

Tobacco Research and Its Relevance to Science, Medicine and Industry*

by

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CONTENTS

Summary	133
Preface	134
Foreword	134
1 The Dawn of Plant Sciences	135
1.1 Plants and Light	135
1.2 Nutrition and Hunger Signs	136
1.3. Genetics	136
1.4 Chemical Growth Control	137
1.5 Physiological Disorders	138
1.6 Organic Metabolism	139
2 Toward Public Health	141
2.1 Radioactive Elements	141
2.2 Mycotoxins	141
2.3 Air Pollutants	142
3 Political Correctness and Academic Research	142
3.1 The Mandate on Tobacco Research	142
3.2 Tobacco Research at Land-Grant Universities and Other Institutions	144
4 The Benefits of Using Tobacco as a Research Tool	144
4.1 One of the Most Valuable	144
4.2 Most Abundant Scientific Information	144
5 Beyond the Frontiers of Science	145
5.1 Tool of Pioneering Science	145
5.2 Food	145
5.3 Medicine	145
Afterward: Treat Tobacco with Respect	146
References	146

SUMMARY

This article is a historical review and a vision for the future of tobacco plant research. This is the perspective of an experienced tobacco scientist who devoted his total professional career to tobacco research. From the very beginning,

pioneering tobacco research was the foundation of plant science at the dawn of modern development, in such areas as light, nutrition, genetics, growth control, disorders and metabolism. Tobacco research led to current advancements in plant biotechnology. In addition, tobacco plant research contributed significantly to public health research in radioactive elements, mycotoxins, and air pollutants. However, public support for tobacco research has today greatly declined to almost total elimination because of a sense of political correctness. This author points out that tobacco is one of the most valuable research tools, and is a most abundant source of scientific information. Research with tobacco plants will contribute far beyond the frontiers of agricultural science: tobacco can be a source of food supply with nutrition value similar to that of milk; tobacco can be a source of health supplies including medical chemicals and various vaccines; tobacco can be a source of biofuel. All we need is to treat tobacco with respect; the use of tobacco is only in its initial stages. [Beitr. Tabakforsch. Int. 22 (2006) 133–146]

ZUSAMMENFASSUNG

Diese Arbeit ist ein historischer Überblick und ein Ausblick auf die Zukunft der Forschung auf dem Gebiet der Tabakpflanze. Es ist die Perspektive eines erfahrenen Tabakwissenschaftlers, der seine gesamte berufliche Laufbahn der Tabakforschung gewidmet hat. Von den ersten Anfängen an war die Tabakforschung Wegbereiter für den Aufbau der Pflanzenwissenschaften, die auf Gebieten wie der Ernährung, des Lichts, der Genetik, Wachstumskontrolle, Krankheiten und des Metabolismus im Entstehen begriffen war. Die Tabakforschung führte zu den gegenwärtigen Fortschritten in der Biotechnologie. Außerdem hatte die Tabakforschung großen Anteil an der Erforschung der Bedeutung radioaktiver Elemente, der Mykotoxine und der Luftverschmutzung für die menschliche Gesundheit. Dennoch hat die von öffentlicher Hand finanzierte Tabakforschung heute stark abgenom-

men und wurde aus Gründen der politischen Korrektheit fast vollständig eliminiert. Der Autor zeigt, dass Tabak eines der bedeutendsten Instrumente der Forschung und eine reichhaltige Quelle für wissenschaftliche Erkenntnisse ist. Die Wirkung der Forschung auf dem Gebiet der Tabakpflanze wird weit über die Grenzen der Agrarwissenschaft hinausreichen: Tabak kann eine Nahrungsquelle darstellen mit einem Nährwert vergleichbar mit dem der Milch; Tabak kann eine Quelle für die Gesundheitsversorgung sein, einschließlich Medikamenten und verschiedenartiger Impfstoffe; Tabak kann als Quelle für Biobenzin dienen. Wir müssen hierzu den Tabak nur mit Respekt behandeln, die Nutzung von Tabak steckt erst in ihren Anfängen. [Beitr. Tabakforsch. Int. 22 (2006) 133–146]

RESUME

Cet article présente une revue historique et prospective de la recherche relative au tabac. Il s'agit de la vision d'un chercheur expert dans son domaine qui a consacré sa vie professionnelle à la recherche sur le tabac. Dès le début, la recherche sur le tabac a été le précurseur de recherches scientifiques sur la plante dans des domaines comme le développement des plantes, la nutrition, la génétique, le contrôle de la croissance, les maladies et le métabolisme. La recherche sur le tabac a conduit à des progrès en biotechnologies. De plus, la recherche sur la plante de tabac a contribué significativement à la recherche des effets des éléments radioactifs, des mycotoxines et des polluants de l'air sur la santé publique. Cependant, le soutien public pour la recherche sur le tabac a fortement diminué jusqu'à une élimination presque totale pour des raisons politiques. L'auteur indique que le tabac est l'un des outils de recherche les plus efficaces et qu'il constitue une source abondante d'information scientifique. La recherche menée avec les plantes de tabac aura une influence au-delà des frontières de la science. Le tabac pourra être une source d'alimentation ayant une valeur nutritive comparable à celle du lait, le tabac pourra être également une source de production de substances d'intérêt pharmaceutique et divers vaccins, et enfin le tabac pourra être une source énergétique. Nous devons traiter le tabac avec respect, l'utilisation du tabac ne fait que commencer. [Beitr. Tabakforsch. Int. 22 (2006) 133–146]

PREFACE

On behalf of the author, Dr. T.C. Tso, I am pleased to provide a few introductory comments and perspectives for the reader on the subject matter and message of this article. Dr. Tso is an outstanding former USDA Agricultural Research Service (ARS) scientist highly regarded and decorated for his contributions to and knowledge of the science of the tobacco plant.

Perhaps the basic theme of this paper is embodied well in the simple question: Has tobacco time come, gone, and come again?

Author Tso reminds us that many of the scientific understandings and technologies we have today on a myriad of subjects were results of fundamental research approaches

and discovery. Tobacco plant research is a case in point. This article illustrates noted cases of scientific findings derived from tobacco studies and that are affecting our daily lives. Such knowledge has not only aided our understanding of the plant kingdom, but has also contributed significantly to our understanding of human health, such as nutrition needs especially micro-elements, air pollutants, mycotoxins, biological pest control, virology, and biotechnology, to name a few. We also now know much about the harmful ramifications of tobacco as a smoke product.

Thomas Jefferson once said: as human mind becomes more developed, more enlightened; as new discoveries are made, new truths developed; manners and opinions change with the change of circumstances, institutions must advance also to keep pace with the times.

Yes, institutions must and do advance with time. USDA and most public institutions have appropriately moved beyond the days of tobacco research as a smoke product. As a result, and perhaps regrettably the use of tobacco as a laboratory tool also has diminished a great deal. It has been superseded to a large extent by the use of other model species, such as *Arabidopsis thaliana*; as many important new advances in understanding plant biology are being made.

Yet there remains considerable opportunity to continue to use tobacco as a research tool by building upon our already extensive fundamental knowledge of its biology. Also, there is great potential for tobacco to become a source of beneficial technologies and products such as food with high nutritional value, and as a factory for producing pharmaceuticals.

This vision is beginning to materialize at a few public and private sector laboratories throughout the country. But it can only be fully achieved when scientists are encouraged and allowed by leaders to explore the tobacco plant in spite of the public perceptions about its mixed past.

In this article, Dr. Tso reflects and expands on these issues, opportunities, and potentials. He puts forth a persuasive case for policymakers to enable tobacco research and for scientists to take up the challenge to unlock the yet hidden essences and understanding of plant biology and beneficial technologies that can be derived from tobacco. In his message the search for these truths is only at the beginning.

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FOREWORD

Politics is not an exact science. — *Bismarck*

Science is the systematic classification of experience. — *George Henry Lewes*

Man does not live by experience alone, but by transcending experience, assured of what he had not seen, as real. — *Sayings of Mencius, in wisdom of China*

As a plant physiologist and phytochemist, I used tobacco as a research tool for almost 60 years. I witnessed the dawn of the field of plant science based on the findings from tobacco research. I conducted or participated in many major

research projects of high scientific value on plant nutrition, organic metabolism, and growth regulators using tobacco during the early development of the sciences of plant physiology and biochemistry

This article is not intended to be a technical report, rather a perspective from one scientist calling attention on many significant contributions generated from tobacco research to the development of plant science, and the great potential tobacco holds for the future.

In 1965, soon after release of the 1964 Surgeon General Report on Smoking and Health, USDA scientists initiated an examination on the presence, source, and possible risk of alpha-particles in the plant; of mycotoxins in its leaf; and of pollutants in the air. They also isolated and purified fraction-1 protein and fraction-2 protein from tobacco, and they conducted animal tests identifying the proteins' high nutritional and medicinal value. The removal of those proteins also resulted in removing the precursors of certain hazardous smoke materials. And USDA scientists observed that interspecific hybridization between certain *Nicotiana* species can develop plant genetic tumors, which is associated with the presence of abnormally high level of polyphenols. As a result of those findings, USDA scientists initiated collaborative studies with several health institutions for in-depth evaluation of the possible risks of this group of compounds.

With authorization from the U.S. Congress and support from the administration beginning in 1966, USDA scientists intensified tobacco plant research in collaboration with other federal agencies, land-grant universities, health research institutions, and industry in and out of the United States. Those projects included intramural, extramural, and cooperative projects aimed at identifying and removing the specific hazardous substances in tobacco plants, either during field production or during post-harvest handling. As a result, a theoretical model of "safer" tobacco was proposed.

It is widely recognized that tobacco is an excellent research tool, similar to that of *Escherichia coli* in biological science. Even as early as the 1940s, geneticists, using tobacco, developed monosomics in studies of gene inheritance, a decade ahead of DNA's discovery. The success with tobacco on parasexual hybridization and the benefit of using tobacco in biotechnology research are well known. Furthermore, there is great potential for using tobacco to produce food, chemicals, medicines, and biomass.

The purpose of this article is to encourage scientists to continue using tobacco as a research tool, because of the wealth of existing scientific information about it and the great potential of its future. There is no need to justify tobacco research in view of the changing political climate. As an experienced scientist, I envision that the use of tobacco is only at its beginning.

This author is indebted to many scientists and staffs in the Agricultural Research Service, U.S. Department of Agriculture, for their wise counsel, especially to Dr. Edward B. Knippling who wrote the Preface. I wish also to express my thanks to Professor Lowell Bush of the University of Kentucky, who kindly reviewed this article and made many valuable suggestions.

T.C. Tso

1 THE DAWN OF PLANT SCIENCES

Biological science is a relatively new chapter of our civilization; in fact its development is only in the last 100 years. Within biological sciences, plant science developed earlier than that of animal science mainly because of nutritional, pharmaceutical, and economical needs. Among plants, tobacco is at the very front in scientific findings and thus led many breakthroughs at the dawn of plant sciences.

1.1 *Plants and light*

Near Washington, DC, where the Pentagon is currently located, there was a farm plot in Arlington, VA, where USDA scientists conducted research. In 1918, two scientists were waiting for tobacco plants to flower in order to collect seeds. However, one special tobacco plant failed to blossom while others did. In late autumn, they moved that particular plant into a greenhouse. That plant finally flowered in December and produced seeds. While others paid little attention to this event, W.W. Garner and H.A. Allard knew they had discovered something of great significance: that the relative length of day and night controls flowering. They called it "photoperiodism". In further observations in 1920, the two found that within *Nicotiana* species, there are day-neutral, day-short, and day-long plants. They extended their tests on many other plant species and observed similar phenomena. Their discovery of photoperiodism revolutionized agriculture and basic science, as well as industry. It led to the essential knowledge on which the year-round, multibillion-dollar horticultural crops industry depends – especially ornamental crops. Most important, it provides plant scientists with a tool for multiple breeding frequencies, resulting in many other beneficial and practical applications. Even in the animal area, photoperiodism is known to affect growth and development – even the human phenomenon of "jet lag". That early tobacco day-length study led to more complicated examinations on the basic science of light and darkness as it applied to plants and other organisms. For example, did the short days make chrysanthemums flower, or long nights? What about a short period of darkness in the middle of a long day? What about a short exposure to light during the long night? How can we save energy and at the same time make plants more productive?

How about the quality of light or wavelength or what kind of lamp to use? It was discovered that in photoperiodism, red light is more effective than that of any other color when used as a dark-period interruption to control flowering. A later study led to another major understanding that the action of red light on flowering is nullified by light having somewhat longer wavelengths in the near-infrared or far-red range.

The occurrence of reversibility in the ability of seed to germinate and of plants to flower suggested that it may also affect other expressions in biological development. Plant system, which prefers red to other colors, must have a system to absorb the energy of red light, a pigment that is blue. Scientists in USDA again discovered the presence of phytochrome, which is another milestone in the advancement of biological science. All this began from studies on tobacco!

1.2 Nutrition and hunger signs

Chemical fertilizer was one of the most important factors in the success of the "Green Revolution". Years ago, people knew that plants had to be fed to make them grow and to grow better. Little understanding was available on when, how, where, and what to feed them. There was minimal knowledge of soil testing, plant analysis, or physiological studies, not to mention the concept of precision agriculture. When we are hungry, we can speak and ask to be fed. Plants can also do so through their "sign language". Those signs were not fully understood until scientists conducted precise studies and detailed observations with tobacco plants. In conducting the studies for each single element, tobacco scientists had to be sure that there was no contamination from any other minor and rare elements, or from the equipment used, or from the environment. In 1933, MCMURTREY led such a study on tobacco plants under specially designed greenhouse conditions with filtered airflow and precise temperature control. Any possible factor which might affect plant growth and development was carefully evaluated, monitored, and controlled to avoid any possible error. MCMURTREY used a calculated amount of chemically pure elements dissolved in doubly-distilled water. The nutrient solution was then placed into specially made quartz glass tanks. Each tank was kept in a closed, wooden box to maintain darkness. Plant tops were supported by frames, and their roots were immersed in the test solution. During the whole period of growth, constant air supply was provided to the root system. That way, scientist could be assured that the plants were free from contamination by other known factors. In addition, both plant tops, as well as the root systems, could be observed in detail during the whole growth period. Based on several years of repeated study, MCMURTREY reported the hunger signs of tobacco plants in a USDA special bulletin. That classical examination was widely recognized as a pioneering achievement in plant science that led to many other similar studies on various crops. It also provided the means for diagnosing any nutrition deficiency in the field. It was one of the most important pioneering research projects in plant science.

It is unfortunate that a recent major agricultural journal which printed hunger signs of six crops on its cover, did not include, or even mention, tobacco. It is indeed regrettable that today's concern for political correctness could blind the scientists – or even worse, that the writer didn't know about those milestone studies.

The early 1930s was the most active era for examining plant nutrition needs and the mechanism of nutrient usage by plants, using tobacco as research tool. For example, nitrogen assimilation was reported in 1931 and 1934; light as a factor for nitrogen utilization in 1933; various weather conditions affecting nutrient absorption and, thus, the chemical composition of plants in 1936. The presence of some micro-elements was reported as early as 1921. Those scientific findings all seem "common sense" today, but they were initially derived from tobacco research.

In 1940, isotope nitrogen, ^{15}N , was first used to establish the absorption and utilization of nitrogen in tobacco plants. It was reported that from root absorption to protein incorpo-

ration took 72 hours. This led to a series of research projects on organic metabolism among amino acids, protein, organic acids, and carbohydrates in tobacco and extended to other plants. In the middle 1950s, research studies with triple labeling of ^3H , ^{15}N , and ^{14}C isotopes in tobacco plants opened the door for intensive studies of alkaloid biogenesis, loci of biosynthesis, and organic metabolism in tobacco, which led the way to other plants. Calcium, commonly known to be essential to cell wall structure, is the most reliable element for calculating dry matter changes on weight basis. Most scientists use calcium as the bench mark, as it is the principle element which does not "lose" or decrease mass in post harvest processing. This was first observed during leaf tobacco fermentation but was used by most scientists studying the postharvest loss of dry matter in crops.

Almost all heavy metals and rare elements are reported to be present in tobacco, including Al, As, Ba, B, Cs, Cr, Co, Cu, F, Au, I, Pb, Li, Mn, Hg, Mo, Ni, Pt, Po, Ra, Rb, Se, Si, Ag, Sr, S, Tl, Sn, U, V, and Zn. The presence of those elements may be accidental, acquired from soil or other sources. However, it led to studies on the elements' respective roles in plant growth and development. For example, the effect of B on plant growth was first noted in 1929, followed by reports on detailed progressive symptoms during various stages of plant growth under B deficiency in 1933. Later studies also reported that B, as well as Ca, plays a major role in organic metabolism. Our knowledge on the effects of B to the protein and other organic metabolic changes in general, and of alkaloid formation, translocation, and translocation of tobacco in particular, is built on the basis of that pioneering research.

Another notable example is Zn, the importance of which on plant growth and development was reported from early tobacco research in 1942. Now we understand that Zn plays a vital role in our own life and health. We need Zn to help decode DNA, to make proteins. In recent USDA research, Zn was found to play an important role in the health of the prostate. By the same token, Cu was found to be an important catalyst in leaf tobacco fermentation. That observation not only advanced catalytic chemistry, it also enhanced industrial progress involving fermentation.

1.3 Genetics

The tobacco plant provides beneficial material for genetic studies because of the availability of many *Nicotiana* species, their abundant seed production, and easy crossing. In addition, there are many distinctive chemical, physical, and botanical characteristics that provide numerous markers for scientific studies. The occurrence of natural mutations in tobacco was observed as far back as 1864.

In 1905, COMES classified existing forms of tobacco into six primary varieties. In 1933, GOODSPEED reported the number of chromosome pairs in 40 species of *Nicotiana* to range from 9 to 32. A plant with variations in its number of whole chromosomes is known as an aneuploid. For example, $2N-1$ is a monosomic and $2N+1$ is a trisomic. The number of chromosomes pairs in commercial tobacco is 24. By genetic manipulation, tobacco scientists in the early 1940s developed 24 monosomics; each monosomics contains 23 pairs plus one single chromosome, or one

chromosome is missing. In doing so, scientists identified which chromosome is responsible for which specific characters. Those studies were conducted long before we knew about DNA, or biotechnology, for plant research.

There are many different botanical classifications for tobacco plants. Commercial tobacco, or *N. tabacum*, is one of the 60 plus established species in the genus *Nicotiana*. This commercial tobacco does not occur naturally in the wild state. To establish the origin of commercial tobacco has been a challenging problem for generations of plant scientists. There were many hypotheses prior to a final conclusion through genetic findings that *N. tabacum* ($N = 24$) is a result of hybridization between two wild species ($N = 12$) after chromosome doubling. This conclusion was later confirmed by taxonomical, morphological, and biochemical examinations.

Tobacco fraction-1 protein is a unique genetic marker. Synthesis of this photosynthetic enzyme is regulated by both the nuclear and chloroplast genomes. Fraction-1 protein is identical to ribulose-1, 5-bisphosphate carboxylase-oxygenase (Rubisco). Analysis by electro-focusing of F-1 protein from *N. tabacum* and the putative progenitor species reconfirmed the origin of this commercial tobacco. Those findings also paved the way for the progress of future plant science.

Nicotine, the unique product of tobacco plants, provides another challenge to plant scientists searching for its biogenesis and biosynthesis, including why, how and where it is produced and what happens after its formation. This search began as early as 1934 and started with tobacco-tomato grafting, which led to broad applications in biochemistry and organic metabolism. Plant-grafting technology is now widely used for scientific studies. A recent application by USDA scientists is the grafting of watermelon onto squash or gourd rootstock to make firmer and healthier fruit. Nicotine is the most important alkaloid produced in tobacco and was investigated since the use of tobacco as smoking material. USDA research scientists examined the nicotine level within *Nicotiana* species and developed plants with various levels of nicotine for health-related studies. In addition, the history of various “family members” of the tobacco alkaloids, the 3-pyridal relatives including nicotine, nornicotine, and many others, was conducted to understand the biochemical pathway and genetic pattern. Such research did much to increase our scientific understanding, as well as contribute to health-related research.

Through advances of plant genetics in tobacco research, breeding tobacco plants for disease resistance became a routine process. For example, tobacco mosaic virus was first observed in 1886, described then as a “contagious living fluid”. The pioneering study on tobacco virus initiated in 1916 by ALLARD led to the virus’ isolation by STANLY in 1937 as a crystalline protein. Those basic studies on tobacco led to the development of modern virology. Because of the crop’s high economic value, many other tobacco diseases were reported as early as 1892, and breeding for resistant varieties started in the middle 1920s. Many distinguished scientists paved the way using tobacco for advancement of our knowledge. Now we are able to make further progress in science and technology because we are standing on the shoulders of those giants.

1.4 Chemical growth control

Tobacco is one of the most labor-intensive and end-use-sensitive crop. From seed germination to commercial products, there are hundreds of processing steps. Each step, acts as a single link in a long chain, may affect the more than 5000-plus chemical components known to be present in tobacco leaf and in smoke – in addition to influencing many critical physical characteristics essential for leaf quality, or even usability. Many chemicals have been developed to control growth and pests and to save labor, but all of them have to be free from any negative effects on leaf usability.

In 1979, research scientists examined 53 chemicals for pest control. Those chemicals included insecticides, acaricides, miticides, and nematocides. All of them were approved for tobacco use. Researchers made specific guidelines on when, how, how much, and where to apply them so as to meet quality and safety requirements. In addition, they also examined 16 others chemicals which were not approved for use on tobacco in the United States, but were used in other countries. All those chemicals were studied extensively for their disappearance and fate in tobacco, but the research findings had broad application on other crops when those chemicals were used.

In the 1970 and 1980, alternative approaches for pest control were first applied to tobacco. One was inoculation to induce plant immunity; another took a biotechnological approach by inserting the *Bacillus thuringiensis* (*Bt*) gene through genetic engineering. The inoculation approach did introduce plant resistance to blue mold and several other diseases, under controlled conditions. Even aspirin was among the compounds tested, but it was discontinued because it required excessive labor and caused some side effects on tobacco. That experience should not prevent scientists testing and applying the same approach to other crops. Insertion of the *Bt* gene is now commonly used for many other crops, especially cotton and maize. Many regulators from different countries are using an “absolute safety” requirement as an excuse for delaying releasing many other *Bt* crops, including tobacco. Viewed academically, an absolute “zero risk” of any new form of life or any new development is not possible.

Chemicals used for growth control usually can be classified into three groups: herbicides; compounds promoting certain physiological or biochemical actions; and compounds inhibiting specific growth at specific times. The most important in tobacco is inhibition of axillary bud growth after “topping”. During tobacco growth, it is necessary to remove the terminal inflorescence, or topping, in order to redirect the energy for leaf development to desired physical and chemical properties. However, because of this removal of apical dominance, axillary buds will grow and develop into branches (suckers) which would defeat the topping purpose unless they’re removed. This procedure, called *suckering*, is usually carried out repeatedly by hand on each tobacco plant. Chemicals used to replace this laborious hand-suckering are called sucker-control agents.

The chemical maleic hydrazide, or MH, was once widely used in the United States and around the world as a sucker-control agent. MH function is the inhibition of cell division, or mitosis, and layered organization in the tunica-carpus of

the apical shoot meristem, as well as DNA and RNA synthesis. However, when MH is applied to tobacco plants, it results in several undesirable effects on leaf quality and, thus, usability. Those effects include changing specific volume (filling power), total ash, and alkalinity of ash, sugar content, and equilibrium moisture content. With special authorization from the U.S. Congress, USDA scientists were requested to find a substitute for MH within a limited time. With more than 3000 compounds on hand, scientists believed it would be easy to screen and select one from them for use on tobacco. However, after repeated laboratory, field, and industry evaluations, none of those known compounds could meet the farmers' production requirement, industry usability requirement, and safety regulations. USDA scientists initiated an intensive search for naturally occurring plant compounds that could serve this special purpose. They finally discovered a new group of compounds that could inhibit axillary growth without causing undesirable side effects: the fatty compounds, including fatty acids, alcohols, esters, and some of their derivatives. This group of compounds is very specific. Even a change of one carbon chain length may change its function. The significance of this finding was of great importance for basic science, as well as practical application. It has been used for fruit thinning and other horticultural practices, and it also has great potential for grass growth control, which is labor-intensive.

Scientific knowledge obtained from tobacco plants has wide application to other areas. Certain natural sugar esters, secreted by the leaf hairs of wild tobacco plants to protect against, insect predators, kills certain insects when they rub against or chew the leaf. Based on this knowledge, analogs, or look-alikes, of the natural sugar esters are being developed as a new class of insecticidal compounds originally described by ARS scientists.

1.5 Physiological disorders

Physiological disorders may be result of many factors. In tobacco, other than those disorders induced by nutrition, disease, and pesticides and commonly known in many other crops, there are certain characteristic disorders that are of importance in plant science. They are physiological disorders generated from genetic and environmental factors. Here two kinds of disorders are reported: frenching, and genetic tumors. Another kind of disorder, air pollutants, will be discussed under public health section.

Frenching: Frenching is a noninfectious physiological disorder of tobacco. It usually appears as a network chlorosis of apical leaves, followed by formation of progressively narrowing leaves. In extreme cases there is no stalk elongation or expansion of lamina in younger leaves, which is known as frenching.

Extensive studies were conducted on the cause of frenching, including pathogenic organisms, soil pH, moisture, temperature, fertilizer elements (thallium, manganese), and chemicals (2,4-D), which can induce certain similar symptoms in greenhouses but not exactly the same as those in the field. Frenching occurs only in certain plots of the field. It cannot be transmitted from plant to plant by grafting, mechanical means, or dodder, but it can be readily transmitted from active to inactive media by

inoculation. Heat and chemical sterilization of active media (leachates, soils, sand, and vermiculite) eliminated their ability to induce frenching in plants.

Organic toxin produced by *Bacillus cereus* produced symptom patterns in seedlings' gross morphology that closely approximated those of frenching. Marginal inoculations of the medium were quite effective and demonstrated that the roots and bacteria need not be in contact to produce the symptoms. It is suggested that diffusates from *B. cereus*, and possibly other soil bacteria, may be the cause of frenching of tobacco in the field. Increased populations of the bacillus have been found in adjacent soil and rhizospheres of drenched plants. Diffusion products of this bacterium and the dead bacteria caused symptoms in aseptically grown tobacco seedlings.

Leaves of frenched plants are high in L-isoleucine. Free L-isoleucine causes frenching in aseptic culture. Subsequently, the effectiveness of various isomers of leucine and isoleucine in producing yellow strap-leaf was evaluated. Listed in order of decreasing effectiveness by soil application, they were: DL-alloleucine, D-isoleucine, L-isoleucine, and D-isoleucine. Although the absolute nature of the toxin has not yet been ascertained, it has demonstrated the complexity of this physiological disorder, as many factors may mask each other and the basic plant science research is being done with tobacco.

Genetic tumors: Certain *Nicotiana* hybrids produce teratoid proliferations of genetic origin. *Nicotiana* tumors may grow spontaneously on stems, roots, leaves, or inflorescence of tumorous F1 progeny or only on part of the tissue. The species involved in the production of tumorous interspecific *Nicotiana* hybrids can be separated into two groups, plus group and minus group. Hybrids among the plus group, or among the minus group, are free from tumors, suggesting that the critical contribution of the plus-parent to tumor production in the hybrid differs from the critical contribution of the minus-parents.

This phenomenon of tumor formation may have similarities in other biological systems. Considerable studies in detailed observation of its genetics, formation, and chemical changes were conducted. Evidence was provided that tumor formation is controlled by "conventional" genes that show segregation, linkage, and mutation, such as: a) tumor-forming *Nicotiana* species hybrids compromise genetic systems that control a precarious balance between normal and tumorous, b) the base factors controlling transformation in the hybrids are genes located in the chromosomes, and c) tumor induction depends on the accumulation of greater than regular amounts of growth-promoting substances.

Various morphological types of tumors were observed by induction of radiation on an interspecific hybrid, *N. glauca* × *N. langsdorffii*. Wounding of leaves of the same hybrid caused tumor formation at the site of the wound. The interaction of IAA, kinetin, and GA on tumor induction in seedlings of amphidiploids *N. suaveolens* × *N. langsdorffii* was observed. Kinetin does induce tumor formation, but IAA does not. However, IAA plus kinetin combined together was the most effective inducer.

Changes in chemical composition of tumorous *Nicotiana* plants are of great interest to scientists. Newly formed tumors accumulated high levels of free amino acids, along with variations in levels of principal alkaloids. Most

important, a sharp increase of scopolin and the new formation of scopoletin in the tumor tissue of *Nicotiana* hybrids were observed compared with the non-tumorous parent material. Observations of an association between the sudden increase of polyphenols and tumor formation was also very important for health scientists, as it is not an isolated case just in *Nicotiana* hybrids.

1.6 Organic metabolism

Extensive studies on organic metabolism were conducted in *Nicotiana* plants because the change of each compound may affect its quality and thus usability. Tobacco scientists closely observed the formation, transformation, and interaction of those organic compounds not only during the plant growth in the field, but during the post harvest handling processes of curing, aging, and fermentation, as well as during manufacturing, including interaction with additives and during blending. Those studies involved all phases of biological and physical sciences, and in turn the knowledge generated is broadly beneficial to all. For example, the tea, food, ornamental horticultural, and poultry industries all share the fruits of research using tobacco as a tool. The use of isotopes in tobacco research--such as triple labeling with ^{15}N , ^3H , and ^{14}C in the early 1950 in USDA was a pioneering breakthrough in the early stage of plant science metabolic research. With this new approach and the science of organic metabolism moving speedily forward, we learned that all organic components are dynamic in nature in any biological system. Following are a few illustrations conducted on organic metabolism with tobacco.

Alkaloids: Alkaloids, especially in the nicotine family, have been the main focus of tobacco research because alkaloids are the characteristic product of tobacco. Scientists always wish to know how they are formed, where they are formed, their function in the plant, what they do after their formation, and their role in the plant's organic metabolism. In other words, do they take an active part in the general organic metabolism among major plant compounds, such as carbohydrates, proteins, and lipids?

Alkaloids are a group of basic substances which contain a cyclic nitrogenous nucleus and are present in plants as well in animals. For example, anabasine, a tobacco alkaloid, is identified as a poison gland product in *Aphaenogaster* ants, in which it functions as an attractant.

Most alkaloids in *Nicotiana* plants are 3-pyridyl derivatives, with nicotine the principal alkaloid in commercial tobaccos. The early studies on the loci of alkaloid formation were mostly dependent on tobacco-tomato, or tomato-tobacco grafting, and they showed that tobacco roots were the loci of alkaloid formation, later translocated to the shoot via the xylem. Studies using isotopes showed that most of the precursors of tobacco alkaloids are formed in the root, but that both the roots and shoots of tobacco plants can form alkaloids independently. Although each *Nicotiana* species may have specific alkaloids, it can metabolize some foreign ones with its own metabolic system. Certain species and varieties are genetically determined to have high or low levels of various alkaloids. However, the alkaloid content in tumors of interspecific *Nicotiana* hybrids may accumulate 3 to 15 times higher than in respective healthy host

tissue. Alkaloids, once formed, are subject to interconversion and degradation, or they take an active part in the plant's organic metabolism. Observations of such degradation were most detailed during leaf fermentation, whether microbial or enzymatic.

Tobacco-specific *N*-nitrosamines have received great attention in laboratory studies, as well as in the production field, to gain insight on the formation of a potentially carcinogenic group of compounds. Various tobacco alkaloids, tobacco types, cultural practices, stalk positions, curing methods, processing, and additives all have important roles in the formation and level of various nitrosamines.

The presence of high concentrations of alkaloids in plant cells which would be toxic to animals, but has no similar effect on tobacco leaf is of great interest to biological scientists. There are many hypotheses regarding their functions, such as a) protection against insects and herbivores, b) detoxification products, c) metabolic reserves, d) regulatory agents, such as nicotinic acid, or e) waste products of metabolism.

Free nicotine is well known to be very toxic. It appears generally in the salt form when used as a pesticide. Spreading tobacco powder around the poultry house or bathing quarantined animals in a solution of "black leaf 40" are some old examples of nicotine use. It is of scientific interest why so many insects that attack tobacco can survive. The adaptation of insects to tobacco alkaloids is a fascinating problem. Green peach aphid is able to subsist on tobacco because it feeds in the phloem of the plant and avoids the nicotine-containing xylem. Tobacco hornworm may ingest and excrete nicotine without degradation. Several other insects, including flies, grasshoppers, tobacco flea beetles, and southern armyworms have been found to metabolize nicotine. The mechanism is not analogous to the production of cotinine when insects were treated with nicotine, nor it is similar to the metabolizing of nornicotine in mammals.

Proteins: Leaf protein is the most important and abundant protein in nature. Tobacco leaf, as well as the leaves of other higher plants, contains two classes of proteins: soluble and insoluble. Of the total tobacco leaf proteins, approximately half are soluble and half insoluble. Fraction-1 protein (F-1 protein) can be as much as 50 percent of the total soluble leaf protein. Soluble leaf protein other than F-1 protein, both chloroplastic and cytoplasmic, is called unfractionated protein. In tobacco, soluble protein can be further divided into two major categories on the basis of molecular size. One with a sedimentation value of 18S, representing a single protein, is fraction-1 protein; all the remaining smaller (4 to 6S) soluble proteins combine together to form the unfractionated, or fraction-2 protein. Generally, there are equal amounts of F-1 and F-2 proteins during earlier stages of plant development. Approaching maturation, however, this proportion is altered due mainly to the degradation of F-1 protein. F-1 protein has a molecular weight of approximately 550,000 and consists of eight large and eight small subunits arranged in two layered structures, each layer consisting of four large and four small subunits.

This F-1 protein is found in all organisms containing chlorophyll a, including the prokaryotic blue-green algae. F-1 protein is now known as ribulose-1, 5 biphosphate carboxy-

lase-oxygenase (Rubisco). This enzyme has a dual function in that it catalyzes the carboxylation and oxygenation of ribulose-1, 5 biphosphate. Therefore, it catalyzes the crucial reactions of both photosynthesis and photorespiration, the ratio of these two processes determining plant productivity. Tobacco leaf proteins contribute little to smoking quality, but they serve as precursors of several harmful smoke components, including quinoline, HCN, and several other undesirable nitrogenous compounds. In addition, several amino acids, especially tryptophan, glutamic acid, and lysine, have been reported to form mutagens when subjected to the high temperature of tobacco combustion. Therefore, removal of proteins from green tobacco leaf prior to the leaf curing processes may result in a better tobacco smoking product.

Through our research, a process was developed for extraction and separation of soluble proteins from green leaf tobacco before curing. Either whole young tobacco plants including stalks, or regular field plant tobacco leaves including midribs were harvested before reaching full maturity and homogenized into slurry that was filtered to collect the liquid portion. The green liquid suspension was centrifuged, and the supernatant was passed through a special column. Crystals obtained were purified and recrystallized to remove any possible bacterial contamination. After the crude F-1 protein had been collected, the mother liquid was processed to precipitate the remaining soluble F-2 protein. It was observed that the ratio of F-1 protein to chlorophyll varied with species, stage of growth, and conditions of development, within the range of 8-10 mg of F-1 protein per mg of chlorophyll. An approximately equal amount of F-2 protein was also present in these leaves. Removing the F-1 and F-2 proteins resulted in decreased precursors of many undesirable smoke products. The remaining solid leaf materials from the slurry were subjected to a newly developed homogenized leaf curing (HLC) process to achieve the required chemical and physical changes desired. The "cured" mess was then reconstituted into a sheet of favorable physical character needed for the making of smoking materials.

The amino acid compositions of the F-1 and F-2 proteins were nearly identical, with their relative amounts suggestive that both F-1 and F-2 proteins had high nutritional value. F-1 could be crystallized, while F-2 protein couldn't be crystallized but could easily be purified from any contaminants. The essential amino acid composition of tobacco F-1 protein was similar to that of egg and milk proteins. In rat feeding studies, we found that the average weight increment expressed as protein efficiency ratio (PER) from tobacco F-1 protein was significantly higher than that of rats fed the diet containing casein. The PER for F-2 protein was similar to that of F-1 protein. Those results showed that tobacco leaf proteins, F-1 and F-2, are at the top of the plant protein range in nutritive quality and thus could provide viable food as a by-product. In addition, soluble tobacco leaf proteins are of high nutritional value for medical use.

Metabolism of other organic compounds: Aside from alkaloids and soluble proteins that are of special importance in tobacco, other major components which are common in plants including carbohydrates, organic acids, pigments,

polyphenols, fatty compounds, phytosterols, and many other primary or secondary compounds were examined in detail by USDA researchers. Many findings are of significance to plant science specifically and to biological sciences in general, such as phenolic and fatty compounds as mentioned in above sections.

However, curing, aging, and fermentation are special processes in tobacco post harvest treatment. Knowledge developed from tobacco research is being widely valued in enzymatic science, catalytic chemistry, microbiology, as well as in the tea and wine industries.

In tobacco technology, curing refers to the changes undergone by harvested fresh leaves under favorable conditions of temperature and humidity. It is a vital process and falls into the category of starvation phenomena, or inanition, of excised plant parts. The purpose of curing is to produce dried leaf of suitable physical properties and chemical composition. Respiratory losses, involving hydrolysis and oxidative deamination may lead to a 20% decrease in dry matter with a slow curing method. Various regimes of ventilation, temperature, and humidity control are employed to achieve results considered desirable for different types of tobacco. For example, it takes several weeks for stalk-cut air-cured tobacco to become dry, but the primed, or picked, leaves of flue-cured tobacco become dry in four or five days at elevated temperatures.

The most conspicuous chemical conversions during curing involve several phases. The first phase is dominated by activities of hydrolytic enzymes and occurs in either flue curing or air curing. In this phase, disaccharides and polysaccharides hydrolyze to simple sugars; proteins hydrolyze to amino acids that undergo oxidative deamination; pectins and pentosans are partially hydrolyzed to pectic acid, uronic acid, and methyl alcohol; and there is little or no loss of dry weight. The second phase of conversion is dominated by oxidative reactions and takes place primarily in air-cured tobaccos. These conversions include the oxidation of simple sugars to acids, CO₂, and H₂O; increased oxidative deamination of amino acids to form ammonia and amides, particularly asparagines; the changes in organic acids, including the conversion of malic to citric acid and decarboxylations; and oxidation and polymerization of phenols to brown products and a small decrease of alkaloids. An appreciable loss of dry weight occurs, particularly in those leaves cured on the stalk, some of which may be due to translocation between leaf and stalk.

The changes in leaf tobacco during curing, aging, and fermentation are results of complicated physical, chemical, and biological processes. Chemical, catalytic, enzymatic, and bacteriological activities may all be involved during the changes from green vegetable materials to aromatic products. According to their relative stability or potential for change, the main components of freshly harvested green leaf may be classified into three groups. The static group are more inert toward changes and includes crude fiber; pentosans; inorganic substances; ether-soluble components; and pectins, tannins, and oxalic acid. The nitrogen group may undertake limited changes and includes insoluble N or protein; soluble N including ammonia, amino compounds, nitrates, amides, and alkaloids. The dynamic group is the least stable and consists mainly of carbohydrates, ether-soluble organic acids, and some yet-to-be-identified compounds.

Freshly cured tobacco leaf is unfit for use because of its pungent and irritating smoke. By the process of aging and fermentation, the leaf delivers mild and aromatic smoke. Aging is generally applied to cigarette tobacco, allowing a mild fermentation. Fermentation, or sweating, is usually applied to cigar tobacco and is characterized by high initial moisture content (may reach 50 percent), by generation of heat, and by 10 to 20 percent loss of dry weight. During the fermentation process there is evolution of CO₂, ammonia and other nitrogenous compounds, and methyl alcohol; an uptake of O₂; a change of pH; a change in water retention; and an improvement of fire-holding capacity.

The aging of tobacco is comparatively a much milder process than fermentation. The first aging process is to redry the cured leaf and bring it to uniform moisture content (9–10 percent). During aging, little self-heating is taking place. Small amounts of CO₂, acetic acid, formic acid, and ammonia are evolved during aging. There is an increase in moisture and a decrease in sugar, total nitrogen, water-soluble nitrogen, amino nitrogen, nicotine, total acids, and pH. It is essentially a chemical process, the main reaction being that between sugars and amino compounds with the formation of melanoidins and CO₂.

2 TOWARD PUBLIC HEALTH

The first report of tobacco smoking contributing to ill health was officially released by the U.S. Surgeon General in 1964. That report led to extensive research on tobacco and tobacco smoke, as well as many other aspects of society health and quality of food and life. In this chapter, however, I will limit my discussions only on three areas which affect all plants and general public health. Those three areas include radioactive elements, mycotoxins and air pollutants.

2.1 Radioactive elements

The presence of radioactive elements, such as beta and alpha emitters, in leaf tobacco and tobacco smoke has been reported in many publications. Naturally occurring beta activities, such as ⁴⁰K and isotopes of rubidium, strontium, and cesium were negligible and believed to be of no significance.

However, the alpha-emitting radioactive isotopes were suggested to be of significance in that they tend to be localized and accumulate in bronchial epithelium. The total activity varies with tobacco types and products. For example, in cigarette tobacco there was reported 0.61–1.88 pc/g, including 0.08–0.22 from ²²⁶Ra and 0.06–0.19 from ²²⁸Ra; in cigar tobacco, it was 0.9–9.75 pc/g, including 0.18–0.74 from ²²⁶Ra and 0.04–1.35 from ²²⁸Ra. The alpha activity also varied widely in tobacco grown in soils with different levels of radioactivities.

Most research efforts conducted by plant scientists in this area are aimed toward identifying the source of radiation and finding the means to reduce or remove it. In addition to ²¹⁰Po, the associated radionuclides ²²⁶Ra, ²¹⁰Pb, and ²¹⁰Bi are found in leaf tobacco. It was believed that the source of ²¹⁰Pb in tobacco is independent of that of ²²⁶Ra or its

daughter ²¹⁰Bi. A series of studies was conducted to examine the source of ²¹⁰Po and ²¹⁰Pb in leaf tobacco. Scientists grew plants under various experimental conditions, including an environmental chamber enriched with ²²²Rn in the atmosphere, or in field and greenhouses with different sources of phosphate-containing fertilizer, and also in nutrient culture containing ²¹⁰Pb. They concluded that the major portion of the ²¹⁰Pb in tobacco plants was absorbed through the root. The polonium seems not to be derived entirely from the radium within plants; plants may take it directly from soil. In one study, ²²²Rn concentration in the chamber was maintained to approximately 50 pc/liter, which is about 500 times the level that occurs in the normal atmosphere. This result showed that ²²²Rn and increased concentration of ²¹⁰Pb in the air is not a major source of ²¹⁰Po in tobacco.

Many agronomic factors were observed to affect the ²¹⁰Po and ²¹⁰Pb levels in tobacco. The amount of phosphate fertilizer applied and the cropping system used contribute to the wide variation of ²²⁶Ra, ²¹⁰Po, and ²¹⁰Pb activities in the soil. Phosphate is the main source of ²²⁶Ra, ²¹⁰Po, and ²¹⁰Pb in fertilizer. The amount of radiation carried by fertilization to the field may not be reflected in the radiation level of the immediate crop. The continued addition of fertilizers with high levels of radioelement would certainly build up these elements in the soil, which would be available to subsequent crops. In addition, the distribution, translocation, and accumulation of those radioelement may be of significance. Tobacco seedlings were found to accumulate ²¹⁰Pb and ²¹⁰Po to a concentration much higher than that in the soil in which they grew. Direct aerial absorption of ²¹⁰Po is considered a major source of ²¹⁰Po supply; in addition to that from soil uptake of ²¹⁰Pb accumulate in leaf tobacco.

In bioassays of tobacco smoke condensate with small animals, some alpha-emitting particles were observed in the lungs. The possible continuous exposure to radiation in a fixed location is of concern to health scientists. However, tobacco crops are different from other crops, as tobacco is used mainly for combustion. If necessary, phosphate fertilizers can be purified to be free from ²²⁶Ra and its daughters but at a rather high cost. On the other hand, food crops grown all around the world in contaminated soils under cultivation for years with phosphate are almost impossible to be clean and free from radioactive elements.

2.2 Mycotoxins

Many microorganisms were found on tobacco plants and leaves during all stages of field production and postharvest processing, including curing and fermentation. It is, therefore, a reasonable concern whether any of the toxins produced by those microorganisms would contribute to consumer health problems.

Generally, tobacco and many other plant systems automatically respond by a mechanism to produce phenolic compounds as a regulatory measure upon unfavorable conditions. The regulatory system governs formation of a particular enzyme in plant tissues. The enzyme, phenylalanine ammonia lyase (PAL), initiates the chemical activities that lead to formation of phenolic compounds. This regulatory system also provides a feedback capacity by the formation of another

protein capable of destroying the PAL enzyme as soon as sufficient phenolic compounds are produced.

When *N. tabacum* or *N. glutinosa* plants were infected with tobacco spotted wilt virus, a fluorescent substance was present in a halo ring around the necrotic lesions. This substance was identified as scopoletin. The accumulation of scopoletin and scopolin in tobacco plants was observed as a response to injury by bacterial, viral, fungal, chemical, or mechanical agents, or even as a consequence of aging or lack of growth. In the tumorous tissue of *N. glauca* × *N. langsdorffii* hybrids, and a sharp increase of scopolin and the new formation of scopoletin was found. This response appears to be associated with tumor formation, which did not occur in either of the parents.

In tissues adjoining infected xylem vessels of tobacco plants infected by the wilt-inducing bacterium *Pseudomonas solanacearum*, an increase of scopolin and scopoletin was found. In stem tissues, a threefold increase of scopolin was found 48 hours after inoculation, and an eightfold increase after 120 hours. Such a rapid increase of scopolin appeared to be associated with rapid multiplication of bacteria in the stem. An increase in scopoletin followed a similar pattern, but it occurred a few hours later at more rapid rate than that of scopolin.

Aspergillus flavus is found in most tobacco leaves. The organism is known to produce aflatoxins that are extremely toxic and carcinogenic. In defined medium, *A. flavus* may use aromatic amino acids as precursors for the formation of aflatoxin B and aflatoxin G. In addition, phenylalanine and tyrosine have also been found to be incorporated into aflatoxin B1 by *A. flavus*. Although tobacco plants are rich in these two aromatic amino acids, there is no evidence that aflatoxins may be synthesized per se in tobacco. In examination of various types of cured tobacco leaves, scientists failed to find the presence of either aflatoxin. Furthermore, researchers injected a high amount of aflatoxin B1 into manufactured cigarettes and found it was destroyed or transformed by elevated temperatures during combustion.

2.3 Air pollutants

Plants are sensitive or more sensitive to air pollutants than animals and can serve as an advance warning system. For example, tobacco is known to be very sensitive to air pollutants, and in particular, to ozone which induces the widely recognizable “tobacco weather fleck” in the open field.

Air pollutants are collectively called “smog”, which is a mixture of gaseous oxidants in the atmosphere resulting from phytochemical reactions of which ozone is the major component. Under normal situations, the concentration of ozone, or O₃, is low by its continuous reaction with NO to form NO₂ and O₂. On the other hand, if gaseous hydrocarbons (exhaust gases) are present in the atmosphere, they become oxidized, which in turn oxidizes NO to NO₂ without destroying ozone. The consequence is that ozone accumulates and thus becomes a major component of smog.

Many factors contribute to air pollutants, but energy conversion is the most significant source. The conversion products include water, carbon dioxide, carbon monoxide, sulfur dioxide, sulfuric acid, hydrogen sulfide, nitric oxide, nitrogen dioxides, hydrogen fluoride, ethylene, ozone, aldehydes, soot, and hydrocarbons. Those pollutants not

only damage plant quality and reduce crop yield, they also are a serious health problem. Symptoms begin to appear on tobacco with a low level of pollutants, which may serve as a warning for factory construction, air movement, and other corrective measures.

Weather fleck is one of the early findings about plants’ sensitivity to pollutants. In general, air pollution causes injuries that are visible either as small patches of brown necrotic areas covering the upper surface of leaves or the margins of leaf tips. Those visible injuries are the result of the localized death of previously living tissues that starts to occur about 24 hours after exposure to air pollutants. “Invisible” injuries, on the other hand, may involve impairments of the photosynthetic system in the mesophyll cells, a change in the stomatal control of gas exchange, or changes in enzymatic activities, such as peroxidases.

3 POLITICAL CORRECTNESS AND ACADEMIC RESEARCH

In 1492, Columbus introduced tobacco to Europe. In 1558, Portugal began to grow tobacco. Commercial production of tobacco soon extended to every corner of the world. Even at the very beginning, tobacco was and still is a controversial commodity politically, socially, and economically. On the other hand, almost every country which consumes tobacco products grows tobacco if its soil and climate conditions allow. In the course of history, tobacco usage has experienced frequent ups and downs, reflecting a changing political climate. This down climate was particularly true in the European countries of the past, and lately it is very strong in the United States.

3.1 *The U.S. mandate on tobacco research; policies change with time*

Tobacco is a highly valued cash crop around the world, especially in the United States because of its ideal soil and climate conditions, plus advanced technology which produces an excellent quality tobacco leaf. In fact, tobacco-producing states and farmers enjoy the abundant revenue and returns, as quality tobacco is a domestic and international commodity in high demand.

In the United States, public support of funding for tobacco research at federal and state agencies, land-grant universities, as well as independent institutes, has been made available from various levels of government in the past. Presently no federal funding and in certain states no state funding is available for tobacco commodity research, but public funding is available for fundamental plant science research. Tobacco-related taxes, even today, are a major source of national and local revenue, and the crop provides increased opportunities for economic growth and development.

The association between tobacco use and health hazard was first suggested and reported in the press many years ago, but it wasn’t seriously considered by the general public or government. One of the earliest suggestions of risk was that tobacco smoking may cause tuberculosis, not lung cancer. In early 1964, the U.S. Surgeon General Committee on Smoking and Health indicted that cigarette smoking contributes significantly to ill health and early death,

particularly in connection with cancer of the upper alimentary and respiratory tracts; chronic bronchitis and emphysema; and cardiovascular disease. The committee summarized its findings in a statement that said: "Cigarette smoking is a health hazard of sufficient importance in the United States to warrant appropriate remedial action." However, following this report, the U.S. Government did not initiate any immediate action or provide special funding for identification, reduction, or elimination of the possible "hazardous" materials in tobacco or in smoke.

On January 30, 1964, USDA's Agricultural Research Service (ARS) administrator led a team of six scientists to a meeting at the National Cancer Institute (NCI) to discuss possible research approaches and cooperation between USDA and the U.S. Department of Health, Education, and Welfare (HEW) on what USDA might do to promote a less hazardous tobacco. Among the major research areas suggested were: examining the mechanism of smoke formation; identifying hazardous smoke compounds and their respective precursors; removing or reducing those precursors in tobacco plant via selecting germplasm, breeding, and cultural practices; controlling the formation of undesirable components during production and post-harvest periods; and developing new curing methods. At that time, ARS plant scientists initiated several important studies with existing funding. Those studies included searching for mycotoxins, searching for the source of alpha-emitting particles, and using labeling technology to identify possible leaf components as precursors of harmful smoke components. Information generated from these early studies in plant science provided important basic information for developing the total U.S. national program on smoking and health.

In 1965, the U.S. Congress reviewed the Surgeon General report on smoking, heard testimony from medical witnesses, determined that cigarette smoking was a significant health hazard, and required a cautionary label to be put on cigarette packages. In addition, in August 1965, the Surgeon General 1964 report on smoking and health was officially released to the general public. Later, the 1967 Surgeon General report on the health consequence of smoking substantiated and expanded on the basic conclusions of the 1964 report.

Two years after the 1964 Surgeon General report, the U.S. Congress began to authorize additional funding through USDA that was earmarked for tobacco research at land-grant universities, especially to the University of Kentucky and North Carolina State. However, there was no significant funding increase for the USDA's in-house tobacco research program.

In 1967, three years after the 1964 Surgeon General report on tobacco smoking, President Johnson asked that a Lung Cancer Task Force (LCTF) be established in his annual Health Message to Congress. That mission fell to the National Cancer Institute (NCI), and the LCTF was established in July 1967. That task force and its subgroups were only concerned with research on various aspects of lung cancer. A separate group, the Committee on Smoking and Health established by the Surgeon General, was concerned with conducting educational and motivational studies related to smoking, plus collecting and disseminating information on smoking and health.

Soon after establishment of the LCTF, a group of leading NCI scientists visited the USDA Beltsville tobacco research site and was briefed on the progress of projects involving alpha-emitting particles, mycotoxins, and other plant-phase research that could serve as a basis for promoting the tobacco group functions of the task force. In addition, USDA scientists discussed with NCI leaders the drafting of a master plan for developing a less hazardous cigarette, from the point of view of plant science, in a programmatic scheme. It was finalized September 30, 1967, and received support from all tobacco-interested institutions, including NCI, industry, and academic institutions.

In the same year, the Committee of Commerce, Consumer Subcommittee, of the U.S. Senate, held a two-day hearing to review progress toward development and marketing of a less hazardous cigarette, heard the testimonies of several expert witnesses, but this hearing did not include any discussion of funding or project authorization.

In late 1967, the National Advisory Council of the NCI concurred that there would be three working groups within the LCTF: one on a less-hazardous cigarette; one on atmospheric-industrial-occupational hazards; and one on clinical management, with emphasis on early detection and diagnosis. The council also recommended that monies be made available for task force activities. This author was invited by the chair of LCTF, and authorized by USDA, to serve as a member of this group in February 1968. The less-hazardous cigarette group, at its initial meeting on March 11, 1968, was initially composed of 10 members, all from government and known health experts. Leading scientists from the tobacco industry were invited to join as members at the first formal group meeting, as this project definitely needed their expertise, as well as the active participation of the tobacco industry. The first meeting also strongly recommended that special federal funding be made available for the program outlined.

Many experimental tobacco samples generated by USDA had to be evaluated through the mechanism and funding of the NCI program, such as the studies of smoke chemistry and various forms of bioassay. In 1972, at the recommendation of the Tobacco Working Group (TWG, originally the less-hazardous cigarette group), the Secretary of HEW wrote to the Secretary of USDA to request more support for the development of less-hazardous cigarettes by supporting tobacco plant research, in addition to projects that were associated with the TWG. Those projects included extremely expensive bioassays using animal inhalation of smoke from plant samples originating from USDA.

The various projects under TWG were proposed and approved by members of this group of experts and health scientists representing the best minds in this area and were funded by the U.S. Congress to the NCI, from 1968 to 1978. During this period, much progress was made toward developing less-hazardous tobacco. However, the political climate changed in 1978 in favor of abolition of smoking, leading to the termination of TWG and all the projects it funded.

In the 14th annual report on the health consequences of smoking from HEW to the Congress in 1981, 3 years after termination of TWG, the Surgeon General concluded that "the single most effective way to reduce the hazards associated with smoking is to quit". While the TWG

projects generated abundant new information, they also generated many additional questions needing answers. Type and nature of the bioassays became significant for the conclusions to be made. There were different systems using different subjects with different smoke products. One can always question where the end point is, and which system is the best model. In addition, animal inhalation takes time and is extremely expensive, in part because there are too many variables in plant samples, as well as too many variables in commercial products, not even considering smoker variables.

At the termination of TWG, there was a need for a new mechanism for communication, especially to exchange information around the world, updating progress on various smoking and health projects still active in different institutes, identifying highest research priorities, developing a list of prospective investigators, and most important, considering which agency might best meet these needs. For this propose, an Interagency Meeting on Smoking-Related Research was initiated and coordinated by the American Cancer Society (ACS), chaired by the former Surgeon General, with only 10 expert members including two from HEW and one from USDA, this author. The first meeting was conducted in September 1978 in Washington, DC; other meetings were held during American Chemical Society meetings in New York City. This effort lasted only for four years, due to lack of funding.

Another Interagency Forum on Health-Related Tobacco Research was organized in 1978 by USDA, mainly for information exchange among federal and state agencies, universities active in tobacco plant research, tobacco-health research institutes, and industry-related research institutes. The forum ended in 1985, prior to the termination of all federal tobacco research programs, including USDA's.

3.2 Tobacco research in land-grant universities and other institutions

Various tobacco research projects were conducted at federal research laboratories, land-grant universities, and independent institutes of an academic or health-related nature. Most of them were production-oriented, some of them health-related, and others totally academic in nature using tobacco as a research tool. Funding sources included Congressional authorization, state or local governments, industry, or independent foundations. Among them, the University of Kentucky was the most active and productive in both production and health-related studies, and both basic and applied research. Most of the research findings were published as scientific reports.

Generally speaking, all tobacco research supported by public funding has been of benefit to the progress of plant science, not limited just to tobacco as a crop. Research facilities of different scales are still available in several tobacco-producing states, even since termination of federal funding. In addition, many research scientists from various independent academic institutions have frequently used tobacco as a research tool and, with it; have made breakthrough findings in life sciences. Use of tobacco as a research tool has been essential to the advancement of science, both in the past, and I predict in the future.

4 THE BENEFITS OF USING TOBACCO AS A RESEARCH TOOL

4.1 One of the most valuable

As is well known, tobacco research has contributed greatly toward the advancement of plant and biological sciences. Examples mentioned above included work on photoperiodism; genetics and breeding, including parasexual hybridization and molecular genetics; growth regulators; pollutants; viruses; nutrition "hunger" signs; photosynthesis and photorespiration; organic metabolism and postharvest physiology; and many other natural processes. Furthermore, since we are still in the dawn of science, the use of tobacco as a research tool is only at its beginning.

Tobacco plants are easy to grow and have a short growing period. Each tobacco plant may produce 14 g or about 150,000 seeds which may provide seedlings for 2 to 5 acres (1–3 ha) of field tobacco, depending on the type. In addition, cell culture or tissue culture technology is well developed for both research and commercial proposes.

Within the genus *Nicotiana* are 60 plus known species, some of them known as ornamentals, but each one well examined as to its genetic, physiological, botanical, and chemical characteristics. The somatic chromosome numbers vary between 18 and 48; the commercial, smoking product species, *N. tabacum*, has 48 or 24 chromosome pairs.

A plant with variations in its number of whole chromosomes is known as an aneuploid. For example $2n-1$ is a monosomic and $2n+1$ is a trisomic. The 24 monosomic lines of *N. tabacum* have been characterized on the basis of their mostly identifiable features. Those monosomics are of special importance for research because they represent ideal material for locating genes on a specific chromosome. In addition, they are of great research value for examining the transfer of single chromosomes between genera, species, or varieties.

Since production of *N. tabacum* is of such economic importance around the world, academic tobacco research is needed to meet the local needs of production, achieve special chemical composition, or as material for academic research on various projects. In almost every phase of plant or biological sciences, tobacco can serve as a valuable tool, in the dawn of science as well as in the frontier of science.

4.2 Most abundant scientific information

Tobacco research has generated abundant scientific publications and a wealth of valuable knowledge. In 1970, when this author was preparing the monograph "Physiology and Biochemistry of Tobacco Plants" only about 9,000 tobacco publications were available for references. However, in 1989, when this author was preparing the second monograph "Production, Physiology, and Biochemistry of Tobacco Plants" almost 60,000 additional publications were found on tobacco and tobacco-related subjects. Those publications were generated during the period between 1971 and 1989. At its height, 60 or more scientific publications on tobacco were published each week. The surge of strong interest in tobacco research during that period may be attributed to many causes, but it mainly falls into the

areas of chemistry, genetics, plant physiology, organic metabolism, plus some biological evaluation of specially-produced plant material. Significant progress was made in pioneering research, especially in the fields of biotechnology, molecular biology, biotic and abiotic stress physiology, and others.

Beginning in 1989, the change of political climate has led to less funding support for tobacco research, and thus reduced the frequency of publications on tobacco. However, today over 120 million sites will be returned on a web search on tobacco, but most will not be associated with plant science. In the peak period of tobacco research, about 3,000 active research scientists were working on tobacco in public and industrial laboratories, not including those at independent academic institutions. Currently, the total number of tobacco scientists is greatly reduced, especially in U.S. public institutions. Many plant scientists in academic institutions cannot obtain grant support for projects using tobacco as a research tool. Some even have to avoid tobacco because of the applying of "political correctness" to academic research. The tobacco plant has served as a valuable tool since the dawn of plant and biological sciences, so it is indeed a great loss to scientific progress that a research tool already invested with so many resources and about which there is such abundant knowledge and such great potential for new advancement is now being wasted.

5 BEYOND THE FRONTIERS OF SCIENCE

Contrary to general public opinion, tobacco has many valuable uses of high economic value other than smoking. As we face the health hazards of smoking, we need to realize the benefits of using tobacco for many other purposes, not only as a tool for pioneering science, but also for uses such as food, medicine, and many others. Following are merely a few illustrations.

5.1 Tool of pioneering science

Molecular biology and genetic engineering offer new and additional tools to meet agricultural needs. Working with single genes rather than a whole plant or animal provides a great advantage in biological science. The most noted advantage is specificity. For example, the insertion of *Bt* genes began with trials on tobacco. SCHELL *et al.*, in the late 1970 and early 1980 suggested three far-reaching major possibilities using tobacco: a) using tobacco cell cultures to produce valuable drugs, exploiting both the available biosynthetic capacity of tobacco cells and specific activities resulting from the introduction of new genes; b) modifying tobacco cultivars through genetic engineering to obtain plants with improved resistance towards various viral, bacterial, fungal, or nematode pathogens; tolerance to biotic or abiotic stresses; or with modified chemical or physical characteristics; and c) breeding of tobacco for production of food.

Of course, there are many other potential applications common to all plants, including tobacco, such as nitrogen fixation, pesticide and herbicide resistance, and tolerance to environmental and physiological stress. The best approach

is to use tobacco as a research tool, based on its abundant knowledge and rich resources to develop new information for all biological sciences.

The extent of the potential benefit of this new technology with tobacco is only limited by the availability of basic knowledge and further development. Once available, it should be easy to extend to other crops. For example, within *Nicotiana* species, there are short-day, neutral-day, and long-day photoperiodic plants as in many other plant or biological systems. These are represented by Maryland Mammoth type, regular cultivated tobacco type, and *N. glauca*, respectively. If the light-inducible genes can be identified and constructed, it would be possible to construct light-inhibiting genes in order to combine those three plant types and create a high-yield, high-quality, and low-labor-intensive plant.

5.2 Food

Rubisco (fraction-1) and fraction-2 proteins isolated from tobacco leaf are of high nutritional value, as well as clinically useful. The biological efficiency of fraction-1 as a mammalian nutrient is very high. Its amino acid breakdown is very similar to that of human and/or cow milk, far superior in this respect to soybean protein. Fraction-1 protein cannot be crystallized out from soybean or indeed from other crops readily. In addition, there are indications that fraction-1 protein can be used for clinical or medical purposes. Fraction-2 protein also has high nutritional value as human food, only a little below that of fraction-1, but is superior to that of soybean protein. Both fraction-1 and fraction-2 are colorless crystals, although in bulk they appear as white powders. They are tasteless and consist entirely of amino acids. The insoluble proteins and some other valuable materials (e.g. xanthophylls) could also be removed in a subsequent solvent extraction and purification process, but further research is needed. To increase protein yield, the desirable direction is a plant with increased photosynthesis and decreased photorespiration. In addition, there is also a need to increase the efficiency of solar energy utilization. The key is to find how to maintain the balance of essential biochemical processes to achieve this goal, since some essential building blocks for protein formation are products of photorespiration. Searching for edible protein from plant sources is not new. Many problems observed in other crops are also found in tobacco during the process of protein recovery. Certain phenolic compounds have to be removed in order to obtain the final protein products suitable for food or feed.

5.3 Medicine

Tobacco plants have long been selected to produce plant-made vaccines. A recent survey from Arizona State University demonstrated strong potential public support for plant-made vaccines. The use of transgenic plants for production and oral delivery of vaccines has been shown effective in animal trials and phase-I human clinical testing, although it is known that public acceptance of genetically-modified (GM) foods is variable on a global scale.

Some of the most recent research conducted by DANIELL at the University of Central Florida, partially funded by

USDA and NIH, demonstrated the benefit of using tobacco as a vehicle for saving lives. One acre of GM tobacco plants was shown capable of producing enough anthrax vaccine to inoculate the entire U.S. population safely and inexpensively. To create the anthrax vaccine, the vaccine gene was injected into the chloroplast genome of tobacco cells. Testing of the vaccine so produced with mice showed it to be very effective, and DANIELL is expanding his research, working with tobacco-grown treatments for type-1 diabetes, hepatitis C, plague, and cholera. Approaches using genetically engineered tobacco plants will greatly reduce the cost of plant-based vaccines.

AFTERWARD: TREAT TOBACCO WITH RESPECT

In this historical review of tobacco research in the U.S., this author has noted numerous academic contributions using tobacco as a research tool since the dawn of plant science development and has reported the changing political climate leading to reduction, even to the near extinguishing, of research using tobacco tissue or tobacco plants. Some anti-tobacco activists, including a few officials from various branches of the U.S. government, ignoring the scientific value of the tobacco plant, have condemned all research related to it.

Knowing that tobacco is a valuable research tool and has great potential for the future, this author recognizes that the use of tobacco is only at its beginning, and that we need to treat tobacco with respect. As we are facing the challenges of environmental damage, disease, hunger, and energy shortages in the future, we will need to have available every means to meet those challenges. Tobacco can, and will, help solve some of these problems. The basic knowledge built on past research on tobacco is already in hand. What we need now is intensive work on transferring this basic knowledge to industrial applications. In addition, we need to formulate a complete infrastructure to make new tobacco-oriented industries economical and beneficial to all concerned.

Conventional tobacco crops are estimated to be capable of producing 2–4 percent of leaf yield as fraction-1 and fraction-2 proteins, which is only 40–80 pounds per acre (lb/A), or 45–90 kilograms per hectare (kg/ha). Plant protein content is at a maximum before the onset of leaf senescence. Preliminary indications suggest that cutting the whole plant when it reaches a height of 12–18 inches (30–45 cm) would optimize protein yields. Increased plant population, plus multiple harvesting of second or even third growth of tobacco leaf, would certainly increase the protein harvest. A reasonable expectation of total soluble protein yields would be in the range of 750–1,200 lb/A, or 840–1,344 kg/ha. Considering the high nutritional and clinical value of soluble proteins and the use of plant residues for energy biomass, further in-depth research on tobacco protein production is highly desirable.

The world is searching for sources of renewable energy, with most attention concentrated on new methods of making ethanol, not just from corn, but from wood chips, plant stalks, or grasses. We need to make this biomass-based ethanol practical and competitive. Obviously corn is needed for food and feed, especially in nations short of arable land or with huge populations. Different countries

may use to their advantage whatever native resources are available. For example, Brazil can use sugarcane-derived sucrose and directly ferment it with yeast to make ethanol. In Europe, wheat, barley, and rye are being used as starch sources. In the United States, we're generally using corn-starch broken down into glucose by amylase and amyloglucosidase enzymes and then fermented into ethanol.

The most practical way, is of course to use "waste" agricultural materials of little or no commercial value, such as residues from tobacco as mentioned above. Those materials are mostly cellulose and would have to be broken down by certain "digestive" processes followed by degradation with cellulose enzymes to obtain glucose and other five- or six-carbon sugars.

Past experience has taught us many other uses for tobacco-derived materials, although currently not in practice or being replaced by newer materials and technology. Some of those applications, however, might show us how we got where we are and also may serve as references for future applications. In the not too distant past, tobacco waste leaves and stems in powder form were used effectively by the poultry industry for pest control. Tobacco "water", a watery solution containing small amount of nicotine ("black leaf 40"), was used to quarantine imported animals and for controlling aphids in orchards and on other plants. This naturally-occurring organic compound can easily be decomposed, unlike some synthetic chemicals that may pollute the environment. In addition, growing tobacco plants to remove heavy metals in polluted area could be an effective and economic approach to bioremediation.

In addition to the need for intensive research, there is a need for a systematic approach to better production of potential tobacco products into commercial and competitive industries, combining all the possibilities mentioned above. A solid infrastructure is, therefore, needed to make tobacco production economically profitable as a commodity, and scientifically respectable as a research tool.

REFERENCES

1. The United States Department of Agriculture: After a hundred years; The Yearbook of Agriculture 1962, pp. 688, The U.S. Government Printing Office, Washington, D.C., 1962.
2. Tso, T.C.: Physiology and biochemistry of tobacco plants; Dowden, Hutchinson & Ross, Inc., Stroudsburg, Pennsylvania, USA, 1972.
3. Tso, T.C.: Production, physiology, and biochemistry of tobacco plant; International Institute of Development and Education in Agriculture and Life Sciences (IDEALS), Beltsville, Maryland, USA, 1990.

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