important economic losses in tobacco crops. They are transmitted by vectors or mechanically, and the battle is difficult due to the lack of chemicals that eliminate them. The most damaging viruses that affect tobacco in Poland are potato virus Y (PVY), tomato spotted wilt virus (TSWV) and tobacco mosaic virus (TMV). Growing resistant cultivars is the most effective method of protection against these viral diseases. The seed collection at the Institute includes more than 60 *Nicotiana* species, which are potential sources of resistance to many diseases. The aim of this study was to assess the resistance of different *Nicotiana* species to PVY, TSWV and TMV using inoculation tests. Diversified effects of virus isolates on the studied species were observed. The species resistant to different PVY strains include *Nicotiana glauca*, *N. knightiana*, *N. benavidesii*, *N. raimondii*, *N. wigandioides* and *N. africana*. The members of the section *Alatae*: *N. alata*, *N. forgetiana* and *Nicotiana* x *sanderiae* displayed a hypersensitive reaction (HR) against TSWV. Within five accessions of *N. alata* and three of *Nicotiana* x *sanderiae* from 6,3 to 50% of plants displayed systemic hypersensitive reaction (SHR) symptoms. In every *N. forgetiana* plant the HR reaction was followed by systemic infection (SHR). SCAR markers associated with a TSWV resistant gene were tested on species from the section *Alatae*. All genotypes which responded with HR or SHR reaction to TSWV infection demonstrated the presence of this SCAR marker. Within the genus *Nicotiana*, only *N. glutinosa* displayed a hypersensitive response to TMV, and some species did not show any sign of infection, despite the presence of the virus in the plant. Species harbouring the resistance genes may be used in resistance breeding.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 42

**Evaluation of nitrogen application timings on yield, quality, and sucker control**

Historically, nitrogen (N) has been applied in flue-cured tobacco in either one or two applications within the first four to six weeks after transplanting. Growers choose a N rate based mostly on soil type and with an average expected amount of total rainfall per season in mind. While this approach is effective, it results in too much N available late in the season if rainfall amounts are below average and N deficiency when above average rainfall amounts occur.

Recently, liquid sources of N such as 30% UAN and 24% S have been used on more than half of the flue-cured tobacco acreage in North Carolina. Use of these sources allows growers more flexibility for N application timing because they can be applied later in the season without damaging the tobacco crop.

This project is analyzing two different studies. The first study looked at different N timing and application rates. Using 28% N we applied 100% of total N needed for crop growth, 75% of total N needed (simulate drought conditions) and 125% total N needed (simulate leaching conditions). Nitrogen was applied in as few as two applications and as many as four applications. Treatments were based on timings of N applied and percentage of total N applied at each time interval. The application timings included combinations of the following growth stages; N application at planting, at two weeks of growth, at lay-by stage, two weeks after lay-by, and at topping. The second study analyses application method. The most production realistic treatments were selected from first study that had N applications at topping. The at topping application in the second study will compare both soil applied N application to a stalk run-down applied N application.

Measurements for yield and quality as well as total leaf tissue N were taken. In the first year few differences were observed for all data taken.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. IG 02

### **Variation in toxicant yields from selected products**

The WHO Study Group on Tobacco Product Regulation (TobReg) have recommended setting simultaneous ceilings on mainstream smoke emissions of nine toxicants expressed as a ratio to nicotine. The proposed ceilings are recommended to be based on the market median (NNN, NNK) or 125% of the market median (remaining seven chemicals). Toxicant measurements on products, and their compliance to a proposed ceiling, is likely to be affected by analytical and product variability over time. In order to contextualise measurement data, it is important to understand the variation of smoke emissions from commercial cigarette products across time.

A study was conducted to provide information on the variability in mainstream smoke yields of these nine chemicals, and their ratios to nicotine, as measured under the Health Canada intense regime, from three large volume commercial cigarette products from a single market (Germany). Tobacco blend chemistry and cigarette physical measurements were also conducted. The three cigarette products were sampled monthly over ten consecutive months in 2010-11. Control data from the 3R4F Kentucky Reference cigarette was also collected at the same interval.

Two main sources of variation were investigated – analytical variability and cigarette product variability. The mean variation for each chemical of interest as a ratio to nicotine will be presented along with the expanded uncertainty ( $k=2$ ) as an estimate for a 95% confidence limit. This within laboratory ‘tolerance’ around measured values for each chemical of interest was found to reach up to 40% in this study. A practical lower limit of variation for a reasonably controlled analysis was identified. Also identified were chemicals which demonstrated larger than average analytical variation and would benefit from improvement in analytical methods.

In summary, measurement of the nine chemicals recommended by TobReg for regulatory ceilings was demonstrated to be affected by significant level of variation.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 13

**Assessing yield loss from hail in Burley tobacco**

A series of conceptual hail damage experiments were done to investigate why yield loss from hail damage cannot be detected until the appraised leaf area damaged is 25-40%. Leaves of individual plants were treated seven days after topping by either removing or breaking one-third or two-thirds of the total leaf area of the plant in several predetermined ways. The leaves from each individual plant were counted and weighed after curing, and yield loss was calculated as a percentage of the undamaged check plants and compared to mathematically modelled loss. The yield loss of individual leaves from which only the mid-leaf lamina was removed was the same as the modelled loss. Cured weight per leaf increased proportionately with the total number of leaves removed from the plant. Where the lamina was severed from the midrib for two-thirds of the leaf length, yield loss was 11% and none if two of every three secondary veins were severed at the midrib. If the lamina of these same leaves were also severed to the margin, the loss was 16%, but there was no reduction in individual leaf weight if two thirds of the leaves on these plants were removed. Up to one quarter of the whole leaves with midribs broken at the axil, but left hanging on the plant reached the stripping table and contributed to yield. The observed discrepancy between actual yield loss and estimated damage from hail is a result of over-estimation of damage, the disproportionate weight contributed by the midrib, the ability of damaged leaf to survive, and compensation for lost leaf. The simplest and most accurate method of assessing yield loss from a hail strike was to determine the proportion of whole leaves lost at the time that the cured tobacco is stripped.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 25

**Wavelength-dependent phototaxis of the cigarette beetle**

Many nocturnal insect pests are attracted by light. Commercial light traps have been used in integrated pest management programmes. The influence of light wavelength on phototactic response has been demonstrated for some of these insects. Nevertheless, few reports describe the wavelength-dependent phototaxis of the cigarette beetle. We therefore examined the phototactic response of the adult beetles in a transparent acrylic tube (100×9 cm ID). Monochromatic light (320–700 nm: 20-nm steps) was radiated from one of the ends. Twenty adults were released at the centre of the tube and their phototactic responses were observed. The adults exhibited positive phototactic responses to wavelengths of 320–620 nm, with the maximum response at 360 nm (UV). No significant response to wavelengths longer than 640 nm was found. Wavelength-dependent phototaxis was confirmed further by release-capture tests. A light trap composed of a monochromatic light source and an 11×9 cm water-pan was placed at a corner of a 2×2×2 m chamber. Then 50 adults were released at the centre of the floor. After three hours, the adults captured in the water pan were counted. The catch was maximised at 360 nm (capture ratio: 39.6%), just as expected. However, strong attraction occurred at a narrower range of wavelengths (320–420 nm). The catch numbers dropped sharply at 440 nm. Results indicate that most commercial light traps equipped with black light bulbs (peak emission typically 370 nm) are effective for capturing adult cigarette beetles.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 40

**The analysis of molecular marker and evolution based on tobacco EST**

Tobacco is an allotetraploid ( $2n = 4x = 48$ ) and most likely derived from a tetraploidization event involving the diploid species *Nicotiana sylvestris* (S-genome,  $2n = 24$ ) and *Nicotiana tomentosiformis* (T-genome,  $2n = 24$ ). Two full length cDNA libraries were constructed using various tissues of *N. tomentosiformis* and *N. sylvestris*. Sequencing was performed and over 20000 EST were obtained. Combining 350000 *Nicotiana tabacum* ESTs from NCBI, all ESTs were assembled using the Program CAP3, resulting in 34450 contigs and 123511 singletons (157961 unigenes). The transcripts from resident T- and S-genomes in the allotetraploid nucleus are more closely related to their diploid parental homologs than they are to each other. Thus, combining two diploid EST sequences with the ESTs of tetraploid species does not introduce appreciable amounts of additional sequence divergence. The UniProt BLASTX hits, GO annotation, and Pfam were analyzed. From the EST alignments, we inferred 127787 single nucleotide polymorphisms (SNPs) and 11869 Microsatellites (SSRs). The *Nicotiana* EST assembly along with SNPs and SSRs will provide a foundation for future investigation of tobacco functional and evolutionary genomics using both long and short oligonucleotide microarrays. The newly identified SNPs and SSRs in our study can be used to generate a genetic map and locate genes of economically important traits within the tobacco genome. These markers will pave the way for successful application of Marker-assisted-selection (MAS) in breeding programmes.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. APPOST 13

**Biological control of *Rhizoctonia solani* in tobacco seedlings with some *Trichoderma* species**

Tobacco production has intentions to promote biological control as a modern and environmentally friendly plant protection measure.

*Rhizoctonia solani* is a very destructive pathogen, the causing agent of damping-off in tobacco seedlings.

Biocontrol activity of some *Trichoderma* species (*T. aureoviride*, *T. hamatum* and *T. harzianum*) was investigated *in vitro* - by dual culturing technique and *in vivo* - in the greenhouse.

The relative development of the pathogen ranged from 33.10% for *T. harzianum* to 41.64% for *T. aureoviride*. The percentage inhibition of radial growth of the pathogen did not show differences among the studied *Trichoderma* species, however, the highest value (66.91%) was in *T. harzianum*.

In conditions of artificial inoculation, the intensity of a disease was reduced when applying the investigated biocontrol agents.

*T. harzianum* has the best reducing effect on damping-off in all application methods of this biocontrol agent. The intensity of disease was only 5.56% in the application 15 days after sowing, while in the application before sowing and mixed with seeds, there was no disease incidence.

Application of these *Trichoderma* species on seeds was confirmed as the best application method of the biocontrol agent.

The biocontrol activity of these fungi enables them to be used in the biological control of *R. solani* in tobacco seedling protection.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 22

**Potential alternative versus standard nematicides for control of *Globodera tabacum solanacearum* for flue-cured tobacco in Virginia in 2010-2012**

*Globodera tabacum solanacearum* (TCN; tobacco cyst nematode) has been an important parasite of flue-cured tobacco in Virginia, and effective nematicides remain necessary when susceptible cultivars are planted and crop rotations are short. Growers are also increasingly interested in alternative nematode management products appropriate for organic or low pesticide residue tobacco production systems. Four field experiments were conducted in Virginia in 2010-2012 to compare TCN control using standard soil fumigants to that from “alternative” nematode management products, such as a mustard (*Brassica juncea*) seed meal or a bionematicide (DiTera). Trials were performed using a randomized complete block design with 4-6 replications. Mustard meal treatments incorporated product 14-21 days before transplanting. Normal fertilization practices were adjusted to account for the N-P-K content (5-1-1) of the mustard meal product. 2010 final TCN population densities after pretransplant incorporation of 280-1,345 kg mustard meal/ha were significantly lower than those after application of 37 L/ha chloropicrin, but apparent reductions in final TCN populations in 2011 were not statistically significant. Final TCN populations after incorporation of 1,681-2,242 kg mustard meal/ha in 2012 were also not significantly different from the untreated control. Application of 17-34 kg DiTera/ha did not reduce TCN population densities or increase mid-season vigor ratings or flue-cured tobacco yield. Incorporating 1,345 kg mustard meal/ha increased yield compared to the 280 kg/ha rate in 2012, but not in 2011. Use of 1,962 kg mustard meal/ha significantly increased yield in 2012 compared to that from two applications of 17 kg DiTera/ha, but not compared to yield from application of 1,3-dichloropropene. Apparent TCN control with mustard meal treatments was highly variable, but consistently poor with DiTera. Additional research is needed to identify nematode management methods and products that consistently reduce plant-parasitic nematode population densities and increase tobacco yield and quality in an economically and environmentally sustainable manner.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 29

**Characterisation of PVY (Potato Virus Y) resistance in tobacco: potential role of an eIF4E gene identified by high throughput sequencing technologies**

Potato virus Y (PVY) is a plant virus belonging to the genus Potyvirus. It is transmitted by aphids, and can cause severe damage in crops.

In tobacco, a large deletion conferring resistance to PVY, the “va” gene, is commonly used. This resistance is associated sometimes with lower quality, maybe due to the absence of several other genes.

In a range of plant species, particularly in Solanaceae, it has been shown that amino acid substitutions in the eukaryotic translation initiation factor 4E (eIF4E) results in recessive resistance to potyviruses.

In the present study, we sequenced, by Illumina, the transcriptome of twelve of tobacco F7 Recombinant Inbred Lines (RILs), segregating for PVY resistance. After comparison with a reference transcriptome and annotation, genes differentially expressed between resistant and susceptible plants were identified. About thirty candidate sequences were selected, among these a sequence annotated as a eukaryotic translation initiation factor was strongly expressed in susceptible plants, and not in resistant ones. Other candidates are mostly related to the photosynthesis process.

The correlation between susceptibility and the presence of this eIF4E sequence was confirmed by PCR in 100 F8 RILs: specific primers succeeded in amplifying this eIF4E in susceptible plants, and not in resistant ones, showing complete correlation.

A range of EMS (ethylmethane sulfonate) mutants in this gene is now being studied to evaluate resistance against PVY. Two mutants with a stop codon in the sequence have already been identified, showing resistance to PVY.

The collection of natural cultivars has been screened with these markers. Natural resistant cultivars without this eIF4E gene but not altered for photosynthesis genes have been identified. Quality could be restored with these new sources of resistance and potential resistance could be increased.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 32

**Potential use of Heavy Metal Atpase (HMA) mutants to reduce cadmium translocation from root to leaf in tobacco**

Heavy metals are naturally present in soils, or can be brought by agricultural practices or spread out by human activities. Some of them are essential for plant development. Cadmium is a non-essential heavy metal which is classified in Group 1 (= known human carcinogen) by the International Agency for Research on Cancer (IARC). *Nicotiana tabacum* possesses the ability to highly translocate cadmium from roots to shoots. Therefore cadmium is accumulated at significant levels in leaves.

The molecular factors involved in cadmium distribution *in-planta* are well described in *Arabidopsis thaliana*. Among those, two Heavy Metal Atpases (HMA) are involved in the loading of cadmium into the xylem: *AtHMA2* and *AtHMA4*. Thus, genes orthologous to *AtHMA2* and *AtHMA4* were looked for in the *N. tabacum* genome.

Two NtHMA genes were identified, originating from *N. sylvestris* and from *N. tomentosiformis*, the two ancestors of the amphidiploid *N. tabacum*. Mutants were identified for each gene in our collection of EMS mutagenised tobacco plants. *N. tabacum* lines expressing artificial micro RNA (amiRNA) constructs were also developed to silence their expression.

Together with the expression pattern of the two *NtHMA* genes, data suggest that the two genes are highly redundant.

The ability of mutant lines to translocate cadmium from roots to shoots was tested. Results with plants grown under hydroponic or greenhouse conditions show a significant reduction of cadmium accumulation in the shoots. However, this reduction could be dependent on the availability of cadmium in the environment.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 30

**Mapping introgression determining potato virus Y (PVY) resistance transferred to cultivated tobacco from *Nicotiana africana***

Potato virus Y (PVY) is one of the most important pathogens affecting tobacco. Since this virus infection cannot be effectively controlled by eliminating its vectors, the main effort of disease prevention is concentrated on breeding tobacco for resistance. However, a high mutation rate of PVY leads to appearance of new necrotic strains which may affect tobacco cultivars previously known as resistant. Therefore, searching for new resistance sources is desirable. Wild *Nicotiana* species, such as *N. africana*, can be a valuable source of PVY resistance.

We studied the genetic basis of resistance in a breeding line BPA, which originates from a cross between susceptible tobacco cultivar BP-210 and *N. africana*. The BPA line does not develop necrotic symptoms when inoculated with necrotic strains of PVY. We crossed BPA line with BP-210 cultivar and then obtained the next generation by self-pollination. This F2 generation of plants segregated in respect of PVY resistance and was used as a mapping population in further analyses.

We used a high density genetic map of tobacco based on microsatellite markers (Bindler *et al.* 2011) to localise an introgression from *N. africana* associated with PVY resistance. In order to screen the whole genome, we tested markers from each of the linkage groups separated by not more than 20 cM. We tested amplification of these markers on the parents of the mapping population (BPA and BP-210) and *N. africana*. Microsatellite markers localised in the region of introgression from *N. africana* amplify in BPA line the same microsatellite allele as in *N. africana*, but different from BP-210. Currently, we are testing amplification of these markers in the segregating mapping population mentioned above.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. APOST 06

**Plant breeding for creation of late-maturing Oriental tobacco genotypes**

Investigations were made with six Oriental varieties of the tobacco sub-types Prilep, Djebel and Yaka (P-23, P-84, P10-3/2, P-76, Xanthi Djebel-1 and YV 125/3) and fifteen F1 hybrids for the characteristics flowering time and length of the growing period of tobacco in the field from transplanting to the end of harvest. The field trial was set up in 2010 and 2011 at the Tobacco Institute – Prilep in a randomised block design with four replications. All appropriate cultural practices were applied during the growing season. Statistical processing of data was performed by using the analysis of variance (ANOVA).

The aim of this work was to study the mode of inheritance and to detect possible heterotic effects for the above biological characteristics, which will allow a selection of lines with longer growing season, higher productivity and some other positive characteristics inherited from the early-maturing parent. They would be promising genotypes intended for arid regions with poorer soils and a longer growing season.

The period from tobacco transplanting to 50% flowering in parental varieties ranged from 45 to 95 days in Xanthi Djebel-1 and P-76, respectively, while the period from transplanting to the end of leaf harvesting in these two genotypes ranged from 70 to 145 days. There were differences in inheritance of the two stages among hybrids. The most common types of inheritance were partial dominance and dominance. The early-maturing parent dominated in the first period, while the late-maturing parent dominated in the second period and there was also some evidence of heterosis.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 15

**Field control efficacy of bio-organic fertiliser fermented with antagonists to tobacco bacterial wilt and its biocontrol mechanisms**

An organic fertiliser made of rapeseed meal and dairy manure with proportion of 1:1 (w:w) was then solid fermented by two antagonists, making it bio-organic fertilizer (BOF). Field experiments were conducted in Anhui Province for two years to investigate the biocontrol efficacy of BOF. Results showed that the control efficacies of the BOF treatment were up to 75.2% for the first year and 95.4% for the second year. Compared with control treatment, the activities of peroxidase (POD), catalase (CAT), superoxide dismutase (SOD) and polyphenol oxidase (PPO) of tobacco plants significantly ( $p<0.05$ ) decreased, while the activities of POD, CAT, PPO and urease in soil significantly ( $p<0.05$ ) increased. Observations of vascular bundles of healthy tobacco plants, wilted plants in control treatment and uninfected plants in the BOF treatment were made with a scanning electron microscope (SEM). The SEM results showed that there was viscous material deforming and blocking vessel of vascular bundles in wilted tobacco while the vascular bundles of healthy tobacco and BOF treatments grew well with a normal shape. The populations of cultivated bacteria, actinomycetes and antagonists were significantly higher than the control, while the counts of fungi and pathogen significantly decreased ( $p<0.05$ ). The functional diversity of the microbial community, that is to say, Shannon Index, Simpson Index and McIntosh Index of soil microbial community, were significantly higher in the BOF treatment than in the control treatment. The DGGE patterns of bacteria and fungi from the soil of BOF and control treatments belonged to two corresponding clusters, suggesting that bacterial species increased and fungi species decreased with the application of BOF. These results suggest that tobacco specific BOF application both in tobacco plants and in transplanted soils can effectively overcome the occurrence of tobacco bacterial wilt, and shows promising potential in controlling tobacco bacterial wilt.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 21

**Differential pathogenicity and genetic polymorphism analysis of tobacco powdery mildew (*Erysiphe cichoracearum*) in China**

This study was carried out in order to identify the physiological races of *Erysiphe cichoracearum* DC, and the genetic diversity of the *E. cichoracearum* DC in China. Firstly, 12 selected varieties of tobacco were inoculated with a single isolate of *E. cichoracearum* DC collected from Shandong Province, to identify the resistance level necessary for discriminating between physiological races. After inoculation, a relative resistance index was calculated based on the disease index of these tobacco varieties. Based on the relative resistance index, the inoculated tobacco varieties were divided into different resistance groups. Using inoculation experiments, seven tobacco varieties chosen from the 12 inoculated varieties were selected as a set of differential hosts for discriminating the physiological races. The flue-cured varieties CF 203 and Zhongyan 90 were the susceptible hosts, the flue-cured variety G140 was the moderately susceptible host, the flue-cured varieties Hicks and Jingyehuang were the moderately resistant hosts, the flue-cured variety NC89 was the highly resistant host and the sun-cured variety Tangpeng was the immune host. It should be noted that these results conflict with those obtained in Zimbabwe and the USA, where Hicks and NC 89 are fully susceptible. Secondly, the seven selected differential hosts were inoculated with 26 isolates collected from the main tobacco production areas in China. Based on the results of this biological test, the 26 isolates were divided into four physiological races.

The genetic polymorphism of *E. cichoracearum* DC for 26 isolates was studied using RAPDs (Random Amplified Polymorphic DNA). Firstly, 14 RAPD primers with strong amplification of polymorphic bands were selected from 50 random primers. One hundred and thirty bands were amplified, including 95 polymorphic bands. Secondly, the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram was obtained using NTSYS-PC. The 26 isolates were divided into four groups using the similarity coefficient of 0.84. The correlation analysis showed that RAPD analysis and biological tests gave similar results.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 43

**A biobed to recover and detoxify polluted external washings of agricultural equipment used for tobacco treatments: a proposal for tobacco farms**

Farms more concerned with social responsibility and environmental protection should not underestimate potential risks related to the dispersion in surface waters and soils of oil and agrochemical polluted waters, deriving from external washing of agricultural equipment. While it is largely established that internal washing waters of sprayers' tanks are reused on the same soil and crop on which a given agrochemical is permitted, much less frequently the same applies to the so-called "external washings". They are generally a mixture of washing water, agrochemical contaminated soil particles which stick to the equipment, diesel and lubricant oil residues. For the proper treatment of these "washings", a project is starting at Fattoria Autonoma Tabacchi of Città di Castello, under the scientific reference of INSTM. An off-set biobed will be built where washing waters enter, after physical removal of oil residues. The agrochemicals are those labelled for tobacco and its rotation crops. This biobed uses materials commonly found at the farm level: soil, de-watered digested phase, cereal straw, and coconut fiber-enveloped drainage pipes. A *Lolium* sp. and *Festuca* sp. turf will be overseeded on the biobed, to increase contaminated water infiltration and detoxification. After turf establishment, washing waters will begin to be sprinkler irrigated onto the turf whenever there is external washing of agricultural equipment. Two sampling wells will be built: one after the oil removing equipment (Enter Well), which will be operated also as a temporary storage tank before sprinkling, and one at the end of the biobed (Recovery Well). During the experimental period well waters will be sampled and multi-residue analysed every 21 days. Tests in similar plants, operating for 4-5 years, have indicated that this kind of biobed works very well on the agrochemicals commonly used by growers, almost reducing agrochemical residues below detection limits in the recovered leachates.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 44

**Effects of legume cover crops on soil characteristics and Virginia Bright tobacco fertilisation**

The High Tiber Valley has sandy-loam to loamy-sandy soils, high in silt and lime, and traditionally low in soil organic matter (OM). Since the late 1980s, *Trifolium squarrosum* L. (TS) has been introduced as a winter cover crop in tobacco rotations, mostly to pursue three important objectives: to protect soil against erosion, act as a catch crop against *Orobanche ramosa* L. (broomrape), and improve general “soil fertility”. The cover crop is controlled at least four weeks before tobacco transplanting, to avoid reduction in early tobacco growth. Over the time, growers using this legume cover crop progressively found their soils more and more “fertile” and productive, but sometimes experienced also increasing difficulties in nitrogen management, especially when irrigation water was in short supply. Extensive soil tests indicated a general soil OM build-up, and less frequent micronutrient deficiencies, especially of zinc. More recently, the agro-environmental measures of Regione Umbria limited the permitted maximum nitrogen rate on tobacco, and this prescription has renewed the interest in legume cover crops with higher nitrogen fixing potential vs. *TS*, e.g. *Vicia faba* L. (broad bean). This presentation describes the changes in soil properties and crop yield potential related to the introduction of legumes as winter cover crops in tobacco during the last 25 years, and how this has affected farm operations, agrochemical and fossil nitrogen use, while striving to improve tobacco yield and quality, and maintaining growers’ returns.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 41

**Approaching the first station on the way to certified organic tobacco: (almost) chemical-free tobacco**

Among EU countries, Italy is the largest producer of certified organic crops, especially in the areas of fresh and processed fruit and vegetable products, meat, and dairy products. Certified organic cropping, however, requires an incentive in order to pay for the increased production costs and the costs related to the full separation between the two, conventional and organic, production lines. This latter requirement is difficult to apply in large production units, and this accounts for most of the lag in certified organic tobacco development. For the last ten years, at Fattoria Autonoma Tabacchi and OPTA, we have been testing several technologies, in order to switch from full chemical to mixed chemical-biological strategies, to feed and protect tobacco, with the aim of producing an almost chemical-free tobacco crop. While some aspects have been successfully solved (nutrition), others (crop protection) still require efforts both at the research and farm operation levels. This presentation outlines what was done, and what is in progress, both concerning the experimental activities under way at OPTA, and the perspectives related to innovative products under development, which may find a niche of use on chemical-free tobacco.

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**MSANGO K.R.; LONGWE L.L.**

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 26

**Efficacy of insecticide-impregnated Carifend® net (ICN) in controlling tobacco beetle (*Lasioderma serricorne*) in Malawi**

The tobacco cigarette beetle, *Lasioderma serricorne*, is a serious insect pest of cured tobacco in Malawi. Fumigation with phosphine is the most common method of control for infested tobacco; however, fumigation alone has not been effective especially when it comes to secondary infestation that requires tobacco to be re-fumigated. This is costly and it increases the chances of beetle resistance to phosphine. An experiment was, therefore, conducted during the 2011 and 2012 seasons to assess the efficacy of the insecticide-impregnated Carifend® net (ICN) against tobacco beetle in warehouses containing tobacco with the goal of widening control options for this economic insect pest. Three separate experiments (each running for a period of thirteen weeks) were carried out in tobacco warehouses at Kanengo in Lilongwe district, Malawi, in collaboration with Alliance One International (AOI). Treatments included tobacco stacks covered with ICN after fumigation with phosphine, tobacco stacks covered with ICN without fumigation, and stacks of tobacco without ICN and fumigation. Results indicated that tobacco stacks covered with ICN after fumigation with phosphine were better protected from tobacco beetle with traps recording a mean of 13 beetles in the first run and none at all in the subsequent runs during the entire thirteen week period under evaluation. The untreated tobacco stacks (without ICN and fumigation) recorded significantly higher beetle counts compared to any other treatment in the trial with average trap catches of 870, 42, and 33 beetles in the three runs, respectively. These findings suggest that the impregnated Carifend® net could be an invaluable tool for managing tobacco beetle in Malawi.

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

**PAN Yihong; ZHOU Lijuan; WANG Juan; ZHANG Xiaolong; ZI Wenhua; CHEN Yunjiao; LI Wenjuan; LI Gang**

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 36

**Effect of harvest maturity on the quality of flue-cured tobacco leaves**

The study was focused on the relationship between flue-cured tobacco quality with different harvest maturity and its industrial utilisation. Appearance quality, physical property, chemical compositions and smoking quality of tobacco with different harvest maturity were analysed. The appearance quality showed no difference in lower leaves with different maturity, while middle leaves and upper leaves with maturity performed better than those with proper-maturity and those over-mature. Upper leaves with proper-maturity, middle leaves with proper-maturity or maturity indicated superior physical properties, as well as the lower leaves with proper-maturity. The content of main chemical components (total sugar, potassium ion, reducing sugars, chloride ion, total nitrogen, sugar-nicotine ratio, potassium-chlorine ratio) in tobacco leaves with different maturity were at optimum range, and the inner quality was relatively satisfactory. The results of smoking evaluation showed that the taste of upper leaves with maturity was pure and mild, little irritation, little strength and comfortable aftertaste. The middle leaves with maturity demonstrated best in aroma quality, with little smoke concentration, irritation, offensive odour and strength. And for the lower leaves with proper-maturity, their aroma volume and concentration were suitable and gave a good taste. In total, the quality of tobacco being harvested in lower leaves with proper-maturity and middle-up leaves with maturity would be much better in Jingdong, Pu'er, Yunnan Province, China.

*Yunnan Reascend Tobacco Technology (Group) Co., Ltd., Kunming 650106, P.R. China*

**PAN Yue; MAO Chuntang; DENG Guobin; ZHANG Xiaolong; ZI Wenhua; YAN Jun; QIAN Zhengqiang**

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 14

**Efficacy and toxicity of several insecticides on *Myzus persicae* (Hemiptera: Aphididae) and its biological control agent *Harmonia axyridis* (Coleoptera: Coccinellidae)**

*Myzus persicae* (Sulzer) (Hemiptera: Aphididae) has become an important and worldwide pest of tobacco causing considerable loss to tobacco quality and yield through direct and indirect harm. The efficacy of several insecticides on *M. persicae*, including botanical and chemical insecticides, was determined. These insecticides were also evaluated for one of the aphid's key biological control agents, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). The results indicated that the control effect of stemonine, melia toosendan, matrine against *M. persicae* was the best, and that of pyrethrins matrine and rotenone took second place. In addition, pyrethrins matrine and matrine had relatively greater safety coefficients to *H. axyridis* of 12.82-15.39 and 5.57-13.48, respectively. In chemical insecticides, the safety coefficient of 70% imidacloprid (7.02-9.36) to *H. axyridis* was the highest, followed by 200 g/L imidacloprid (3.90-5.85), 5% imidacloprid (3.21-5.35), 1.7% avermectin imidacloprid (1.05-1.32) and 3% acetamiprid (0.36-0.48). Our results suggested that pyrethrins matrine, 70% imidacloprid, 5% imidacloprid and 200 g/L imidacloprid may be useful for *M. persicae* due to efficacy against the aphid with differential non-target effect on *H. axyridis*. Implications of the study are discussed in integrated pest management.

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**PAPENFUS H.D.**

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. IG 01

**Sustainability in leaf tobacco production**

Sustainability in the leaf production sector of the industry aims at long-term farming. As such, it not only embraces all aspects of optimising yield, quality and profitability of the crop, but also of conserving the environment and improving quality of life for farmers, their workers and surrounding communities. Sustainability is not a new concept and has been a part of the philosophy and practice of agriculture since the start of settled farming. In recent years, however, it has been receiving heightened attention because of increasing concerns for the environment and security of agricultural products attributed to burgeoning population growth on the one hand and loss of arable land by erosion, industrialisation and accelerated climate change on the other. At the same time, there is a greater insistence by the public that agricultural products should be produced ethically, taking into account environmental and sociological issues, and that they comply with strict standards of integrity in terms of quality, traceability and purity. The industry is responding vigorously to these challenges to long-term farming. For most members, it is a critical part of their business policy. In support of this, sustainability has been a major topic at meetings of the CORESTA Agronomy/Phytopathology Study Groups for some time now, culminating in the setting up of a Task Force which is actively identifying the principal issues associated with sustainability in the leaf production sector with a view to publishing appropriate guidelines for ensuring its long-term future.

This presentation introduces the subject of sustainability in the leaf production sector, defines its key elements and illustrates how some of these are being implemented in practice.

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**PAPENFUS H.D.(1); JACK A.(2)**

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 11

**Crop rotation practices in tobacco**

Crop rotation has long been an important practice for maintaining and improving soil fertility and condition, minimising erosion and slowing down the build-up and spread of pests, diseases, and weeds. It is therefore a corner-stone of IPM programmes and for achieving sustainable crop production.

A survey was conducted on current rotation practices in tobacco to provide information for the “Rotations” chapter of the CORESTA IPM manual. Information was obtained from 21 countries, including all the major producers and covering both small and large scale production systems. It indicated that rotation is practised mainly to assist in the control of the common soil borne fungal diseases and nematodes and, to a lesser extent, to assist in control of insect pests, broom rape and weeds.

Intervening crops vary widely between countries. Some are chosen because they are not hosts of tobacco pests, others for purely economic purposes.

Length of rotation varies from short term with one crop alternating with tobacco in one year, such as rice and tobacco, to long term with 3-4 years between tobacco crops rotating with a grass pasture on its own or supplemented with crops such as maize or groundnuts.

Crop rotation is not practised universally, especially in the case of small scale farmers, in the main because of issues of land tenure, small farm size with little opportunity for expanding, limited farm income and the need to be self-sufficient in food. Also, because of the high cost of seed for suitable alternative crops, availability of resistant varieties and practices that enable the crop to be grown when pest pressures are small and conception that pest control by crop protection agents is adequate and more economical.

Details of the findings are presented and discussed in relation to implementation of IPM programmes, achieving sustainable production, education and research.

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**PEEK D.R.(1); DENTON P.(1); CLICK C.(2); WHITLEY S.(3); PEARCE R.C.(4)**

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 03

**Effect of reduced rates of maleic hydrazide on sucker control and residues in regional Burley sucker control tests**

Maleic hydrazide (MH) is widely used for sucker control in US Burley tobacco production. Tobacco manufacturers have expressed concern about MH residues in Burley and requested that producers reduce MM rates. Generally, it is assumed that reduced application rates should result in lower residue in cured leaf, but few studies have actually investigated this relationship in Burley. Universities in the US Burley growing region annually conduct a regional sucker control test at five locations. In recent years (2009-2012) these studies have examined the efficacy of varying rates of MH and cured leaf samples analyzed for MH residue. Rates evaluated were 1.68, 2.52 and 3.36 kilograms/hectare active ingredient of MH. Samples were taken from three stalk positions - cutter, leaf and tips. MH residues in 2009 were generally low, less than 25 ppm in all cases except one, and generally below the detection limit of 10 ppm at the lowest rate. In 2010, MH residues were much higher. There was a very clear trend of reduced MH residues from reduced MH rates. At one location with dry conditions after topping, residues from the standard recommended rate of MH (2.52 kg/ha a.i.), exceeded 100 ppm. MH residues were below 80 ppm at the two lowest rates at all stalk positions. At the highest rate, residues exceeded 80 ppm in at least one stalk position at four of five locations. In 2011, MH applied a rate of 2.52 kg/ha a.i. resulted in residues of 80 ppm or more at one location. However, when MH was applied at the higher rate (3.36 kg/ha a.i.) residues exceeded 80 ppm at three locations and exceeded 200 ppm at one location. Across all locations and years, sucker control was not different between MH rates of 1.68 and 2.52 kg/ha a.i. when MH was combined with a local systemic material.

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**PRZYBYS M.; DOROSZEWSKA T.**

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. APPOST 12

**Effect of weather conditions on PVY infections**

Potato virus Y (PVY) is one of the main tobacco pathogens in the world. The main aim of this study was to determine the effect of weather conditions (temperature, humidity, precipitation, insolation) on infection of tobacco plants with PVY. To determine the relationship between results of field observations and meteorological data, Spearman's rank correlation coefficient was used. Only temperature had a statistically significant effect on PVY infection of tobacco. The number of infected plants do not depend directly on the maximum or minimum temperature, but the duration of the time period with high temperature. Only in the case of the temperature range  $T > 20$  °C, statistically significant result  $R^2 = -0.561487$  was obtained ( $\alpha = 0.05$ ), which means that the long period with average temperature above 20 °C is negatively correlated with the level of PVY infections. In the years 2003, 2006 and 2010, there were low levels of PVY infections (below 30%), as there were limited by high temperatures during the growing season. A different situation was observed in 1996, 2004 and 2009, when the cool summer led to a large increase of PVY infections.

Despite previous assumptions that cold winters can affect the population of aphids – the PVY vector – and thus lower the number of infected tobacco plants, results obtained revealed that there was no significant effect on PVY infections.

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**REED T.D.**

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 02

**Factors impacting maleic hydrazide residues on flue-cured tobacco**

Maleic hydrazide (MH) remains an important compound for efficient, cost effective sucker control for flue-cured tobacco production in the U.S. Minimizing MH residues in cured tobacco is critical to maintaining the demand for U.S leaf in international markets. The objective of on-going research at the Virginia Tech Southern Piedmont Center is to develop best management practices (BMPs) for the use of MH on flue-cured tobacco. Such strategies include: the application of reduced MH rates, better understanding plant factors impacting MH residues, low volume wash-off treatments, and alternative application techniques. One study investigated the impact of time-of-day for MH application (8:00 a.m., noon, and 4:00 p.m.) and timing of the application in relation to first priming (prior to, shortly after, and delayed). Green leaf tissue was periodically sampled to quantify the natural weathering of MH residues from rainfall as well as the corresponding cured leaf. A second study investigated the impact of low volume wash-off application on MH residues. Wash-off application volumes of 560 and 1680 L/ha were compared at both 2 and 6 hours after MH application. Data from 2012 demonstrated significantly lower MH residues following a wash-off rate of 1680 L/ha applied at 2 or 6 hours. Sucker control was not adversely affected by the low volume wash-off applications. The “conveyor” sucker control application technique produces a concentrated spray pattern of very coarse droplets. Comparisons were made of three rates of MH made with and without the conveyor spray hoods. Treatments were evaluated for green leaf residues the day after application, cured leaf residues on both cutter and tip leaves, and sucker control. Results of the above research should provide BMPs necessary to allow growers to continue to use MH for effective sucker control and provide tobacco minimal residues.

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

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 27

**Preliminary evaluation of correlation between within-barn curing environment and TSNA accumulation in dark air-cured tobacco**

It is not uncommon to see significant variability in TSNA content when sampling cured leaf within dark air-curing barns. This variability may be due to subtle changes in the curing environment between different areas of the barn. A graduate student project was initiated in 2012 through support from a CORESTA Study Grant to evaluate if differences in microenvironments during curing within designated areas of curing barns can be correlated with differences in TSNA accumulation. Experiments are being conducted in barns near Princeton and Lexington KY where curing environments (temperature and relative humidity) are being monitored at 27 locations within each barn for the entire cure and compared to TSNA content from samples of low converter (LC) and high converter (HC) selections of TR Madole dark tobacco collected from those same locations. During the initial 2012-2013 curing season, average TSNA accumulation across each barn and selection was nearly twice as high at Lexington compared to Princeton (3.61 µg/g at Lexington and 1.88 µg/g at Princeton). Within TR Madole LC, average TSNA content was 0.39 µg/g at both locations, but TSNA within TR Madole HC was 6.82 µg/g at Lexington and 3.37 µg/g at Princeton. Within TR Madole HC at Lexington, TSNA content averaged 5.86 µg/g in the top tier, 7.19 µg/g in the middle tier, and 7.41 µg/g in the bottom tier. Within TR Madole HC at Princeton, TSNA content averaged 4.40 µg/g in the top tier, 2.92 µg/g in the middle tier, 2.80 µg/g in the bottom tier. Differences in average TSNA between Lexington and Princeton is likely associated with the average number of hours during the cure that exceeded 80% relative humidity, which was 407 hours at Lexington and 131 hours at Princeton. Correlation between TSNA and relative humidity between individual locations within each barn will be discussed.

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**SCHOLTZ A.**

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 46

**Sustainable of tobacco production in Africa**

Tobacco is produced in Africa by small scale growers and commercial farmers and the sustainability of tobacco production in these farming systems is discussed. The author will refer to tobacco production factors that improve farming productivity and profitability, the development and implementation of training programmes for small scale farmers and leaf technicians, the implementation of minimum standards and best practices in small scale production programmes, the optimisation of land use and food with tobacco programmes for small scale growers and the harmonisation of tobacco farming with environmental issues.

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**SHI Hongzhi(1); WANG Ruiyun(1); BUSH L.P.(2); ZHOU Jun(3); FANNIN F.F.(2);  
BAI Ruoshi(3); YANG Huijuan(1)**

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 28

**Changes in TSNA contents during tobacco storage and the effect of temperature and nitrate level on TSNA formation**

Samples of Burley, sun-cured and flue-cured tobacco from the main producing areas of relevant tobacco types in China were collected to study the changes in tobacco-specific nitrosamine (TSNA) contents during storage and to investigate the effect of storage temperature and tobacco nitrate level on TSNA formation in cured tobacco. Contents of TSNA in Burley and sun-cured tobacco increased substantially during one-year under natural storage environment, with total TSNA content increasing about 215% for both tobacco types. The most rapid increase occurred during the high temperature season. Temperature had significant promoting effect on TSNA formation during storage. Storage temperature as high as 27 °C for 12 days was enough to induce the increase of TSNA formation, while the most significant effect was shown when the temperature was above 30 °C, and the increased rate of accumulation became greater as the temperature increased. Total TSNA content in air-cured Burley tobacco after the treatment of 60 °C for 24 days was 772% higher than that in the low temperature control. Different types of tobacco showed different results in terms of the response of TSNA formation to high temperature. TSNA formation in flue-cured tobacco did not increase after high temperature treatment for 36 days, while Burley and sun-cured tobacco saw dramatic increase of TSNA content. This difference could be explained by the fact that Burley tobacco and sun-cured tobacco usually had more than 10-times the nitrate content found in flue-cured tobacco. Addition of nitrate to flue-cured tobacco to the level equivalent to Burley tobacco followed by high temperature treatment increased the TSNA concentration to a level comparable to Burley tobacco. The interaction between high temperature and abundant nitrate content in cured tobacco could be responsible for TSNA formation during storage.

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VANN M.C.

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 04

**Current status of the Field Testing Program for pesticide residues in North Carolina flue-cured tobacco**

Pesticide residues on tobacco have received increasing attention in recent years. While pesticide residue evaluations have been conducted for many years in tobacco, recent legislation granting the Food and Drug Administration regulatory oversight of tobacco products has brought the need to determine expected residues from pesticides used in tobacco production into focus. As part of an on-going program at North Carolina State University, several pesticides are evaluated over a three-year period. The objective of the research is to establish the maximum expected residues on cured leaf that would result from a maximum labeled application and minimum pre-harvest interval (PHI).

Residues of four pesticides (bifenthrin, chlorantraniliprole, clothianidine, and flubendiamide) were measured on flue-cured tobacco at two locations in North Carolina in 2010, 2011, and 2012. Each product was applied according to maximum usage and minimum PHI according to the current or most recent US label. Treatments were applied to flue-cured tobacco grown on research stations near Rocky Mount, NC, and Kinston, NC. Tobacco was managed according to Extension recommendations and harvested four times by individual stalk position. Cured leaf pesticide residues were determined by Global Laboratories in Wilson, NC.

General observation shows that bifenthrin, chlorantraniliprole, and clothianidin had very low residues that decrease from the lower stalk position to the middle and upper stalk positions. Many results for these two pesticides were below detectible limits. Alternatively, the results for flubendiamide are relatively high when compared to the other compounds. Across all stalk positions and all locations; the highest mean residues observed for bifenthrin, chlorantraniliprole, clothianidin, and flubendiamide were 3.745, 7.425, 1.014, and 25.835 mg/kg, respectively. The presentation will further summarize the testing results from the entire research cycle of 2010-2012 with data presented by individual year, location, compound, and stalk position. Additional information will be presented regarding changes to and the future of the Pesticide Residue Testing Program.

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VANN M.C.

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 05

**The United States tobacco GAP program: an overview from creation to implementation**

Good Agricultural Practice (GAP) programs have become extremely common for much of the fruit and vegetable industry in the United States; however, programs similar in nature have never been required for the tobacco industry. In 2012, a standardized GAP program was created for U.S. tobacco producers with the intention of implementation for the 2013 growing season. The GAP program was created through the joint efforts of the tobacco industry (leaf dealers and manufacturers), tobacco interest groups, the Cooperative Extension Services of tobacco producing states, and other governmental and non-governmental organizations. The intention of the program is to train and certify every contracting tobacco producer, regardless of tobacco type, on GAP initiatives in order to ensure that tobacco is produced in a manner that demonstrates sound agronomic practices, environment stewardship, and that protects workers' rights. This presentation will give a brief overview of the formation of the U.S. Tobacco GAP Program, U.S. Tobacco GAP initiatives, GAP implementation and record keeping on-farm, and how the North Carolina Cooperative Extension Service provided training to over 2,000 tobacco producers in the state of North Carolina.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 07

**The evaluation of alternative fertilizer rates and programs in the northwestern Piedmont region of North Carolina**

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

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

Two on-farm evaluations were conducted in 2012 in Forsyth and Davie County, North Carolina. Nitrogen was applied at two rates: 65 and 85 lbs. N/acre (from 28% UAN) and phosphorus (from 0-46-0) was applied at three rates: 0, 25, and 50 lbs. P<sub>2</sub>O<sub>5</sub>/acre. Potassium was applied to each plot at a rate of 75 lbs. K<sub>2</sub>O/acre (from 0-0-22, K-Mag). All possible combinations of nitrogen and phosphorus rates were used. An additional treatment of 500 lbs. 8-8-24/acre at transplanting + 10 lbs. N/acre, from 15.5-0-0, at layby was included as a conventional check. Plant height at layby, flower emergence prior to topping, and yield/quality/value were unaffected by fertility rate and fertilizer source. Early results demonstrate that reduced nitrogen rates, reduced phosphorus rates, and liquid nitrogen sources are acceptable in this region.

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## VANN M.C.

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 12

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

In recent years, the glyphosate resistant weed Palmer amaranth (*Amaranthus palmeri*) has become a major production issue in agricultural systems in the south-eastern United States. The continual use of glyphosate tolerant crops, such as corn, cotton, and soybeans, in succession has allowed the weed to spread with little to no control. However, it has been observed that when flue-cured tobacco is used in a cropping rotation the populations of Palmer amaranth are greatly reduced. Tobacco, in general, is considered to be a useful tool for the reduction of certain weed species populations because of field preparation methods, the use of secondary cultivation post transplanting, differing herbicide chemistries, and the implementation of hand labor which can be utilized to manually remove large weeds not adequately controlled with other management strategies.

Research was conducted in 2012 at the Upper Coastal Plain Research Station near Rocky Mount, North Carolina, to determine the effects of tillage (deep versus shallow) in field preparation, herbicide combinations (clomazone versus clomazone+sulfentrazone), and hand weeding (hand weeding versus no hand weeding) on weed species populations. Weed species identification and quantification were conducted using a 1.21 m by 1.21 m sampling square. Weed sampling occurred three times between transplanting and layby in each plot with hand weeding occurring once around topping in designated plots. Weed species counts, total time to hand weed plots, yield, quality, value, total alkaloid, and reducing sugar data were recorded.

Preliminary results favor the use of deep tillage in combination with clomazone+sulfentrazone. Hand weeding was beneficial when shallow tillage was utilized and/or clomazone was applied alone. The effects of each treatment will be measured in subsequent rotational crops of cotton and soybeans.

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## VANN M.C.(1); ELLINGTON G.H.(2)

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 45

### **Reducing curing costs and alternative fuel sources for U.S. flue-cured tobacco production**

With significant increases in both the price of fuel and building materials as well as an increased focus on environmental sustainability, U.S. flue-cured tobacco producers have begun to consider the entire curing process in greater detail. Management practices as simple as using a wet-bulb thermometer during curing (58% of producers) to reinsulating barns and new barn pads have been implemented. Additionally, major retrofits such as the use of automatic ventilation controls (43% of producers) or alternative fuel sources are being used as well (15% of producers). With the implementation of the U.S. Tobacco Good Agricultural Practices (GAP) Program the subject of curing efficiency and sustainability will continue to receive major emphasis.

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

CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 09

**ECLT Foundation's work and a current focus on promoting decent youth employment in tobacco agriculture in Malawi**

In agricultural supply chains, if smallholder/family farmers can be supported in making basic improvements in safety and health standards, then much of what is currently regarded as “hazardous child labour”<sup>[1]</sup> could be reclassified as “decent youth employment.” Simple improvements in safety and health risk management can be made to address the hazards of certain tobacco and agriculture youth employment activities currently classified as illegal on the national (government) hazardous child labour lists for any child under 18 (and where the farmer using/employing children on such activities - could be subject to criminal sanction, i.e. fines and/or imprisonment).

The Eliminating Child Labour in Tobacco Growing Foundation (ECLT), based in Geneva, currently operates five comprehensive projects in tobacco-growing areas in Uganda, Mozambique, Malawi, Tanzania, and Kyrgyzstan, assisting communities and district governments to eliminate hazardous child labour, and prevent all forms of child labour. The ECLT Foundation has added policy and advocacy activities to its project implementation agenda. Recently, the Foundation sponsored a national conference on child labour in agriculture in Malawi, a visible event convened by government, including labour, agriculture, social protection, ILO, International organisations, trade unions, tobacco growers associations, tobacco companies, and civil society.

The proposed session focuses on one of the on-going efforts following on the agreements of the national Malawi conference, aimed at influencing public policy: the promotion of Youth Employment in agriculture by addressing conditions that contribute to hazardous child labour. The session will also describe ECLT Foundation's approaches to improving livelihoods of farmers, ensure education for children, and build capacity in governments, multiple stakeholders, and local communities to sustain systems and practices that protect children in tobacco-growing areas.

[1] Employers' and Workers' Handbook on Hazardous Child Labour. ILO ACTEMP and ILO ACTRAV, Geneva, 2011

*Eliminating Child Labour in Tobacco Growing Foundation, 14 Rue Jacques Dalphin, 1227 Carouge, Geneva, Switzerland*

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 39

**Characterisation of two members of the AN subfamily, NtANs, from *Nicotiana tabacum***

Tobacco (*Nicotiana tabacum*) is an important leaf economic crop. Narrower and thicker upper leaves are the primary cause of its lower usability in southwest China. ANGUSTIFOLIA (AN) gene controls the polar elongation of leaf cells in leaf width direction and the trichome-branching pattern in plants, which will play an important role in improving width and thickness of leaves. Therefore, characterisation of a member of the AN subfamily, NtAN, from *Nicotiana tabacum* will be crucial to increase usability of upper leaves. In this report, we cloned two cDNAs and the genes of tobacco (*Nicotiana tabacum*) AN (NtAN) which originated from diploid species *N. tomentosiformis* (NtAN-T) and *N. sylvestris* (NtAN-S), respectively. The full-length cDNA of NtAN-T is 2,288 bp and contains a 1,893 bp ORF that encodes a protein of 630 amino acids. The 5' and 3' UTR are 132 and 262 bp in length, respectively. NtAN-T gene has a length of 5,537 bp, and consists of seven exons and six introns. They are 2,800 bp in length cloned in the gene's 5' flanking region. The full-length cDNA of NtAN-S is 2,410 bp and contains a 1,899 bp open reading frame (ORF) that encodes a protein of 632 amino acids. The 5' and 3' untranslated regions (UTR) are 127 and 384 bp in length, respectively. NtAN-S gene has a length of 5,529 bp, and consists of seven exons and six introns. They are 2,322 bp in length cloned in the gene's 5' flanking region. The NtAN-T and NtAN-S shows 54.9% and 67.8% identity to an AN orthologue from *Arabidopsis thaliana* (L.), respectively. These results will provide important an base for the AN gene's function on leaf width regulation in tobacco.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 18

**Regional characteristics for flue-cured tobacco at the Tropic of Cancer, Yunnan Province**

Tobacco planting is especially responsive to weather fluctuations mainly due to the impact of the environment on quality and quantity. The availability of environment realisations with an adequate accuracy is an essential component of tobacco production. This paper sought to determine the relationship between chemical components and environmental varieties of flue-cured tobacco under the relative similar latitude at the Tropic of Cancer, located in the southern Yunnan Gold Corridor Tobacco Cultivation Region. The result demonstrated the regional characteristics of major chemical components and environmental factors. They showed that there were three chemical types of flue-cured tobacco in this region, which were associated with high total nitrogen, high Cl as well high total sugar and K<sub>2</sub>O but low total nitrogen and Cl content. Four environmental-type areas were also revealed including humid-warm-short solar duration, dry-hot-long solar duration, humid-warm-median solar duration and dry-warm-median solar duration. The findings underscored the importance of environment effects on major chemical components. The total sugar and K<sub>2</sub>O contents increased while total nitrogen and Cl decreased when the precipitation, average temperature and solar duration increase. It might be possible to adjust critical technological decisions for flue-cured tobacco cultivation that would result in significant improvement in quality control efficiency.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 35

**High-capacity processor for air-cured, whole-plant tobacco**

A prototype high-capacity processor for air-cured, whole-plant tobacco has been designed and tested by the University of Kentucky. Whole, air-cured plants are cut into axial segments by pairs of counter rotating disks spaced 10 cm apart. The segments, consisting of stalk and detached leaf components, are grouped into three (3) grade categories by axial stalk position. A combination of mechanical and pneumatic action is used to separate stalk and leaf materials.

Approximately 80 Mg of air-cured Burley tobacco was processed during January-February, 2013 at the Doug Langley farm near Shelbyville, KY (USA). Ten workers processed and baled 10-15 300 kg bales per work day. Design capacity for the system, designated as the Cured Plant Segmenting-Separation System (CP3S), is targeted as 2.5 plants/sec using 6 workers.

Tobacco processed by the CP3S was found to include excessive stalk material (1.66% by weight, versus 0.03% expected). Design revisions are underway to address insufficient stalk fragment removal while increasing processing capacity. Approximately 8% of leaf weight remained attached to stalk segments which were discarded by the processor. However, discarded leaf fragments included only 25% lamina, being comprised by the largest segments of midribs.

Development and testing will continue through the 2013-2014 U.S. Burley processing season. The work is sponsored by Philip Morris International Management, LLC and Dr. Donald Fowlkes, Manager, Agronomy, serves as project advisor.

A U.S. Provisional Patent Application (no. 61/750,575) has been filed pursuant to intellectual property rights claims for the CP3S.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 24

### **Colonisation of *Pseudomonas aeruginosa* SXYC15 in tobacco via EGFP**

*Pseudomonas aeruginosa* SXYC15 (the SXYC15 strain) was isolated from fresh leaves of tobacco, which had considerable antagonism to *Ralstonia solanacearum*. The stability of the control efficacy mainly depends on whether antagonistic microbes could effectively colonise the plant. Based on molecular biology method, a recombinant plasmid vector pEGFP was constructed by *egfp*, pBBR1MCS-5 and pME6010. The plasmid vector was transferred into the SXYC15 strain by electroporation. Antibacterial activity was detected by the plate assay method and the genetic stability was screened by an antibiotic marker. The pattern of colonisation of the tobacco was observed with fluorescent confocal microscopy and control efficacy was calculated after the tobacco seedling being inoculated the labelled stain on tobacco bacterial wilt. The results showed that *egfp* was successfully inserted into the SXYC15 strain and the labelled strain was stable after 25 generations. Antibacterial activity testing proved that there was no obvious difference between the original strain SXYC15 and the labelled strain. The SXYC15 strain could be colonised in tobacco rhizosphere soil, root tip and the central root; Four doses of pEGFP-SXYC15 ( $1 \times 10^3$  cfu·g<sup>-1</sup>,  $1 \times 10^4$  cfu·g<sup>-1</sup>,  $1 \times 10^5$  cfu·g<sup>-1</sup>,  $1 \times 10^6$  cfu·g<sup>-1</sup>) showed different control efficacies (which were 46.21%, 49.65%, 53.24% and 68.45% respectively) on tobacco bacterial wilt. The study provided a scientific basis for the SXYC15 strain on controlling tobacco bacterial wilt.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 19

### **Comparative shotgun proteomic profiles in response to topping in *Nicotiana tabacum* leaves**

To explore how the differentially expressed proteins during leaf development respond to topping, shotgun proteomic investigations were carried out and a total of 198 and 193 proteins were identified from tobacco, respectively. Among them 64 and 59 proteins were identified uniquely in pre- and post-topping leaves, respectively, of which 46 and 40 proteins had functional annotations. According to the annotations of the proteins, functional categories were analysed during the specific proteins identified in the two groups. The most abundant categories of the proteins identified in the pre-topping leaf group were those related to photosynthesis and ATPase energy, while the most abundant categories identified in the post-topping leaf group were resistance/defence related. The differentially expressed proteins between pre- and post-topping leaves were involved in different metabolism pathways. In pre-topping leaves, the identified proteins participated mainly in highly active energy and synthesis metabolisms, such as photosynthesis, energy metabolisms and biosynthesis. At the same time a high level of gene and protein expression related proteins were identified in pre-topping groups. Large amounts of resistance/defence and secondary metabolism related proteins were identified in post-topping leaves. Present proteomic results indicated that topping with the removal of plant apex may trigger the defence and secondary metabolism in plant leaves. Energy synthesis and resistance proteins were the most significant differences at the protein expression level between leaves pre- and post-topping.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 17

**Alleviative effects of exogenous calcium salts on the decline of photosynthetic capacity in flue-cured tobacco under different light environments**

Experiments were carried out to investigate the regulating effects of exogenous calcium salts on the photosynthetic capability of flue-cured tobacco under different conditions of light intensity. The experiments were also carried out to elucidate the feasible type of calcium salt that could improve flue-cured tobacco growth and to ascertain the mechanism of calcium salts on light use efficiency under low light intensity. With flue-cured tobacco (*Nicotiana tabacum*) cv Zhongyan 100 as experimental material, a pot experiment was carried out during 2012-2013. Two studies including four kinds of light intensities and three types of calcium salts were studied to evaluate the effects of the cooperation of light intensity and calcium salts on the photosynthetic physiology indexes, chlorophyll fluorescence characteristics and the accumulation of dry matter in flue-cured tobacco. The content of pigments which included chlorophyll and carotenoid in flue-cured tobacco leaves were significantly increased with the increase of shading and the application of calcium salts. In the early stage of growth, the values of net photosynthetic rate (Pn) decreased with shading, however, the rate of decrease was slowed down in the shading treatments during the maturation stage. The highest content of Pn was recorded in the L2 levels during 75 days after transplant. The photochemical quenching coefficient (qP), maximum photochemical efficiency (Fv/Fm) and the PSII electron transmit rate (ETR) were increased with the application of calcium salts, however, the original fluorescence (Fo) and non-photochemical quenching coefficient (NPQ) was decreased. As a result of acclimatisation for low light environment, the photochemical efficiency was promoted when the light level was at 70% of natural light transmittance. The results also showed that the photosynthetic efficiency and the absorption ability under low light conditions could be improved by appropriate application of calcium salts; thereby the photosynthetic rate could be promoted. Generally, the combination of 70% natural light intensity with proper application of calcium salt (Ca(AC)<sub>2</sub>) in this experiment could improve the degree of utilisation of light energy and increase the adaptability of flue-cured tobacco to adverse situations.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. APPOST 03

**Judgment of aroma types of middle and upper flue-cured tobacco leaves based on proportions of aroma components**

To establish mathematical models for judging the aroma types of upper and middle flue-cured tobacco leaves, 128 samples (63 C3F and 65 B2F) from 11 main tobacco production provinces of China were selected as materials. Stepwise discriminant analysis was applied to samples with different aroma types and discriminant function was expressed with the proportions of 67 aroma components in total aroma constituents as the index. The results showed that the proportion of most aroma components in clear and full aroma tobacco leaves was higher than that in middle aroma leaves. The proportions of 51, 43 and 40 aroma components of clear, middle and full aroma tobacco leaves were higher in upper leaves than those in middle leaves. Aroma components dominated certain aroma types and differed between middle and upper leaves. The proportions of 18 and 11 aroma components in upper and middle leaves were led in the stepwise discriminant function respectively. Self-validation and cross-validation methods were applied to evaluate the original samples, and the accuracy rates reached 100% and 98.6% on middle leaves, 96.37% and 94.4% on upper leaves. The accuracy rates on some other samples reached 100% on middle leaves and 91.7% on upper leaves as predicted by the model. In conclusion, the proportion of aroma components as discriminant index could improve discriminant accuracy significantly in the middle and upper leaves. It could be used to analyse aroma types objectively, accurately and quickly.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. APPOST 02

**Effect of flue-cured transplanting-date on leaf growth dynamics and establishment of a simulated model**

Using K326 as materials, flue-cured tobacco with four leaf positions (the fifth, tenth, fifteenth and twentieth leaves) in three sowing dates (May 4<sup>th</sup>, May 14<sup>th</sup>, and May 24<sup>th</sup>) were used in field experiments where parameters of dynamic leaf growth including length and width of leaves were measured. The results showed that: (1) A Richard curve equation,  $y=a/(1+e^{b-cx})^{1/d}$ , was developed and suitable to relationships between leaf growth or growth days and active accumulated temperature; (2) The basic parameter of ultimate growth (a) and growth rate parameter (c) changed slightly, but initial growth parameter (b) and shape parameter (d) changed dramatically. Transplanting dates regulated the equation of the dynamic mainly by controlling parameters b value and d value. The dynamic model could make a good estimation for leaf growth dynamics with the accuracies (0.9966-1.1114), and the precisions ( $R^2$ ) of above 0.9577. The differences reached an extremely significant level; (3) The sequences of growth days with different transplanting date corresponding to leaf length maximum rate showed the following trends: May 4<sup>th</sup> > May 14<sup>th</sup> > May 24<sup>th</sup>. Those of growth amount of the fifth and tenth leaf corresponding to maximum rate of leaf growth showed the same trends, while those of growth amount of the fifteenth and twentieth leaf corresponding to maximum rate of leaf growth showed the opposite trend. Growth days of flue-cured tobacco in rapid growth stage with the same transplanting period are less than those of the initial growth stage and the stable growth stage. Active accumulated temperature of three growth stages among different leaf positions performed the sequence: initial growth phase < rapid growth stage < stable growth stage.

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CORESTA Meeting Agro.-Phyto Groups, Brufa di Torgiano, 2013, abstr. AP 37

**Study on aging quality index based on ratio of free basic amino acids and free acidic amino acids of flue-cured tobacco**

In order to judge leaf ageing quality, an analysis was made of free amino acid changes. The sensory quality of 30 flue-cured tobacco produced in 2006, 2007 and 2008 from 10 regions was analysed during ageing by HPLC with precolumn derivatisation and using the rating scale method for sense-evaluation. The results showed that:

1. Ageing leaf sensory quality gradually increased in the start period. After achieving peak quality, sensory quality gradually declined. The best ageing times were different for same position samples from same regions among production years.
  2. The level of free amino acid gradually declined with the increase in aging time. The level of free basic amino acid (CFBAA) and of free acidic amino acid (CFAAA) reduced, increased, then decreased during ageing.
  3. There was a good linear relation between the ratios of CFBAA and CFAAA and aging times. The values of ratio gradually reduced with the increase in ageing time. There was a binomial relation between the ratios of CFBAA and CFAAA and sensory quality.
  4. The ageing quality index of Jinan was studied and established by the regression equation of 2007, 2008 samples and best sensory quality aging times. Test results indicated the indexes were right. The index partly imaged a chemical component equilibrium state of aging leaf.
  5. Sensory quality peaks of ageing leaf in 2006 were forecasted by quality aging index. The result indicated that the accuracy was 80%.
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