Conference Report

52nd Tobacco Science Research Conference (TSRC), September 13-16, 1998, Atlanta, Georgia, USA

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The 52nd Tobacco Science Research Conference (TSRC) was held from 13th to 16th September 1998 in Atlanta, U.S.A., at the invitation of the University of Georgia, Atlanta. The sponsor of the conference was Schweitzer-Mauduit International, Inc. Two parallel sessions were held during which 64 papers were presented dealing with the results of recent biotechnological research with respect to the tobacco plant, as well as research into the manufacture and analysis of tobacco products, human smoking behaviour and the effects of pyrolytic products on the environment and the organism.

The Conference was attended by some 300 delegates from the industry as well as from private and state research institutes, showing great interest in the event.

The title of the 52nd TSRC Symposium was "Responding to Change in Our Industry".

As LESLIE LEWIS (RJR, Winston-Salem, USA) who chaired the symposium, pointed out in her introduction, the four invited papers indeed should have covered the interests of all participants. E.C. JONES (with his coauthor ALAN NORMAN, RJR, Winston-Salem, USA) presented a comprehensive review on the 'History of Cigarette Design'. CHERYL E. JOHNSON (Celanese Acetate, Charlotte, USA) gave an informative overview entitled 'Development of Cigarette Components to Meet Industry Needs'. In both papers, apart from the achievements in cigarette design elements in the past (1950–1970), in-

cluding cigarette papers and filters, filter ventilation, reconstituted tobacco and expanded tobacco, also future challenges for the industry were considered. These include: Developing products with selectively reduced smoke constituents, improved degradability of butts and reduced ignition propensity and reduced sidestream smoke yields as well as ultra-light cigarettes with increased consumer acceptance.

PATRICK J. DUNN (Imperial Tobacco, Montreal, Canada) described 'International Tobacco Control Legislation and its Effects' with special emphasis on the situation in Canada. A. BLAKE BROWN (North Carolina State University, Raleigh, USA) discussed 'Long Term Effects of An Increase in Cigarette Prices With and Without the Tobacco Program'.

For the first time in the history of this Conference Symposium, a panel discussion was arranged. The lively discussion revealed that particularly the recently enacted Tobacco Act in British Columbia (Canada) and the pending US tobacco legislation creates a significant extent of uncertainty in the industry and for the US tobacco growers. As usual, the papers presented at the TSRC Symposium have been published in the Symposium Proceedings (*Recent Advances in Tobacco Science, Volume 24*).

Part of the scientific program of the 52nd TSRC also reflected response of the industry to change, particularly the current legislation in the US and in Canada. From the scientific point of view, one of several problems is that the analytical data required by the

legislators have to be generated by methods which have not been validated. For example, state regulation in Massachusetts (1997) and Texas (1998) require "smoke pH" measurements. Papers from MICHAEL BORGERDING (RJR, Winston-Salem, USA), DENNIS WINGATE (RJR, Winston-Salem, USA), SERBAN MOLDOVEANU (B & W, Macon, USA), CHARLENE CALLICUTT (PM, Richmond, USA) and RANA WALKER (Lorillard, Greensboro, USA) dealt with methods for the determination of "smoke pH". It became clear that not only a standard method is lacking, but also the theoretical concept of "smoke pH" is not defined. "Smoke pH" does not appear to be a very informative parameter since the values of commercial cigarettes are in a relatively narrow range (pH 4.6–5.5) and are not correlated with the smoke nicotine yields. It should be also noted that SINCLAIR, FROST and MARINER (Rothmans International, Milton Keynes, UK)¹ have recently shown that, contrary to expectation, higher "smoke pH" values reduce rather than increase the pulmonary nicotine absorption.

According to the new legislation in Massachusetts and in Canada, the industry has to report cigarette mainstream smoke yields obtained with the established standard method (ISO, FTC) and with an 'intense' puffing regimen with partial vent blocking. The intention behind this is to provide the consumer with more realistic information on the smoke intake. However, it appears to be doubtful whether this goal is achievable. Studies on human puffing profiles (DONALD W. EDWARDS and JOHN H. ROBINSON, RJR, Winston-Salem, USA), filter analysis after human smoking (F. KELLEY ST. CHARLES, B & W, Macon, USA) and a bulk of published data show that there is a large inter-individual variation in human smoke intake, even when smoking the same brand. Furthermore, the filter analysis data revealed that the estimated average yields when smoking 4–12 mg 'tar' cigarettes were closer to the common FTC-yields, whereas when smoking 1–3 mg 'tar' cigarettes, estimated average yields were closer to yields obtained with the 'intense' smoking regimen. This result hints at compensational smoking behaviour. It should be noticed that none of the proposed changes in machine smoking parameters take into account the phenomenon of partial compensation which has been demonstrated in many investigations. Therefore, it appears appropriate to state that the standard smoking methods (ISO, FTC) still supply the consumer with a useful information, namely the basis for a rank order of 'tar' and nicotine yields.

The Canadian Tobacco Act requires, apart from lots of other information, the reporting of fifty toxic

mainstream smoke constituents under standard and 'intense' smoking regimens. The way of communicating the resulting bulk of data to the public in a simple and scientifically correct way is important and certainly not an easy task. BILL RICKERT (Labstat Inc., Kitchener, Canada) proposed two methods for this purpose: A starplot which allows to quickly identify differences between and peculiarities of brands; (ii) a relative exposure index (REI) which is a summation of the ratios between the calculated 15 minute weighted average exposure (based on the smoke concentration rather than the yield of a substance) and the corresponding short-term exposure limit value for (presently) 28 smoke constituents. There are a number of serious problems with this REI and the future must show whether this approach is suitable.

M. CUI and X. WEI from the research groups of L.P. Bush and H.R. Burton (University of Kentucky, Lexington) presented the results of joint research projects on tobacco-specific nitrosamines (TSNA) accumulation in air-cured tobacco leaves and on the isolation and identification of a new pyridine alkaloid. M. CUI explained the influence of environmental factors, such as rain, temperature and air humidity on the formation of nitrite and TSNA during the air-curing process of Burley and dark tobaccos in barns. According to CUI *et al.*, the beginning of TSNA formation can be observed in the mid rib after a drying period of 14 days, showing a marked increase depending on the prevailing environmental conditions and the water content of the leaf. In general, it was found that high air humidity during the curing process slows down the water loss of the leaf, thus leading to increased microbial activity. This promotes nitrite formation in the mid rib and increased amounts of TSNA may be formed.

In years with high rainfall (1996), for instance, approximately ten times the amount of TSNA (20 µg/g) was measured after a curing period of 56 days as compared with a dry year, such as 1994. Temperature was not found to have a significant influence on TSNA formation during the curing process.

X. WEI presented the biosynthesis and quantification of the pyridine alkaloid 3,5-bi(*N*-methyl-2-pyrrolidinyl) pyridine (MPN) in tobacco. The endogenous formation of this recently discovered compound in the plant seems to occur via 3,5-dinicotinic acid.

A purification method was developed to identify and quantify MPN in tobacco tissue. By extracting tobacco using an acid buffer solution (pH=3) and in subsequent purification steps by means of methylene chloride and a GC/MS analysis of tobacco specimens, MPN concentrations of up to 100 µg/g d.w. were found in the roots. As reported by WEI, MPN concentrations in dried lamina were about 47 µg/g. The smoke of a 2R1 Reference cigarette was

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¹ Paper presented at the 1998 CORESTA Congress, Brighton, UK, October 11–15, 1998

found to contain 5 µg MPN/cigarette. The transfer rate from the cigarette into the smoke was 10 %.

H.-J. EBERHARDT (VdC, Bonn, Germany) presented recent biotechnological methods and ways of using biotic and abiotic activators of the plant's defence systems in order to provide effective protection for the tobacco plant against pathogen infestation.

One of the plant activators already used commercially, which are applied to induce resistance against infection by viruses, bacteria and fungi, is benzothiadiazole (BTH). This plant activator, for instance, is highly effective in protecting the tobacco plant against blue mold infestation. BTH imitates the natural induction properties of the endogenous signal substance salicylic acid which has been found to be synthesized more intensely in numerous plants in case of infection or lesion. In view of its phytotoxic properties and its quick deactivation when sprayed onto the plants at higher concentrations, salicylic acid (SA) is not suitable for exogenous application. BTH and SA differ from conventional plant protective agents in that they do not exhibit direct antimicrobial effects but promote the development of resistance against pathogens by activating the defence systems of the plant.

The elicitors cryptogein and capsicein as well as various oligosaccharides, such as glucans, oligogalacturonides and chitosans were also discussed as further candidates for possible use as plant activators in the future.

Cryptogein and capsicein are proteins secreted by fungi of the genus *Phytophthora*, which develop their resistance-inducing activity by binding to plasma membrane proteins of the tobacco cell.

The oligosaccharides may derive from the cell wall of the pathogen or may occur as a cleavage product of the cell wall of the plant cell. However, due to a number of unfavourable molecular properties (e.g. necrotising activity of cryptogein, the refractory effect of the oligogalacturonides, i.e. a second exposure to the same stimulus is without effect), direct use of the elicitors and oligosaccharides as plant activators does not seem advisable. In order to weaken the necrotic activity of the elicitors or eliminate the refractory effect of the oligogalacturonides, the molecules need to be modified into a form in which their practical use as plant protective agents appears feasible.

Finally, the abiotic stimulus UV-B and UV-C was mentioned by the authors EBERHARDT and WEGMANN as the last example for induction of the plants' defence systems against TMV infections. UV light at certain wavelengths, intensities and irradiation periods may promote the development of resistance against certain pathogens in tobacco. While this effect has, unfortunately, not been sufficiently investigated so far, it would represent a new

approach to plant protection which does not use chemicals and is completely residue-free.

M.H.M. OLIVEIRA (Souza Cruz, Rio de Janeiro, Brazil) presented a method for detecting 23 different organophosphate pesticide residues in dried tobacco. It is based on ethyl acetate as extractive agent, followed by a cold coagulation cleanup at a temperature of 10 °C. The actual processes of detection and quantification are carried out by means of high resolution gas chromatography (HRGC) coupled with a phosphorus-sensitive flame photometer detector (FPD). This method was presented by M.H.M. OLIVEIRA as a selective and sensitive procedure for the detection of organophosphorus residues on tobacco, allowing good recovery rates, and covering a sensitivity range of 5–40 µg/kg, in relation to the organophosphorus compound to be detected.

W. WEEKS (Department of Crop Science and Entomology, North Carolina, USA) presented a number of studies on the determination of aroma components in stored flue-cured tobaccos, which are responsible for attracting the tobacco beetles. The tobacco beetle, *Lasioderma serricorne*, one of the most feared storage pests of tobacco, primarily reacts to specific volatile aroma components of the tobacco. This is the initial hypothesis of the authors WEEKS and KEEVER on which their experiments are based. Steam distillations were carried out in order to isolate and purify these volatile aroma constituents. The distillate was then collected and extracted using various solvents, such as methylene chloride, ethyl ether or pentane. After denicotinisation of the extracts and the addition of cellulose powder, 50 tobacco beetles were exposed to the treated samples and their reaction was tested.

The tobacco beetles showed different reactions to the aroma fractions obtained using various extractive agents. Thus methylene chloride preparations tended to develop a deterrent effect on the insects, whereas pentane extracts attracted the pests. Further research is needed to find out whether these effects are attributable to one single substance or to the synergistic effect of several aroma components.

An interesting paper on the possible release of filter fibres during the smoking of filter cigarettes was presented by R. SHEPHERD (Filtrona International, UK). The methods used by the working group for collecting all free fibres which were presumed to be released at the filter end are based on two methods which had already been thoroughly tested by the Reemtsma Company and which were presented by W. RÖPER at the Vienna Meeting of the CORESTA Study Groups Smoke & Technology in 1995. One of these was an agar test involving repeated dabbing of cigarettes on the agar surface. The adhering fibres were then determined by microscope. The other method attempted to collect possible fibre fragments

on the Cambridge filter during simulated machine smoking under standardized conditions (ISO).

All sorts of filter types were investigated in this study, such as monoacetate filters, dual, triple or PFC filters. The number of fibres released during simulated smoking was reported to be 0.3 fibre per filter. The lengths of the identified fibre fragments exceeded a value of 35 μm for all filter types. For the triple filter, for instance, the lengths were approx. 157 μm and for the dual filter approx. 426 μm . Fibres of this order of magnitude are generally not considered to be respirable, and can therefore not be absorbed into the lung tissue of the smoker.

This year's Philip Morris research prize for outstanding achievements in the field of tobacco research was awarded to B.A. FORTNUM (Clemson University, South Carolina, USA) in recognition of his scientific contributions to combatting root nematodes of the tobacco plant.

In general, the scientific quality of the papers presented at the 52nd TSRC was good to excellent and it is certainly worth attending the 53rd TSRC which takes place in Montreal, Quebec, Canada, September 12–15, 1999. It will be hosted by the Canadian Tobacco Manufacturers' Council.